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(INCLUDING ABSTRACTS)

INVESTIGATION OF THE CLAY MINERAL PROTECTION THEORY FOR NON-HYDROLYSABLE NITROGEN IN SOIL

By J. R. FRENEY and RAYMOND J. MILLER

The stability of clay minerals and synthetic clay mineral–nitrogen complexes in boiling 6 N hydrochloric acid was studied to determine whether clay minerals in soil could protect nitrogen compounds or ions from solution.

The crystal structure of uncomplexed clay minerals, such as montmorillonite, appeared to be destroyed by boiling acid. Interlamellar ammonium did not reduce the solubility of these clay minerals in boiling acid, nor was the ammonium ion protected from solution. However, a number of organic nitrogen compounds (in particular quaternary ammonium salts) markedly reduced the solubility of montmorillonite. In addition the clay mineral reduced the solubility of organic nitrogen compounds in boiling acid. This reduction in solubility did not appear to be due to humin formation or to adsorption of nitrogen compounds on the amorphous residue produced from acid treatment of montmorillonite. The results suggest that a clay mineral protection theory for non-hydrolysable nitrogen is tenable.

Introduction

Most of the recent information concerning the chemical nature and biological stability of organic nitrogen in soil has been obtained from hydrolytic studies using boiling acid or alkali.[1,2] These treatments do not dissolve all of the soil nitrogen,[3,4] and few attempts have been made to study the chemical nature of the nitrogen in the insoluble residues.

A number of hypotheses have been advanced to account for the nitrogen in the acid-insoluble residues.[2,5] These suggest that it may be present as: (a) acid-insoluble humin, produced by condensation of amino acids (mainly tryptophan) and furfural released by acid hydrolysis of protein–carbohydrate mixtures; (b) heterocyclic nitrogen compounds; (c) acid-insoluble complexes formed by reaction of phenolic compounds with inorganic nitrogen or amino compounds; or (d) it may be trapped in clay mineral lattices. Published results[3,4] suggest that little nitrogen is converted to an acid-insoluble form by humin formation during acid hydrolysis, and no evidence is available to support hypotheses (b) and (c).[2]

Although published work[6–12] on the stability of clay minerals in boiling acids suggests that uncomplexed clay minerals which could trap nitrogen (e.g. montmorillonite) are rapidly degraded, Freney[13] found that a fraction of soil nitrogen appeared to be protected from acid solution by silicates. The present paper attempts to account for the apparent contradiction by reporting a study on the solubility of uncomplexed clay minerals and synthetic clay mineral–nitrogen complexes in boiling acid.

Experimental

Clay minerals

The montmorillonite clay was prepared by saturating Wyoming bentonite with sodium. Excess sodium was removed by repeatedly washing with distilled water and separating the two phases by centrifugation. Other clay minerals were obtained from Ward's Natural Science Establishment, Rochester, New York. Those which were not of clay size were ground with mortar and pestle and the clay fraction (< 2 μm) was separated by sedimentation. These clays were not sodium saturated before use.

Preparation of organic nitrogen–montmorillonite complexes

These complexes were prepared by addition of saturated solutions of the organic compound in water to clay sus-

pensions (7 g clay/100 ml water) in amounts sufficient to flocculate the clay. In some cases, it was necessary to add a small amount of acid to bring about coagulation. Excess of the organic compound was removed by washing the flocculated material with distilled water, and the resulting product was dried at 100°. This material was ground with an automatic mortar and pestle for 20 min and then sub-samples were subjected to the various treatments described below.

As no information was available concerning the chemical nature of non-hydrolysable nitrogen in soil, the choice of nitrogen compounds was arbitrary. Some compounds were selected because they had been isolated from soil, and others because it was thought that they should form stable complexes with the negatively charged clay mineral, montmorillonite.

An attempt was made to prepare complexes which contained organic nitrogen compounds in the interlamellar spaces only of the clay minerals, by treatment of complexes, prepared as described above, with alkaline potassium hypobromite solution. The procedure used was the same as that proposed[14] for the removal of organic nitrogen and readily exchangeable ammonium from soils for the determination of fixed ammonium. It was thought that the presence of the potassium would prevent reaction of the hypobromite with the interlamellar organic nitrogen compounds in the same way as it prevents reaction with interlamellar ammonium ions.

Preparation of interlamellar ammonium–clay complexes

These were obtained by saturating the clay minerals with N ammonium chloride solution, centrifuging to remove the excess solution, heating the residue in an oven at 105° for 24 h and then leaching the dried clay with N potassium chloride solution to remove ammonium ions from the external exchange sites. The resulting products were dried at 105° and ground with an automatic mortar and pestle for 20 min. The resulting products were analysed for nitrogen and subjected to 6 N acid treatment as described below.

Acid treatment

The sample (0·5 g clay or clay complex) was boiled for 24 h with 20 ml 6 N hydrochloric acid in a 100 ml round-bottom flask, attached to a water-jacketed reflux condenser, on an

electric heater. The solution was filtered off through a Whatman No. 42 filter paper and the residue was well washed with 6 N hydrochloric acid. The filtrate and washings were combined, diluted to a known volume and analysed for aluminium and nitrogen. The weight of the residue was determined in other experiments by filtration through a washed, weighed, sintered glass crucible. Complete transfer of the residue from the flask to the crucible was effected with the aid of a polyethylene policeman. The residue was washed, dried, weighed, analysed for nitrogen, and subjected to X-ray diffraction analysis.

Control experiments were also carried out in which the organic compound alone was boiled with hydrochloric acid, and then filtered as described above. The filtrate was analysed for total nitrogen.

To determine the capacity of the residue from 6 N-HCl treatment of montmorillonite for adsorption of nitrogenous compounds 1 g of the residue was boiled with 100 ml of 6 N-HCl containing 10 mg N as trimethylcetylammonium bromide (Cetavlon) or cetylpyridinium chloride for 24 h. The resulting mixture was filtered through a Whatman No. 42 filter paper and well washed with 6 N-HCl. The residue on the paper was analysed for total nitrogen by the micro-Kjeldahl method.

X-ray diffraction analysis

The clay minerals, clay–mineral complexes, and residues from acid treatments were subjected to X-ray analysis. Efforts were made to obtain oriented specimens for analysis by allowing the samples to dry slowly and by addition of sodium oxalate solution to the slide before drying. A Norelco diffractometer and goniometer, with copper radiation operated at 50 kV and 15 mA, or cobalt radiation at 40 kV and 24 mA, were used for the diffraction analyses.

Chemical analysis

Total nitrogen was determined by a micro-modification of the method described by Piper.[16] Nitrogen present in residues from potassium hypobromite and hydrochloric acid treatments was also determined by shaking the residue (1 g) with 20 ml of 5 N-HF/1 N-HCl solution at 20° for 24 h. The mixture was filtered through a Whatman No. 42 filter paper and nitrogen was determined on an aliquot of the filtrate by the micro-Kjeldahl method.

Organic carbon was determined by a dry combustion method[16] and aluminium was determined by the Eriochrome Cyanine R method[17] or by the emission spectograph.

Results and Discussion

Reaction of uncomplexed clay minerals with hydrochloric acid

Comparison of the X-ray diffractograms obtained for the clay minerals montmorillonite (see Fig. 1), meta-bentonite, nontronite, vermiculite and chlorite with their acid-treated residues showed that, apart from the presence of small amounts of a micaceous impurity in some samples, the crystal structure of the clay mineral appeared to be destroyed. All of these minerals had their characteristic d spacings before acid treatment, but exhibited no such spacings after acid treatment.

Some workers[18,19] have used extractable aluminium as an index of the solubility of clay minerals, and it has been shown that the crystal structure of montmorillonite is destroyed when 75–85% of the aluminium is removed from the lattice.[19]

TABLE I

Dissolution of clay minerals in boiling 6 N hydrochloric acid* as indicated by aluminium analyses

Clay mineral	Total aluminium, mg Al/g clay	Aluminium dissolved mg Al/g clay	% of total Al
Montmorillonite	115	100	87
Kaolinite	170	170	100
Biotite	150	114	76
Muscovite	189	20	11
Illite (Beaver's Bend)	108	63	58
Illite (Fithian)	126	105	83
Illite (Morris)	118	107	91

* 24 h digestion

The data presented in Table I support and extend published reports on the instability of many minerals in boiling acid. More than 75% of the aluminium was dissolved from montmorillonite, biotite, Morris illite, Fithian illite and kaolinite but considerably less from Beaver's Bend illite and muscovite (see Table I).

Solubility of clay mineral–organic nitrogen complexes

Comparisons were limited to the solubility of a sized clay mineral with and without organic nitrogen compounds or ammonium ions, to avoid effects of complicating factors such as structure (whether dioctahedral or trioctahedral), or particle size, on solubility in acid.

Using the amount of aluminium dissolved as an index of the amount of clay dissolved, it can be seen from Table II that the presence of organic nitrogen compounds reduced the solubility of the clay mineral. This finding was also supported by the dry weight data. The clay–organic complexes did not dissolve to the same extent as the montmorillonite alone (see Table II). In addition, Table II shows that some of the nitrogen was not dissolved from the montmorillonite-organic complexes by the acid treatment. The amount of nitrogen retained in the residue varied from complex to complex and was greatest for the trimethylcetylammonium (Cetavlon)– and cetylpyridinium–montmorillonite complexes.

Control experiments showed that in the absence of clay mineral the organic nitrogen compounds were completely soluble in boiling hydrochloric acid and that there was no detectable change in the solubility of the nitrogen compounds when boiled with 6 N hydrochloric acid for 24 h. It appears unlikely that the insoluble nitrogen remaining was due to the formation of organic polymers (humin-like material) during acid treatment, unless the montmorillonite catalysed the production of such insoluble organic polymers.

The residues from hydrochloric acid treatment of trimethylcetylammonium– and cetylpyridinium–montmorillonite complexes (i.e. those which retained most nitrogen) were shaken with 5 N-HF/1 N-HCl solution for 24 h and filtered. Aliquots of the filtrate were tested for the original organic nitrogen compounds by descending paper chromatography and Dragendorff's reagent.[20] These results showed that considerable amounts of the original trimethylcetylammonium and cetylpyridinium ions were still present in the 6 N hydrochloric acid-treated residues. In addition, the filtrates from these residues, after solution in 5 N-HF/1 N-HCl solution still retained the surface active properties of the original com-

Solubility of montmorillonite and montmorillonite–organic nitrogen complexes in boiling 6 N hydrochloric acid* as reflected by aluminium, nitrogen and dry weight determinations

	Total N, μg N/g clay or complex	Aluminium dissolved, % original Al	% of original material dissolved	N remaining	
				μg N/g residue	% of original N
Montmorillonite (M)	63	87·0	45·2	21	33·3
Creatinine–M	32,420	72·8	42·1	2,390	7·4
Citrulline–M	11,000	72·7	32·7	885	8·0
Ethylenediamine–M	18,800	71·4	35·8	705	3·8
Arginine–M	16,260	71·1	35·0	641	3·9
Pyrrolidine–M	16,650	69·5	28·8	1,110	6·7
Glycocyamine–M	11,160	68·0	38·5	765	6·9
Urea–M	16,360	67·6	29·7	790	4·8
Cetavlon–M	4,790	65·4	31·8	4,160	86·8
Ornithine–M	4,925	65·0	37·1	459	9·3
Histidine–M	6,245	64·8	36·3	162	2·6
Cetylpyridinium–M	2,940	59·0	26·5	2,730	92·8

* 24 h digestion

pounds. The results suggest that the nitrogen retained was associated with the clay mineral and was not present as acid insoluble organic polymers because these would have been removed by filtration of the hydrofluoric acid residue mixtures.

X-ray diffraction studies of the organic nitrogen–clay mineral complexes after acid attack showed that the association of trimethylcetylammonium and cetylpyridinium ions with montmorillonite protected part, at least, of the crystal structure of the clay mineral from destruction (Fig. 1). The association of organic nitrogen compounds (other than quaternary ammonium) with montmorillonite resulted in residues which contained nitrogen, but no crystal structure could be detected in these residues by X-ray diffraction.

An attempt was made to prepare clay mineral–organic nitrogen complexes which contained (i) organic nitrogen in the interlamellar spaces only or, (ii) organic nitrogen on the exterior surfaces only, to determine whether the positioning of the organic compound affected the solubility of the complex in acid. The results in Table III show that considerable nitrogen remained in the clay complex after the potassium hypobromite treatment and it is assumed that this must be interlamellar nitrogen, as the organic compounds, in the absence of montmorillonite, were soluble in potassium hypobromite. The resulting products were relatively stable in boiling 6 N hydrochloric acid, and the results in Table III show that nitrogen was retained in the acid-treated residues.

As kaolinite and muscovite do not have expanding lattices little organic nitrogen should penetrate the lattice and, thus, most of the organic nitrogen associated with these minerals should be on the exterior surfaces. The presence of pyridinium ions, equivalent to the nitrogen added to the montmorillonite complex, did not reduce the solubility of these two minerals in boiling acid. These results suggest that the decreased solubility of the clay–organic nitrogen complex was due to the presence of the organic compound in the interlamellar spaces of montmorillonite.

The amorphous residue from 6 N-HCl treatment of montmorillonite was reacted with Cetavlon and cetylpyridinium chloride in boiling 6 N-HCl for 24 h to determine if it could absorb nitrogenous compounds. Total nitrogen determination showed that the residue had no capacity to adsorb nitrogenous compounds.
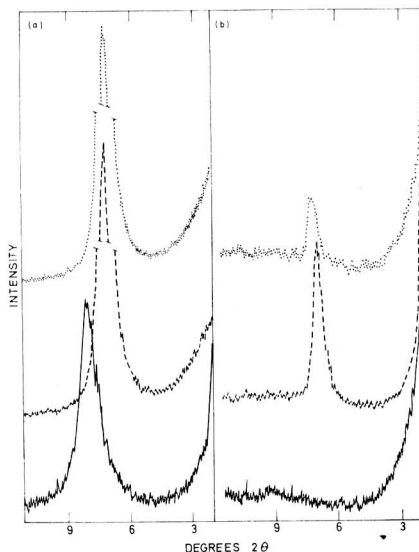
FIG. 1. *Effect of acid treatment on X-ray diffraction patterns of montmorillonite (———), cetylpyridinium–montmorillonite complex (– – – –), and trimethylcetylammonium–montmorillonite complex (. . . . .)*
Cobalt radiation
(a) Untreated; (b) boiled with 6N-HCl for 24 h

TABLE III

Total nitrogen in montmorillonite–organic nitrogen complexes after treatment with potassium hypobromite and 6 N hydrochloric acid

μg N/g complex or residue

	Untreated complex	Complex treated with KOBr	KOBr-treated complex boiled with 6 N-HCl for 24 h
Montmorillonite–Cetavlon	4790	4590	4312
Montmorillonite–cetylpyridinium	2940	2370	1974

TABLE IV

Solubility of clay minerals and clay mineral–interlamellar ammonium complexes in boiling 6 N hydrochloric acid*
as reflected by aluminium, nitrogen, and dry weight determinations

Clay or complex	Total N, μg N/g clay or complex	Aluminium dissolved, mg Al/g	% of original material dissolved	N remaining, μg N/g residue
Vermiculite	112	56	53·5	63
Vermiculite + interlamellar NH_4^+	5180	63	54·4	98
Nontronite	168	29	59·8	70
Nontronite + interlamellar NH_4^+	2670	33	54·4	19
Meta-bentonite	616	90	33·0	35
Meta-bentonite + interlamellar NH_4^+	1140	89	33·7	28
Fithian illite	1120	77	35·3	390
Fithian illite + interlamellar NH_4^+	1386	80	34·3	370

* 24 h digestion

Methylpyridinium bromide, cetylpyridinium chloride, and trimethylcetylammonium bromide (Cetavlon) all formed acid-stable complexes with montmorillonite and thus it appeared that the stability was related to the strongly basic character of the cations and not to the length of the side chain (cetyl vs. methyl) or the to type of nitrogenous radical (pyridinium vs. ammonium).

Solubility of clay mineral–interlamellar ammonium complexes

The presence of inorganic ammonium in the interlamellar spaces of vermiculite, nontronite, meta-betonite or Fithian illite did not reduce the solubility of these minerals in boiling hydrochloric acid, as judged from the amount of the solid (see Table IV). The nitrogen results in Table IV suggest that all of the added ammonium was dissolved by the boiling acid as there was no significant difference between the results obtained for the residues from individual pairs of minerals. However, some nitrogen was left in the residues and this was greatest for the illite residues. Nitrogen, equivalent to about a third of the indigenous nitrogen (1120 μg N/g clay), was not dissolved by the boiling acid. The reason for the retention of nitrogen by the illite is not known but it may be due to the presence of organic compounds in the clay which impart some stability to the clay, similar to that shown above. Samples of illite were analysed for organic carbon and were found to contain 0·36%. X-ray diffraction patterns for the untreated and acid-treated clay complexes also suggest that interlamellar ammonium did not impart any stability to these clay minerals.

Conclusions

The above results show that it is possible to form clay mineral–organic nitrogen complexes which are relatively stable to attack by boiling acid. The association of quaternary ammonium compounds with montmorillonite results in mutual partial protection of the clay mineral and of the nitrogen from solution in acid. The results suggest that nitrogen was not retained in the residue by humin formation or by adsorption on the amorphous residue produced by 6 N-HCl treatment of montmorillonite.

The results obtained lend support to a clay mineral protection theory for the occurrence of non-hydrolysable nitrogen in acid-treated soils. Some of the clay residues

which contained nitrogen did not appear to be crystalline when subjected to X-ray analysis and it is possible in these cases that nitrogen is trapped in some manner in the siliceous residues.

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EFFECTS OF GAMMA IRRADIATION ON THE AVAILABLE NITROGEN STATUS OF SOILS

By B. R. SINGH* and Y. KANEHIRO

Five Hawaiian soils were irradiated at various levels of gamma irradiation and analysed for available (NH₄ and NO₃) nitrogen content immediately after irradiation, and after periods of incubation. The release of NH₄-N generally increased with increasing dosages in all soils, while NO₃-N decreased in some soils. Incubation studies showed that the loss of NO₃-N continued even after several days of irradiation. The rate of NH₄-N mineralisation in irradiated soils was highest during the first seven days and declined later on.

Introduction

Subjecting soils to gamma irradiation has been a recent approach in the study of changes in available nutrient status of soils, of which change in mineral N content is of considerable importance. Gamma irradiation is reported to increase N mineralisation in soils.[1-8] Bowen & Cawse[1] noted that the release of NH₄-N was directly related to dosages of gamma radiation. They believed that N comes from decomposition of organic matter present in soils. Popenoe & Eno[9] reported that nitrate production in a 14-day period was progressively reduced by such treatments. Their finding was later supported by Bowen & Cawse[10] who observed a decline in the amount of nitrate in the solution of soils irradiated at 5 Mrad. At lower levels of irradiation, however, Cawse[2,3] noted a faster rate of nitrification. Recently, Cawse & White[11] reported an increase in nitrate content of soils irradiated over the range of 0·25–2·4 Mrad, maximum accumulation being between 0·25–0·75 Mrad.

The work reported here was undertaken with two major objectives: firstly, to study the changes in available N status of soil as a function of gamma radiation dosages, and secondly, to study the effect of incubation on the rate of mineralisation and accumulation of NH₄- and NO₃-N in irradiated soils.

Experimental

Five Hawaiian soils, Akaka silty clay (Typic Hydrandept), Wahiawa silty clay (Tropeptic Eutrorthox), Paaloa silty clay (Humoxic Trophohumult), Koko silty clay loam (Ustollic Eutrandept), and Lualualei clay (Typic Chromustert) were used in this investigation. The soils differ widely in physical, chemical, and mineralogical properties owing to differences in their parent material and the environment under which they developed. Soil samples from the surface of these soils

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were collected and placed in double polyethylene bags and brought to the laboratory. All the soils were passed through a 20-mesh sieve, except the Akaka which was passed through a 10-mesh sieve because of its high water content. These soils have been described in an earlier paper[12] and some of the properties are included in Table I.

Soil samples (25 g for the Akaka and 50 g for other soils on an oven-dry basis) were placed in polyethylene bags (4 × 3 × 8 in). The soils in the bags were brought to the moisture equivalent (except the Akaka soil to which no water was added) and mixed well. The bags were sealed with an electric sealer and irradiated with a ⁶⁰Co gamma radiation source at 0, 0·5, 1, 3, and 5 Mrad. Available N was determined immediately following irradiation (about 15 min post-irradiation) by the extraction-distillation procedure of Bremner[13] using MgO for NH₄-N and 100-mesh Devarda's alloy for NO₃-N.

In another experiment the Akaka, Wahiawa, and Lualualei soils were irradiated at 0, 0·5, 1, and 3 Mrad as above and were subsequently incubated in the laboratory for 0, 7, 14, 21, and 28 days. Available N was determined at the end of each period.

Results

Effect of varying radiation dosages on NH₄-N

The data in Table II show that NH₄-N content generally increased with increasing dosages of radiation in all soils. The greatest release was in the Akaka followed by Lualualei and Koko, the radiation effect being significant at the 1% level in all cases. The differences in the magnitudes of mineralised NH₄-N between 0 and 5 Mrad in these three soils were 32, 17, and 14 ppm N, respectively. Although there was an increasing trend in the Wahiawa soil, the differences in the magnitudes of mineralised NH₄-N owing to successive dosages were statistically non-significant, but they were significant at the 5% level in the Paaloa soil. On the whole the NH₄-N content in these soils increased with

TABLE I

Some properties of the experimental soils

Soil type	Elevation, ft	Rainfall, in	Soil pH	Total N, %	Organic C, %
Akaka silty clay	1500	225	4·7	0·81	14·60
Wahiawa silty clay	700	50	4·9	0·17	1·44
Paaloa silty clay	1000	75	4·5	0·25	3·26
Koko silty clay loam	50	25	7·2	0·29	3·12
Lualualei clay	20	20	7·6	0·10	1·06

TABLE II

Effect of gamma radiation dosages on soil available nitrogen

Soil		Dosage, Mrad	ppm N		
			NH_4	NO_3	$NH_4 + NO_3$
Akaka		0·0	3	308	311
		0·5	8	307	315
		1·0	16	291	307
		3·0	24	247	271
		5·0	35	210	245
	F value		**	**	
	L.S.D. at 5%		8·1	7·7	
Wahiawa		0·0	21	25	46
		0·5	21	28	49
		1·0	24	28	52
		3·0	26	25	51
		5·0	28	20	48
	F value		n.s.	n.s.	
Paaloa		0·0	98	112	210
		0·5	111	113	224
		1·0	101	111	212
		3·0	104	99	203
		5·0	104	87	191
	F value		*	**	
	L.S.D. at 5%		6·8	4·6	
Koko		0·0	50	97	147
		0·5	52	95	147
		1·0	57	107	164
		3·0	64	105	169
		5·0	64	108	172
	F value		**	*	
	L.S.D. at 5%		6·1	6·5	
Lualualei		0·0	19	9	28
		0·5	24	11	35
		1·0	26	14	40
		3·0	34	13	47
		5·0	36	12	48
	F value		**	n.s.	
	L.S.D. at 5%		4·4	—	

n.s. = non-significant
*   = significant at the 5% level
** = significant at the 1% level

irradiation in Paaloa, Wahiawa, Koko, Lualualei, and Akaka, in order of increasing magnitude.

Effect of varying radiation dosages on NO_3-N

Table II shows a consistent decrease in NO_3-N content with increasing dosages in the Akaka and Paaloa soils, and a slight decrease at 5 Mrad in the Wahiawa soil. The decrease in magnitudes of NO_3-N owing to successive dosages were highly

significant in the former two soils, but non-significant in the latter soil. A small increase was noted in the Koko (significant at the 5% level) and Lualualei (statistically non-significant) soils.

Effect of radiation dosages after periods of incubation

Data pertaining to this aspect of gamma irradiation study are given in Table III for the Akaka, Wahiawa, and Lualualei soils. Since irradiated soils behaved differently, they will be discussed individually.

Effect on the Akaka soil

The data in Table III show that NH_4-N content was more in irradiated than unirradiated Akaka soil. The magnitudes of mineralised NH_4-N were higher in samples irradiated at 0·5 Mrad at respective periods of incubation. This was followed by those irradiated at 1 and 3 Mrad. The rate per day of NH_4-N mineralisation was highest for the first seven days. Irradiation at 0·5 Mrad was very effective, giving a maximum ammonification rate of 8·2 ppm N during the first seven days. This was followed by treatments at 1 and 3 Mrad where the rates were 7·0 and 5·9 ppm N per day, respectively, during the same period. The ammonification rate declined during subsequent incubations in all cases, although the overall magnitude of NH_4-N owing to a particular radiation dosage increased with the length of incubation.

The nitrification was much higher in the control than irradiated soil. It is interesting to note from the data in Table III that, except for the control, the magnitude of NO_3-N decreased with the length of incubation in irradiated Akaka soil at all dosages. Evidently, the loss of NO_3-N continued even after several days of irradiation.

Effect on the Wahiawa soil

The overall magnitude of the mineralised N in this soil (Table III) was very low compared to that in the Akaka soil. The irradiated soil followed the same trend as the Akaka with a few exceptions, but the rate of ammonification or nitrification was too low to draw any plausible conclusion. In fact, the effect of radiation dosages or periods of incubation on NH_4- or NO_3-N release was not very appreciable.

Effect on the Lualualei soil

The behaviour of this soil was different than the former two soils. Almost all released NH_4-N was nitrified in un-irradiated samples (Table III). Nitrification also proceeded in samples irradiated at 0·5 Mrad where magnitude of NO_3-N was highest at all periods of incubation. This shows the stimulative effect of gamma radiation to nitrifying organisms

TABLE III

Effect of incubation on available nitrogen content (ppm N) of gamma irradiated soils

Soil	Dosage, Mrad	Days							
		7		14		21		28	
		NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Akaka	0·0	5	328	3	335	7	348	6	358
	0·5	60	273	93	244	129	244	154	207
	1·0	52	270	74	249	95	230	121	221
	3·0	44	291	51	283	70	283	81	280
Wahiawa	0·0	24	31	24	26	27	27	24	28
	0·5	25	22	25	24	29	25	29	25
	1·0	22	24	24	24	25	25	29	22
	3·0	24	22	27	19	29	23	27	22
Lualualei	0·0	1	33	1	40	2	47	1	45
	0·5	20	31	19	43	15	59	11	62
	1·0	36	31	40	30	42	38	39	43
	3·0	39	11	47	10	59	11	64	12

in the soil However, higher dosages of radiation were detrimental to nitrifying organisms present in this soil as is indicated by an increase in the magnitude of NH_4-N and decrease in that of NO_3-N at all periods of incubation of samples irradiated at 1 and 3 Mrad.

Discussion

The NH_4-N mineralisation generally increased with increasing dosages of radiation in all soils, and this is consistent with results of Bowen & Cawse[1] who reported that the release of NH_4-N was directly related to dosages of gamma radiation. There are three possible ways in which NH_4-N may be released from soils by irradiation. Firstly, a chemical action of irradiation could produce ammonia from nitrogenous organic compounds by a variety of processes, notably deamination of amino acids[14,15] and protein.[16] Nitrogen release from the fractionation of organic molecules containing N is possible because ionisation is thought to split molecules in random locations throughout the organic fraction of the soil.[5] Secondly, McLaren and co-workers[17,18] have shown that several enzymes, including urease which produces ammonia as a decomposition product, are functional in irradiated soils. Thirdly, some N is thought to be released from the dead organisms owing to subsequent lysis of microbial cells.[4,5] Relative to the theories mentioned above, the greater release of NH_4-N in the Akaka soil could be attributed to its high organic matter content (Table I).

On the basis of their NO_3-N content following irradiation, the experimental soils can be grouped in two categories: (i) soils exhibiting nitrate loss – Akaka, Paaloa, Wahiawa; and (ii) soils exhibiting nitrate gain – Koko, Lualualei. A consistent decrease with increasing radiation dosages in the NO_3-N content of the Akaka, Paaloa, and Wahiawa soils was noted immediately following irradiation. In other words, there was a loss of the initial NO_3-N in these soils during the process of irradiation. Cawse & Crawford[19] noted nitrite accumulation in irradiated soils, and concluded that nitrate reduction is the principal cause of nitrite formation. Also, they observed that nitrate reduction occurs even during irradiation. Allison[20] proposed the formation and decomposition of NH_4NO_2 as a mechanism in gaseous N losses from soils. If nitrite had formed in the present study, then a similar reaction might have occurred during irradiation of

soils. The other possibility might be the decomposition of so formed nitrite at low pH (Table I) of these three soils. In other words, the soil pH might also be involved in nitrate loss from irradiated soils. While studying the recovery of added NO_3-N from the experimental soils, Singh et al.[12] noted decreased recovery from all the five soils used in this investigation, regardless of their pH, after irradiation. It may be noted that the recovery of NO_3-N was almost 100% from unirradiated soils. Therefore, the possibility of low soil pH being involved in NO_3-N loss from irradiated soils seems to be doubtful. Bacterial enzymes have been reported to be very radioresistant[21] and function in irradiated soils.[18] It is possible that these enzymes are involved in NO_3-N loss from experimental soils either by reducing nitrate or by other pathways. It is further speculated that certain chemical reactions rendering NO_3-N loss are enhanced by irradiation. The accumulation of NO_3-N in irradiated Koko and Lualualei soils is supported by the finding of Cawse & White[11] who found an increase in nitrate content of soils irradiated over the range 0·25–2·5, with maximum accumulation between 0·25–0·75 Mrad.

In the experiment involving incubations, the maximum rate of ammonification during the first seven days following irradiation could be attributed to the rapid ammonium release from nitrogenous organic compounds owing to deamination of amino acids[14,15] and protein.[16] This could also be due to fractionation of N-containing organic molecules.[5] The most contributory source could be dead organisms that release N during their lysis.[4,5] As pointed out previously, the high magnitude of NH_4-N in the Akaka compared to the other two soils could be attributed to its high organic matter content.

The reduction in ammonification rate during successive incubations after seven days in Akaka and Wahiawa soils was due to the corresponding increase in the nitrification rate by recovering nitrifiers.

The reduction in the native nitrate content of irradiated Akaka, Paaloa, and Wahiawa soils analysed immediately after irradiation was pointed out earlier. Similar results with irradiated Akaka soil were also observed in this set of experiments where NO_3-N loss continued even after several days of irradiation. The loss of nitrate from the Akaka soil could be attributed to the causes discussed earlier.

The Lualualei soil has been reported to possess a very high nitrifying capacity.[22] This property explains the complete nitrification of NH_4-N in unirradiated, and very good nitrification in samples irradiated at $0 \cdot 5$ Mrad. This is substantiated by the report of Cawse & White.[11] They noted increased nitrate accumulation in soils irradiated over the range $0 \cdot 25$–$2 \cdot 5$ Mrad. However, this was not the case in the Akaka and Wahiawa soils. A dosage of 3 Mrad certainly inhibited nitrifiers because nitrification was almost completely arrested at this rate. It can be concluded that the response of soils to gamma radiation dosages is greatly dependent upon the type of soil and its physical and chemical composition.

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RHEOLOGICAL PROPERTIES
OF WHEAT FLOUR DOUGHS

I.—Method for determining the large deformation and rupture properties in simple tension

By N. W. TSCHOEGL,* J. A. RINDE† and T. L. SMITH**

A method and an apparatus are described for determining the large deformation and rupture properties of wheat flour doughs in simple tension. Dough rings are submerged in a liquid of matching density to prevent the dough from deforming under its own weight and are stretched at constant rates of extension until rupture occurs. Stress–strain curves obtained at different temperatures and extension rates on doughs prepared from a medium strength flour and a weak flour are presented, and the effects of rest period, mixing time, and mixing atmosphere on dough properties are discussed.

Introduction

Wheat flour doughs are subjected to considerable deformation during the make-up and baking process. Although this is commonly recognised, few attempts have been made to describe the large deformation behaviour of doughs in terms of fundamental quantities. Almost nothing is known about the fundamentals of the ultimate (rupture) properties of dough, even though extensibility and strength are important in the baking process.

Many industrial dough testing machines (e.g., the Brabender extensograph, the Chopin alveograph, and others) subject the dough to large deformations until rupture occurs, but the test records cannot be interpreted in terms of stress and strain.[1-4] The problem arises because of the difficulty in devising a suitable procedure to deform a dough piece while it maintains a well defined geometry so that the load–deformation record can be translated into a stress–strain relation.

To obtain stress–strain data on doughs, a method similar to that commonly used[5,6] to determine the large deformation and rupture properties of elastomers in simple tension can be used. In this procedure, rubber rings are stretched at constant rates until they rupture. To apply this method successfully to a ring of wheat flour dough, two main modifications are necessary. First, the dough rings must be tested while submerged in a liquid of matching density to prevent sagging of the ring under its own weight. Secondly, an 'effective circumference' must be determined, since (contrary to rubber rings) dough rings do not slip freely around the supports on which they are suspended.

In this paper, an apparatus and a procedure for determining the large deformation and ultimate properties of dough rings are described. In addition, the selection of the buoying liquid and the effect of rest period, mixing time, and mixing atmosphere on dough properties are discussed. Also, stress–strain curves obtained at different temperatures and extension rates on doughs from a medium strength and a weak flour are presented. A detailed analysis of the stress–strain and ultimate property data for these doughs under a variety of test conditions have been given in another paper.[7]

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Experimental

Flours

Doughs from two flours were studied. A major portion of the study was made on a medium-strength unbleached commercial bakery flour milled from Kansas hard red winter wheat and a small percentage of hard red spring wheat. It had a protein content of 12·0% (14% moisture basis), and contained 0·45% ash and 13·5% moisture. The baker's water absorption was 60%. Because it was experimentally more convenient to work with relatively stiff doughs, the tests were made on doughs with considerably lower water absorptions than those normally used in baking. Quoted water absorptions are on the basis of 14·0% moisture.

To contrast the properties of doughs from the medium strength flour with those from a weak flour, tests were also made on doughs prepared from a low-protein Lemhi flour – a straight-grade commercially milled unbleached flour of relatively poor baking quality. It had a protein content of 8·0% and contained 0·55% ash and 12·6% moisture. Protein and ash contents are on a 12·6% moisture basis.

Because of the weak character of the Lemhi flour, it was much more difficult to obtain reliable tensile date on dough rings of it than on Kansas flour. Often a test had to be repeated several times owing to the propensity of the ring to stretch non-uniformly. Weak doughs are apparently more sensitive to small heterogeneities caused perhaps by incomplete mixing and by small variations in ring dimensions. Both factors may cause non-uniform stretching of a ring, giving an unsatisfactory test. The weak character of the Lemhi flour doughs also made it necessary to adjust the density of the supporting fluid rather accurately.

Apparatus

The apparatus, shown in Fig. 1, consisted essentially of two specimen supports between which a dough ring was stretched vertically. The upper (stationary) support was attached to a 16 oz Statham load cell (No. G1-16-350) at the top of the apparatus. The lower (movable) support was attached to a crosshead, capable of 24 in of vertical motion along a ½ in half-round guide shaft. A wire fastened to the crosshead passed under a freewheeling pulley at the bottom of the guide shaft, up around the driving pulley, and back to the crosshead, thus forming a closed loop.

The supports, crosshead, guide shaft, and lower pulley were surrounded by 4 × 4 in square transparent Lucite
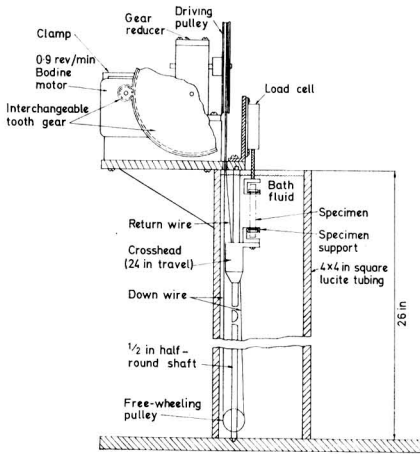
FIG. 1. *Schematic diagram of apparatus to test dough rings at a constant extension rate*

FIG. 2. *Diagram of the dough ring and cutter*
(a) Front view of cutter; (b) section of dough ring

tube filled with a liquid whose density matched that of the dough (selection of the liquid is discussed later). The temperature was controlled by circulating the liquid through heat-exchange coils immersed in a constant temperature bath. The buoying liquid not only facilitated temperature control but also prevented the dough from drying out.

The driving system was mounted on the same platform as the load cell. The drive consisted of an $0 \cdot 9$ rev/min constant-speed Bodine motor, an interchangeable gear train, a speed reducer, and the driving pulley. By using different combinations of gear trains, crosshead speeds between about $0 \cdot 2$ and more than 50 in/min can be obtained. The output from the load cell was recorded on a Hewlett-Packard Model 7100B Moseley Autograph with Model 1750 1A input amplifier. The load cell was calibrated by dead weights.

Preparation and dimensions of ring specimens

Doughs were mixed in a Swanson mixograph to standardise the mixing conditions as much as possible. First, 30 g of flour were placed in the mixograph bowl, the desired amount of a $2 \cdot 0\%$ salt solution was added from a burette, and a mixogram was recorded. Doughs were subsequently mixed to the maximum development time indicated on the mixogram, and sheets of uniform thickness were then prepared with a sheeting roll. A dough sheet was placed on a Teflon slab in a plastic bag containing a moist sponge, not in direct contact with the dough, and rested for not less than 45 min. Rings were then cut from the dough sheet with the specially constructed dough cutter shown in Fig. 2. By pressing the spring-loaded bolts on the release plaque, the dough ring was readily ejected from the cutter. Mineral oil (standard white oil No. 9) was used to facilitate release. Each ring was immediately placed in a small tared dish filled with mineral oil, and the whole was re-weighed to determine the weight of the ring. The rings in the dishes were rested for a minimum of 75 min before being tested.

In tests on elastomers, circular rings are commonly cut from rubber sheets using a fly cutter. Owing to the distortion (straightening out) of circular rings during the initial portion of a test, a 'toe' appears in the load-deformation curve for
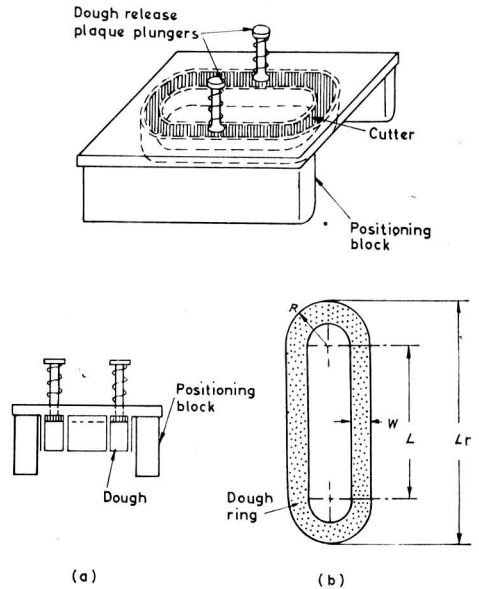
which a correction must be made.[6] However, the 'race tract' specimen (Fig. 2) can be prepared from dough sheets as easily as a circular ring, and the 'toe' is thereby avoided.

For the calculation of stress and strain, it is necessary to know the thickness T of the ring, its width W, and either its outer circumference C_o or its inner circumference C_i.

If, as shown in Fig. 2, R is the radius of the curved portion and L_r is the length:

$$C_o = 2\pi R + 2L = 2\pi R + 2(L_r - 2R) \dots \dots (1)$$

The inner circumference is $C_i = C_o - 2\pi W$, and the average circumference is $C_a = C_o - \pi W = C_i + \pi W$. (The actual dimensions of the cutter used were $W = 0 \cdot 250$, $L_r = 1 \cdot 560$, and $R = 0 \cdot 405$ in.) Simple geometric considerations show that the volume of the ring is $C_a WT$; hence, the thickness is given by:

$$T = \frac{M_r}{\rho W C_a} \dots \dots \dots \dots \dots \dots (2)$$

where M_r is the weight of the ring, and ρ is the density of the dough. As this method gives an average thickness, the dough must be rolled out as uniformly as possible. Typically, the sheet was rolled to a thickness of about $0 \cdot 25$ in. The densities were obtained by hydrostatic weighing or by equilibrium flotation in a suitable liquid mixture of known density.

Testing procedure

To install a ring in the apparatus, one of the oil-filled dishes containing a weighed ring was lowered into the apparatus, and the ring was then transferred to the specimen supports while remaining submerged under the buoying liquid at all times. After the ring had attained the bath temperature, it was stretched at the pre-set crosshead speed. When a ring stretched unevenly, as sometimes happened, the data were discarded and the test was repeated.

Results and Discussion

Calculation of stress and strain

The stress and strain in the dough ring must be calculated from the record of the load (force) against time. The (nominal or engineering) stress σ is the force f per initial (undeformed) cross-sectional area, i.e., $\sigma = f/2WT$. Upon substituting for T from Equation (2):

$$\sigma = \frac{f}{2WT} = \frac{f_\rho C_a}{2M_r} \quad\ldots\ldots\ldots\ldots\ldots (3)$$

The denominator of Equation (3) contains the factor 2 because the force is supported by both sides of the ring.

When the deformations are large, the nominal stress differs considerably from the so-called true stress, i.e. the force divided by the area of the deformed specimen. Since for all practical purposes no volume change accompanies the stretching of dough, the true stress in simple extension equals $\lambda\sigma$, where λ is the extension ratio defined by $\lambda = l/l_0 = 1 + \varepsilon$; l and l_0 are distances between two points on the deformed and undeformed specimens, respectively; the strain $\varepsilon = \Delta l/l_0$.

When a circular rubber ring is stretched, the stress and strain on the inside circumference are greater than on the outside circumference. The average strain can be calculated from the crosshead displacement using a cubic equation,[8] based on the assumption (verified by experimental results) that the rubber ring slips uniformly over the supports during a test. As is mentioned below, the dough rings do not slip over the supports, and the extension of the dough occurs primarily in the two straight portions of the ring. Hence, the strain was calculated from:

$$\varepsilon = \frac{2\Delta L}{C_e} \quad\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where ΔL is the crosshead displacement, which equals the crosshead speed multiplied by the time, and C_e is the 'effective circumference'. If the strain in the centre (or gauge) section of a ring (approximately distance L in Fig. 2) increases in direct proportion to the time during a test at a constant crosshead speed, an effective circumference (or effective gauge length) can be defined such that the strain can be obtained from the crosshead displacement using Equation (4).

Effective circumference

A special study was made to determine whether the strain in fact increased in proportion to the time and to obtain a value for the effective circumference. A number of rings on which 8 bench marks had been placed, as shown in the lower right corner of Fig. 3, were tested. A photograph was taken before the crosshead was started and periodically thereafter until the ring broke. The distance between marks was read in arbitrary units on a Model 29E-39 Telereadex at $20\times$ magnification. The observed strain, ε_{obs}, was obtained from the separation of the bench marks. The calculated strain, ε_{calc}, was obtained from the equation $\varepsilon_{calc} = 2\Delta L/C_a$. If the strain (extension) rate in the gauge section remains constant (i.e., if the ring stretches evenly) a plot of ε_{calc} against ε_{obs} should yield a straight line. Further, the line should have a unit slope if $C_e = C_a$.

Fig. 3 shows the results obtained on a Kansas flour dough with $39\cdot2\%$ water absorption at crosshead speeds of $2\cdot63$ and $26\cdot3$ in/min, and also on a dough with $45\cdot8\%$ water absorption at a crosshead speed of $26\cdot3$ in/min. The difference between the strains derived from the inner and outer pairs of marks at the higher extensions for the dough of
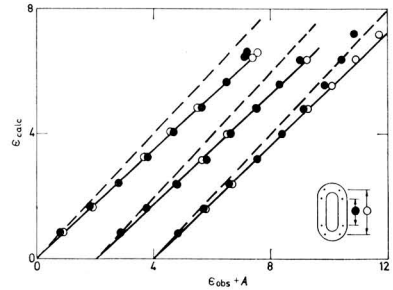
FIG. 3. *Comparison of the strain calculated from the crosshead displacement assuming that the dough ring slips freely over the supports, with that obtained from the separation (determined photographically) of bench marks (●, ○) placed on the ring as shown*

The lines are offset by arbitrary amounts, A

A	Water absorption, %	Strain rate, min⁻¹	
		Observed	Calculated
0	$39\cdot2$	$1\cdot84$	$1\cdot61$
2	$39\cdot2$	$18\cdot2$	$16\cdot1$
4	$45\cdot8$	$17\cdot5$	$16\cdot1$

$---$ Unit slope

$45\cdot8\%$ water absorption arose because this specimen broke near a support and thus the inner marks gave a lower strain during the period immediately before rupture. All other points fell on straight lines, indicating that the dough stretched uniformly between the supports. As the slope of each line was less than unity, the strain rate in the gauge section was larger than in the overall ring. As the calculated values of strain rate were lower than the observed values (cf. Fig. 3), it was concluded that a ring does not slip freely around the supports and the dough in the gauge section stretches significantly more than that in contact with the supports.

Because, however, the plot of ε_{calc} vs. ε_{obs} was essentially linear up to the rupture point, the strain in the gauge section can be calculated from the crosshead displacement by replacing the average initial circumference, C_a, with an effective circumference, C_e, obtained by multiplying C_a by the slope of the lines in Fig. 3. Before this procedure can be applied generally, it is necessary to determine whether C_e depends on temperature, crosshead speed, and dough consistency.

Effects of temperature, crosshead speed, and water absorption on the effective circumference

To determine whether C_e depends on crosshead speed, temperature, water absorption, and the nature of the flour, tests were made as described above at crosshead speeds of $0\cdot53$ and $12\cdot9$ in/min at 10, 25, and $40°$ on Kansas flour doughs with water absorptions of $39\cdot2$, $45\cdot8$, and $52\cdot5\%$; tests were also made at a single crosshead speed of $10\cdot5$ in/min at 10, 25, and $35°$ on Lemhi flour doughs of $44\cdot3$ and $49\cdot2\%$ water absorption. The distance between bench marks was plotted against time. The slope of each resulting straight line gave ε_{obs}, the strain rate observed over the gauge section of a ring. Values of C_e were obtained from:

$$C_e = \frac{2(XHS)}{\varepsilon_{obs}} \quad\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where XHS is the crosshead speed.

The mean value of C_e obtained from all 18 determinations on the Kansas flour doughs was 2·75 in with a standard deviation of 0·176 in. For the Lemhi flour doughs, the mean value of six determinations was 2·68 in with a standard deviation of 0·120 in. The average circumference calculated from the ring geometry was 3·26 in.

Analysis of variance indicated that neither temperature, crosshead speed, nor water absorption had a statistically significant effect at the 5% confidence level on C_e for either the Kansas or Lemhi flour doughs. The ratio of the variances of the effective circumferences for the Lemhi and Kansas flour doughs was not significant at the 5% confidence level; therefore, the values can be regarded as coming from the same population and $C_e = 2·75$ in, established for the Kansas flour doughs, was also adopted for the Lemhi doughs.

Effects of water absorption, crosshead speed, and temperature on stress–strain behaviour.

The curves in Fig. 4 illustrate the effect of water absorption on stress–strain curves of Kansas flour doughs. The top curve for the dough of 39·2% water absorption is typical of 'strong' behaviour while the bottom curve for 45·8% absorption exemplifies 'weak' behaviour. The 'slackening' effect (i.e. the reduction in stress for corresponding values of strain) caused by an increase in water content is clearly visible. Under the several test conditions, an increase in the water absorption from 39·2 to 45·8% produced a stress reduction of about 50%. It may be noted, however, that the total elongation, although slightly higher at the lower absorption, was not strongly dependent on water content.

Figs 5 and 6 show the effect of crosshead speed, and Figs 7 and 8 show the effect of temperature on the stress–strain curves of Kansas and Lemhi flour doughs. The weaker character of the Lemhi doughs is readily apparent in the stress values even after considering the different water absorptions. The Lemhi flour doughs also showed a more pronounced first maximum than did the Kansas doughs, but rarely a second maximum before break. The decrease after the maximum was greater for the Lemhi doughs.
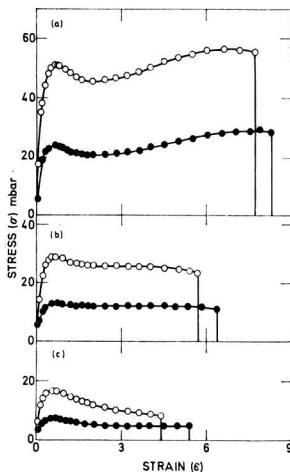
The mechanical properties of doughs are clearly dependent upon conditions of the test which are normally not varied in industrial dough testing, resulting in a loss of important information on the characteristics of doughs from different flours. Thus, the effect of a variation in strain rate and temperature shown in Figs 5–8 clearly demonstrates differences in the shift from weak to strong behaviour with either an increase in strain rate or a decrease in temperature.

Qualitatively, the curves are similar to the load-deformation records obtained with the Brabender extensograph or the Chopin alveograph. In Fig. 4 the down-line at the break point is shown to emphasise the resemblance of these curves to extensograms and alveograms. The curves in Fig. 4, however, represent the behaviour in terms of the fundamental quantities – stress and strain – and thus are capable of meaningful interpretation.

As an example, it can be mentioned that the maxima in the stress–strain curves, even for the Lemhi flour doughs, disappear when (as discussed in detail elsewhere[7]) the nominal stress (the force divided by the initial, undeformed cross-sectional area) is replaced by the true stress (the force divided by the instantaneous, deformed cross-sectional area). This raises the question of whether any basic significance can be
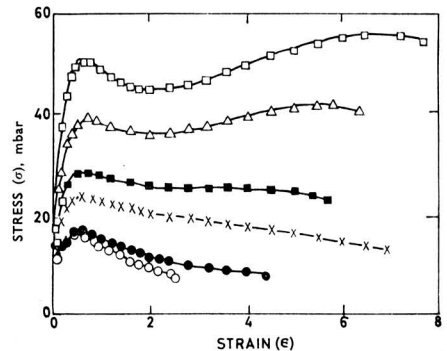
FIG. 5. *Stress–strain curves determined at 35°C on a Kansas flour dough at various crosshead speeds (XHS)*

Water absorption 39·2%
○ $XHS = 0·132$; ● $XHS = 0·263$; × $XHS = 1·32$; ■ $XHS = 2·63$; △ $XHS = 13·2$; □ $XHS = 26·3$ in/min

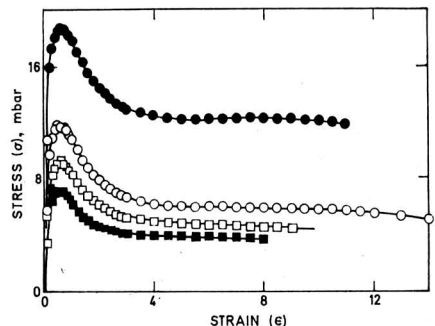FIG. 6. *Stress–strain curves determined at 25°C on a Lemhi flour dough at various crosshead speeds (XHS)*

Water absorption 49·2%
● $XHS = 52·6$; ○ $XHS = 26·3$; □ $XHS = 12·9$; ■ $XHS = 5·26$ in/min

FIG. 4. *Stress–strain curves determined at 35°C at 3 crosshead speeds (XHS) on two doughs of Kansas wheat flour*

(a) $XHS = 26·3$; (b) $XHS = 2·63$; (c) $XHS = 0·263$ in/min
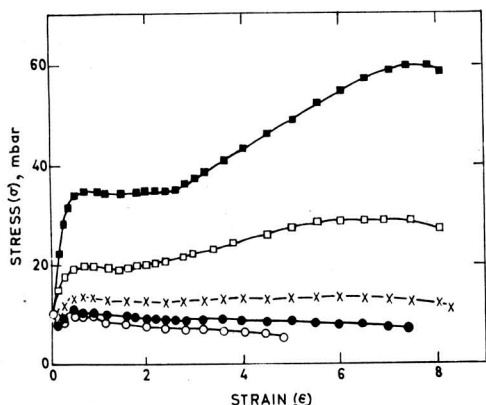○ Water absorption 39·2%; ● water absorption 45·8%

FIG. 7. *Stress–strain curves determined at a crosshead speed of 1·32 in/min on a Kansas flour dough at various temperatures*

Water absorption 45·8%
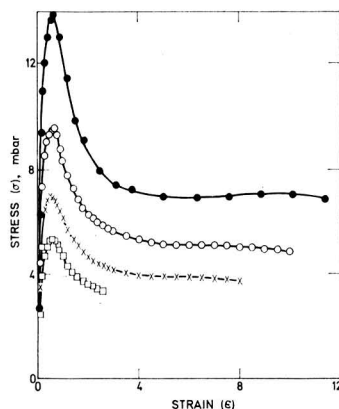
■ 5°; □ 15°; × 25°; ● 35°; ○ 45°C

FIG. 8. *Stress–strain curves determined at a crosshead speed of 5·26 in/min on a Lemhi flour dough at various temperatures*

Water absorption 49·2%

● 5°; ○ 15°; × 25°; □ 35°C

attached to these maxima or to the similar maxima observed in extensograms and alveograms.

Buoying liquid

A typical dough ring used in these experiments weighed about 3·5 g and the cross-sectional area of each side of a ring was about 0·4 cm². If the ring is suspended from a support, the gravitational force will produce a stress at the ring's midplane of about $1·75/(2 × 0·4) = 2·2$ mbar. As shown by Fig. 8, the maximum stress produced during the stretching of a Lemhi flour dough at 35° and 5·26 in/min is 5·3 mbar. Thus, the stress produced by gravity can be quite comparable to that developed during a tensile test. A buoying liquid is clearly required to counterbalance the sizeable and non-uniform stress produced by the gravitational force.

A suitable buoying liquid not only must have essentially the same density as the dough but also must not alter the properties of the dough. For this work, a mixture of mineral oil (standard white oil No. 9) and Freon TF was selected. The Freon, whose density is 1·564 g/ml, was added to the mineral oil to increase its density to that of the dough. After preliminary tests indicated that the liquid mixture had no detrimental effect on dough properties, except possibly on prolonged contact, a rather extensive study to verify this finding was made. Rings of Kansas flour doughs with 45·8% water absorption, prepared in the usual manner, were rested under Freon for periods up to 4 h. As a control, rings were concurrently rested under mineral oil for the same period. After different rest periods, the rings were tested at a crosshead speed of 12·9 in/min while submerged in the mixture of mineral oil and Freon.

The results showed that prolonged exposure to Freon had no significant effect on dough properties. In later work, however, the density of a dough ring was observed to increase slightly when the ring was submerged in the buoying fluid for long periods. The increase apparently resulted from a slight penetration of Freon into the dough. Another possible disadvantage of Freon is its relatively high vapour pressure, causing it to evaporate preferentially from the liquid mixture. Consequently, the density of the mixture gradually decreased with time, and Freon had to be added periodically.
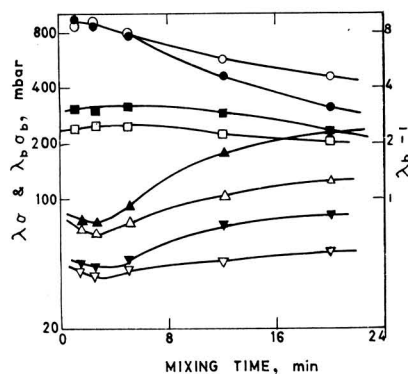
FIG. 9. *Data obtained on Kansas flour dough mixed for different periods in air and under nitrogen*

Open symbols, N₂; solid symbols, air

○, ● λ_b-1; □, ■ $\lambda_b\sigma_b$; △, ▲ λ-1 = 3·0; ▽, ▼ λ-1 = 1·0

Because of these undesirable features of the mixture, a liquid having a low vapour pressure and a density close to that of dough was sought. Two candidate liquids, with densities closely matching those of the doughs studied, were dimethyl phthalate and Arochlor 1221,.whose densities are 1·178 and 1·187 g/ml, respectively. (Arochlor 1221 is one of a series of chlorinated biphenyl liquids manufactured by Monsanto Chemical Company.) These liquids were subjected to the procedure used to evaluate Freon. Dimethyl phthalate had an adverse effect on the dough properties; Arochlor 1221 did not. Because the Arochlor is mostly a mixture of closely related compounds having low vapour pressures, its density should not change appreciably upon standing. Hence, it should be a more convenient buoying liquid than the mixture of mineral oil and Freon.

Effect of rest period and mixing time

Doughs at 49·0% water absorption were mixed from the Kansas flour for 2·5, 4·0, 6·0, 8·0, and 10·0 min and from

the Lemhi flour for $1\cdot5$, $2\cdot5$, $4\cdot5$, $6\cdot5$, and $9\cdot0$ min. For the Kansas flour, $4\cdot0$ min was the mixing time used in previous experiments; this corresponds to the peak development time shown by the mixogram. For the Lemhi flour, this optimum mixing time was $2\cdot5$ min. Thus, apart from the doughs mixed to the optimum, one undermixed and three overmixed doughs were prepared. The mixogram indicated that at the longest mixing times the doughs were severely overmixed. These doughs were quite sticky but could be handled satisfactorily after short waiting periods.

Rings prepared in the usual manner were rested under mineral oil for periods of approximately 45, 90, 135, and 180 min; they were then stretched at $25°$ at a crosshead speed of $12\cdot9$ in/min. Tests were also made at 15 and $35°$ on Kansas flour doughs mixed for $2\cdot5$, $4\cdot0$, and $6\cdot0$ min. The rings were held at the test temperature during each rest period. To obtain some information on the interaction between the level of water absorption and the effect of mixing time and rest period, tests were made at $25°$ on Kansas flour doughs of 44% water absorption mixed for $3\cdot0$, $4\cdot5$, and $6\cdot5$ min. As in the experiments to determine the possible deleterious effect of the buoying liquid on dough, the effect of mixing time and rest period was investigated by considering the true stress at a fixed extension. This method is essentially similar to the structural relaxation method of Hlynka.[9] However, while Hlynka and his co-workers were interested in studying the structural relaxation immediately after mixing and moulding, the emphasis here was on determining the rest period needed for virtually complete relaxation of what Hlynka has called structural activation, i.e., relaxation of stresses induced during the mixing and shaping operations.

Analysis of the data showed that after about 1 h the internal stresses in both doughs had relaxed completely, for all practical purposes, although the relaxation appeared to occur somewhat more slowly in the doughs from the stronger flour. There was no clear evidence that the relaxation rate is greater at higher temperatures. The stronger nature of the Kansas flour was indicated in higher stress levels compared with the Lemhi doughs. For both doughs at 49% water absorption the stress at fixed extension first decreased and then increased on prolonged mixing. In the experiments at 44% water absorption there was no decrease. The rate of stress rise on prolonged mixing was about the same for the doughs from both flours in all experiments. The increase in dough strength appears to result from progressive oxygen uptake during mixing, as discussed below. No correlation was found between the stresses measured at fixed extensions as a function of mixing time and the optimum mixing time as indicated on the mixogram.

Properties of doughs mixed in air and under nitrogen

To ascertain whether the observed increase in strength on prolonged mixing is due to oxygen uptake, tests were carried out on doughs mixed for periods between $1\cdot5$ and 20 min in air and also under nitrogen. The doughs, prepared from the Kansas flour with $45\cdot8\%$ water absorption, were mixed in a farinograph mixing bowl having a tight fitting cover. After mixing, the doughs were rested for a short time and then sheeted out. Rings were cut in the usual manner, rested under mineral oil, and then tested in the tensile tester at $25°$ at a crosshead speed of $12\cdot9$ in/min. At least 90 min elapsed between the time a ring was cut and the time that it was tested. As is discussed in the preceding section, this period was more than sufficient for relaxation of any stresses induced during

the mixing and forming operations. Four rings were prepared and tested from each dough. Average values of true stress at 100% and 300% extension, along with the rupture data are plotted against mixing time in Fig. 9.

Fig. 9 shows that the stress at a fixed extension increases with mixing time, except for a slight minimum at a mixing time of 5 min. The stress increase for the doughs mixed in air is considerably greater than for those mixed under nitrogen. Before mixing the latter, the flour was purged under nitrogen (dry mixed) for 5 min. However, it is known[10] that flour lipids may retain oxygen, and thus retained oxygen is possibly responsible for the stress increase observed on the doughs mixed under nitrogen.

Fig. 9 also shows that the rupture strain, $\lambda_b - 1$, decreases markedly with mixing time; the decrease is somewhat less for doughs mixed under nitrogen than for those mixed in air. Although the true stress at break, $\lambda_b\sigma_b$, decreases somewhat with mixing time, the percentage change for doughs mixed in air is about the same as for those mixed under nitrogen.

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DIGESTIBILITY *IN VITRO* OF DRY MASHED POTATO PRODUCTS

By E. W. HELLENDOORN, M. VAN DEN TOP and J. E. M. VAN DER WEIDE

The *in vitro* digestibilities of potato granules and flakes were compared. The digestibility of flakes was superior to that of granules. By drum-drying mashed potatoes, as is customary in the manufacture of flakes, the digestibility was improved, whereas by repeatedly drying and wetting mashed potatoes, as in the manufacture of potato granules, digestibility was diminished.

The dry matter content of mashed potatoes obtained by rehydrating potato granules was higher than that of rehydrated flakes.

The influence of digestibility and dry matter content of rehydrated potato granules and flakes on digestion in humans is discussed.

Introduction

In the Netherlands Army, potato granules, having a higher packing density than potato flakes, are used not only for making mashed potatoes to serve with meat and vegetables, but also in mixed dehydrated meals consisting of potato granules and dehydrated vegetables, such as carrots, onions, spinach, curly kale. The amount of granules in these dry compositions is about 70%.

These rehydrated mixed meals, when served with sausage or meat and meat gravy, are consumed in quantities equivalent to the consumption of similar meals prepared from the fresh ingredients. However, it was observed, and particularly when the meal was eaten after strenuous exercise in a state of exhaustion, that an unusual number of complaints were made of stomach distension as if from overeating, followed by flatulence, in comparison with remarks made after eating a similar meal prepared from freshly cooked ingredients. The complaints had nothing to do with enteritis or food poisoning. After a few hours rest the well-being of the men was restored completely. Mashed potatoes, when given as a separate serving, are consumed in smaller quantities than in the case of mixed meals and give less rise to complaints. The disturbed digestion might be effected by a reduced digestibility of the dried potatoes, caused by the drying process itself. Moreover the dry matter content of the rehydrated meal might be higher than that of a freshly prepared meal. This investigation was carried out to test these possibilities.

Dehydrated mashed potato products are obtained in two ways.[1] Potato granules (powders) are usually made by an add-back process, i.e. recycling a portion of dehydrated material by mixing it with freshly mashed cooked potatoes, to reduce its water content, and finally drying the mixture with hot air. Flakes are made by dehydrating the mashed cooked potatoes on a drum dryer.

Processing of carbohydrate-rich foods can influence the enzymic digestibility of the starch.[2,3] Retrogradation of the starch occurring during drying[4,5] is expected to have a diminutive effect on digestibility.[6] It is well known that by dehydrating food products with air the rehydration properties are adversely affected.

As potato starch was the main component of the served meals this investigation of the enzymic digestibility was confined to dehydrated mashed potato granules and flakes.

Experimental

Measuring the digestibility of potato starch

Apart from starch, potatoes contain a variable amount of sugars, such as glucose, fructose and sucrose which are easily digested and crude fibre, cellulose, hemicellulose, pectic substances and other polysaccharides which are not digested.[1] The potato starch occupies an intermediate position, being difficult to digest when uncooked,[7] but the digestibility increases with cooking time. In this investigation starch is defined as the digestible part of the carbohydrates, including the sugars. If under certain circumstances, e.g. with potatoes after insufficient cooking to softness, only a limited amount of the starch present was susceptible to amylolysis, this part expressed as percentage of the total amount of starch, was called the digestibility of the starch.

To measure starch digestibility with pancreatic amylase a 250 ml double-walled glass reaction vessel was used. Water at a thermostatically controlled temperature circulated through the double glass wall, maintaining a temperature of $37 \cdot 0 \pm 0 \cdot 2°$ of the liquid in the reaction vessel. A magnetic stirrer provided a vigorous turbulence of the reaction mixture.

2–$2 \cdot 5$ g of the starch-containing product in the reaction vessel was dissolved in a solution of 180 ml phosphate buffer, pH $6 \cdot 8$ (according to Sörensen equal amounts of 1/15 M-KH_2PO_4 and 1/15 M-Na_2HPO_4. H_2O) 15 ml $0 \cdot 3$ M-NaCl, 20 mg sodium merthiolate, and 5 ml pancreatin 4.NF solution* (200 mg in 50 ml distilled water). This made a 1% solution of the substrate and gave a ratio of 1% of pancreatic amylase with respect to substrate.

The starch-containing product was introduced into the buffer solution and after 10 min stirring to obtain complete wetting, the pancreatic solution was added to start the digestion.

5, 10, 20, 30, 60 and 120 min after starting the digestion, 25 ml of the reaction solution was pipetted into a 100 ml measuring flask, 5 ml Carrez I solution ($10 \cdot 6$ g $K_4Fe(CN)_6 \cdot 3$ H_2O in 100 ml distilled water) were added and after shaking, 5 ml Carrez II solution ($23 \cdot 9$ g $Zn(CH_3COO)_2 \cdot 3$ H_2O and 3 g glacial acid in 100 ml distilled water), were added. The reaction stopped at this stage and the mixture could be set aside. After neutralisation, the mixture was made slightly acid, made up to 100 ml with distilled water and filtered through a folded filter paper; 25 ml of the clear filtrate, containing dextrins and maltose as digestion products of the starch, were boiled for $1\frac{1}{2}$ h with $2 \cdot 85$ ml 25% HCl under reflux to hydrolyse these intermediate products to glucose.

The solution was then neutralised and made up to 50 ml with distilled water. 5 ml of this solution were used for the semi-micro estimation of glucose,[8] using the copper reagent of Luff-Schoorl.[9]

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*The amount of pancreatin (Miles) can be substituted by an equal amount of Amylase BDE (Société Rapidase) or by the double amount of Rhozyme 33 (Rohm & Haas)

By multiplying by a factor of $0 \cdot 9$ the glucose values were converted to amounts of starch.

Before estimation of total amount of starch present, first the uncooked starch has to be made available to enzymic digestion. This can be done by normal cooking or pressure-cooking. In this investigation the susceptibility to amylolysis was obtained by grinding the product with cold hydrochloric acid in a mortar to a paste with an end concentration of 4 M.[10] This paste was left for 1 h at room temperature before analysis. To ensure total digestion of the acid-converted starch within 2 h, the pancreatin content of $1–1 \cdot 2$ g of the dry product was taken and the pancreatin content was increased tenfold (200 mg); in this case 50 ml was pipetted for sugar analysis.

All the experiments were carried out in triplicate and the figures obtained were averaged.

Results

Dry matter content and starch content

For the experiments 6 samples of dry mashed potato products, obtainable on the Dutch market, were used, viz. 3 products in flake form and 3 products in powder form.

The dry matter content and starch content of these products together with that of cooked potatoes are given in Table I.

Product 6(b) represents a product being used for several years. Product 6(a) represents the latest product of the same factory.

In the experiments potatoes of the Dutch stock Bintje, which are in most cases preferred as starting material for industrial processing, were used. A quantity of these potatoes that had been cooked for different periods of time showed a water content increasing with cooking time.

Owing to the high water content, the experimental error in determining the starch content was relatively large, rendering it impossible to draw a conclusion about a possible change of starch content of potatoes cooked for a certain period of time.

Digestibility of dry mashed potato products

The digestibility of starch in dry mashed potato products as function of time of digestion is given in Fig. 1.

The digestibility curves of the flaked products 1, 2 and 3 lying closely together, indicated a rapid digestion. The granular products 4 and 6 possessed a distinctly inferior digestibility. Product 6(a), replacing the older product of the same factory, occupied an intermediate position. Product 6(b), which was supplied to the Army, appeared to have

an inferior digestibility compared with 6(a) (not given in Fig. 1).

In this connexion it is interesting to know whether the digestibility of these dehydrated products would be comparable to that of freshly made mashed potatoes.

Digestibility of cooked potatoes

The results of measuring the digestibility of cooked potatoes of the Dutch stock Bintje as a function of cooking time are given in Fig. 2.

It was found that potatoes not cooked at all but disintegrated in a blender prior to enzymic digestion possess only a slight digestibility. After cooking the potatoes, cut in parts of about 30–40 mm dia., with a small amount of water for 15, 25 and 40 min respectively, the digestibility increased to high values. After 15 min the potatoes were still underdone and rather hard. After 40 min the potatoes were overcooked and soft, while after 25 min they were quite cooked. From an organoleptical point of view 20–30 min would be sufficient, giving a broad band for the digestibility of properly cooked potatoes (represented by the shaded area in Fig. 2).
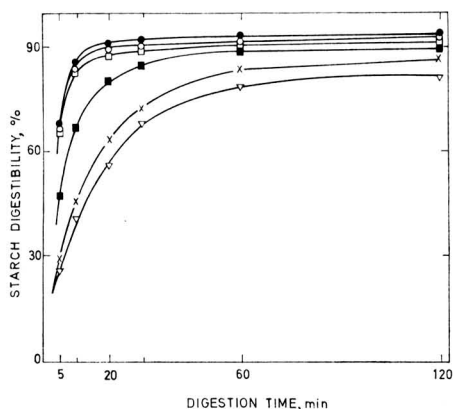
FIG. 1.   *Digestibility of the starch of 6 samples of dry mashed potato products*

●, ○, ▢ Potato flakes; ×, ▽, ■ potato granules
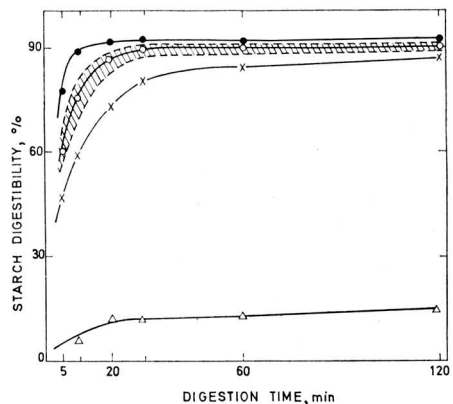
FIG. 2.   *Digestibility of the starch of potatoes in relation to cooking time*

▨ Properly cooked potatoes; ● 40 min; ○ 25 min; × 15 min; △ 0 min

TABLE I

Dry matter content and starch content of dry mashed potato products and of cooked potatoes

No.	Product	Dry matter content (24 h/70° c/vac.), %	Starch content (by dry matter), %
1	Flakes	91·0	75·3
2	Flakes	92·3	78·6
3	Flakes	91·7	75·0
4	Granules	93·8	76·3
5	Granules	92·7	73·7
6(a)	Granules (new item)	92·4	75·4
6(b)	Granules (old item)	93·3	72·4
	Potatoes cooked 15 min	21·9	75·0
	Potatoes cooked 25 min	19·6	76·1
	Potatoes cooked 40 min	16·2	75·3

Comparison of Fig. 2 with Fig. 1 leads to the conclusion that all the digestibility curves of the flaked products can be found lying beyond the upper limit of the shaded area. The potato products in powder form, however, were all below this area. That means that the digestibility of the flakes was superior and that of the granules was inferior to the digestibility of potatoes cooked in a household manner.

The following experiments show that by making potato flakes on a drum dryer the digestibility of the starch improves; on the other hand repeated drying and wetting of mashed potatoes had an unfavourable influence on the digestibility of starch.

Digestibility of mashed potatoes during the flaking process

Potato flakes were manufactured on the experimental single-drum drier of the Institute for Storage and Processing of Agricultural Produce at Wageningen (The Netherlands), according to the following process.

Potatoes (stock Bintje), peeled with caustic soda, were cut into parts of 10–12 mm thick, heated in water at 70° for 20 min and immediately cooled in running water at 2° for 15 min. The potato parts, partly 'cooked' in this way, were then fully cooked for 30 min by steaming. The steamed parts were mashed by pressing them through a rotating perforated metal cylinder, the holes of which had a diameter of 6 mm. Before drying $0 \cdot 2\%$ monopalmitate (by dry matter) was mixed with the mashed potatoes. Neither pyrophosphate nor sulphite was used in this experiment. The drum (50 cm dia.) was heated with steam at a pressure of $5\frac{1}{2}$ atm. The layer on the drum was built up by feeding with 3 unheated rolls. The drying time was about 20 sec.

During the manufacturing process samples were taken after cooling the partly cooked potatoes (samples were taken after heating and cooling in three separate experiments); after mashing the fully cooked potatoes (before and after the addition of monopalmitate); and after the drying operation of the mashed potatoes (with and without added palmitate).

The rate of digestion of each sample was determined three times and the figures obtained were averaged. The results of the experiments without palmitate are given in Fig. 3.

The first sample taken during the manufacturing process had already been submitted to two important operations,
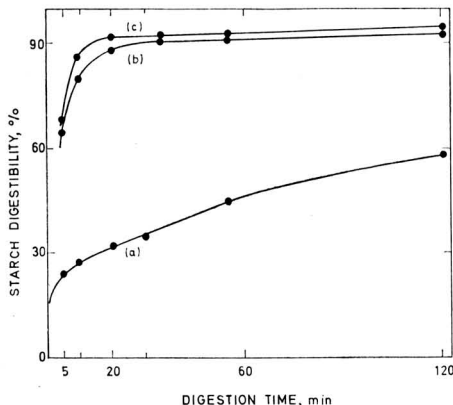
viz. a heat treatment bringing the starch above its gelatinisation temperature, followed by rapid cooling, both treatments achieving a diminution of amylose solubility by which an end-product with improved texture can be obtained.[11-13] The careful cooking process of the potatoes in two steps may help to avoid a too rapid water absorption of the starch accompanied by damage of cell structure liberating the starch.[14]

As expected, the gelatinised starch of the partly cooked potatoes did not appear to be completely digestible. Cooling after pre-cooking decreased its 'blue value'[11] indicating some retrogradation of starch. According to Radley[6] retrograded starch shows an increased resistance towards hydrolysis by amylolytic enzymes. Curve (a) in Fig. 3 only gives the net digestibility after pre-cooking and chilling, thus some lowering influence of cooling on starch digestibility remains undetected.

Pre-cooking and cooling of raw potatoes was repeated three times, each sample giving a slightly different digestibility curve. This could be expected as a slight variation in cooking time may result in a relatively large difference in digestibility of the semi-cooked product.

Curve (b) in Fig. 3, obtained from a sample of the fully cooked and mashed potatoes ready for drying, shows that the product had a very good digestibility. This product is comparable with homemade mashed potatoes.

To a part of the mashed potatoes monopalmitate was added. Glycerol monopalmitate,[15] monostearate[16,17] or monolaureate[18] serve to improve the texture of the finished product by complexing with liberated starch, making it less pasty. The 'blue value' of the starch is diminished by the addition of such substances; however, no change in digestibility was found.

More interesting than the rate of digestion of the mashed potatoes ready for drying was the improvement in digestibility by the dehydration process as shown by comparing curves (b) and (c) of Fig. 3. This operational step is not a single one. When coming into contact with the drum the mashed potatoes were heated to cooking. This heating process was so intense, that even raw potato starch from intact cells was fully gelatinised. In this case the properly cooked mashed potatoes were cooked to an even higher degree, consequently the digestibility of the starch, already being good, was still further improved. Moreover, the percentage of broken cells will be increased by the drying process as a whole, thus making the starch more easily available to amylolysis.[2,3] A negative influence of dehydration on the digestibility, caused by the retrogradation of starch taking place during drying was expected.[4,5] On the other hand the drying rate in drum drying was too fast to be responsible for any retrogradation. Guilbot & Mercier[2] found an increased digestibility by carefully drying wheat starch with air or by freeze-drying. Whatever the influence of drying on starch digestibility may be, the net result of the drum drying process appeared to be a positive one. The flakes dried after the addition of mono-palmitate showed almost the same digestibility as the dry sample without the addition, so that in this phase too, mono-palmitate appeared to have only a minor influence on digestibility.

Digestibility of mashed potatoes after repeated drying and wetting

During the production of potato granules, mashed potatoes are mixed with a portion of dry granules. The mixture with a reduced water content, obtained in this way, is

FIG. 3. *Digestibility of the starch of samples during the flaking process*
(a) Partly cooked and cooled; (b) fully cooked and mashed; (c) dry flakes

dried. As a result of this procedure part of the dry granules are wetted and dehydrated more than once. Moreover, mere holding or conditioning of the moist mix markedly influences retrogradation of starch and the structure of the dry product.[1]

In order to study the influence of repeated wetting and drying on starch digestibility a simple laboratory experiment was chosen, because it was not possible to study the phenomena on a factory scale. A potato flake product was used as starting material in view of its high rate of digestion.

A thin layer of flakes having a moisture content of 10% was wetted with a water spray, resulting in a 40–50% moisture content. The flakes were then placed in a laboratory oven with forced draught at a temperature of 48°. After 2 h drying the moisture content had decreased to 8–10%. This procedure was repeated 5 times. During this experiment two samples were taken to determine the starch digestibility, the first after 3 times wetting and drying, the second at the end of the experiment.

Fig. 4 shows the digestibility curves of the 2 samples of wetted and re-dried product as compared to the digestibility of the original potato flakes.

The rate of digestion of the potato starch diminished appreciably after 3 times wetting and redrying and decreased still further as a result of the 2 succeeding steps of wetting and drying.

These laboratory operations are certainly not comparable to factory practice; however, the observed tendency of starch digestibility to decrease on repeated wetting and drying may explain the lower digestibility of the potato granules in comparison with flakes.

Dry matter content of rehydrated potato products

Earlier it was suggested that mashed potatoes made by rehydrating dried potato flakes or granules might have a higher dry matter content than mashed potatoes made from potatoes freshly cooked in a household manner. In order to study whether this was true or not, portions of mashed potatoes were prepared according to the instructions for use on the packets. It was significant that the amount of granules to be mixed with the same quantity of water and milk was higher than that of flakes, viz. for $\frac{1}{2}$ l of water and $\frac{1}{4}$ l of

FIG. 4. *Digestibility of the starch of potato flakes after repeated wetting and drying*
(a) Original potato flakes; (b) after 3 times wetting and drying; (c) after 5 times wetting and drying

TABLE II

Dry matter content of 250 ml portions made from rehydrated mashed potato products and freshly cooked mashed potatoes

Mashed potatoes made from	Dry matter content of 250 ml portions, g
Cooked potatoes	41
1 Flakes	35
2 Flakes	33
3 Flakes	33
4 Granules	43
5(a) Granules (new item)	45
5(b) Granules (old item)	50

milk the packets contained 145–165 g of granules compared with 125 g of flakes. In addition freshly cooked potatoes were mashed and so much milk was added to give about the same consistency as that of the rehydrated products.

250 ml portions were dried and the amount of dry matter was determined. Results are given in Table II.

Compared with the mashed potatoes made from cooked potatoes the rehydrated potato flakes had a lower dry matter content; the rehydrated potato granules on the other hand, have a higher dry matter content.

The 5(b) granules, representing the product used by the Army, gave a rehydrated product with the highest dry matter content of all the dry potato products investigated.

Discussion

The two species of dry mashed potato products, viz. granules and flakes, were digested with pancreatic amylase at a different rate, the rate of digestion of the flakes being the highest of the two. By the flaking process on a drum dryer the rate of digestion of potato starch was improved; on the other hand, it was diminished by repeated wetting and drying, which occurs during the manufacturing process of potato granules.

Moreover, the dry matter content of rehydrated granules was higher and that of rehydrated flakes was lower than that of the mashed freshly cooked potatoes.

The problem was now with the men who complained of a disturbed digestion after eating a meal of rehydrated potato granules and dried vegetables.

Normally starch is digested by enzymic hydrolysis in the small intestine, while the degradation products, which are of low molecular weight, are absorbed in the intestinal wall and metabolised.

Some people only tolerate potatoes that are cooked to total softeness. They react to partly cooked potatoes with a stuffed feeling and formation of intestinal gas, caused by an insufficient enzymic digestion of the potato starch in the small intestine. Part of the carbohydrates that are not absorbed in the ileum will give rise to bacterial fermentation and formation of gas in the lower ileum and in the colon by the micro-organisms which are always present in these parts of the intestines.[19,20]

The men complaining of digestive disorder after eating the rehydrated meal must have had a more or less disturbed starch digestion. This may have been caused by an inborn abnormality, but may have been only temporary owing to exhaustion or nervous stress. Furthermore, the fat meat gravy served with the meals may have had an unfavourable influence on the intestinal starch digestion, and the portions
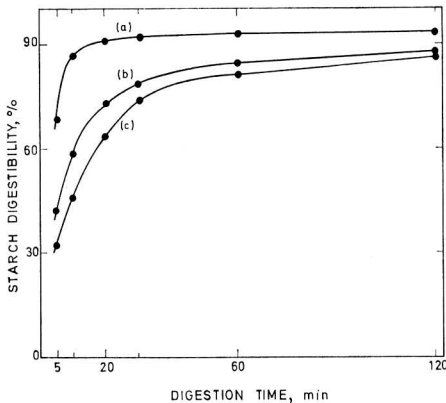
eaten by the tired men after a strenuous exercise may have been too big for a number of them. The number of complaints must have been caused by the higher dry matter content of the meal in combination with the lower rate of digestion of starch of the potato granules compared with a freshly cooked meal. On the other hand mashed potatoes from flakes may be beneficial to people suffering from a disturbed starch digestion.

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THE SIRO GRITOMETER FOR DETERMINATION OF THE GRIT CONTENT OF DRIED VINE FRUITS

By J. S. HAWKER and M. GRNCAREVIC

A relatively simple and inexpensive apparatus has been developed for the rapid determination of the surface contamination of dried vine fruits by grit. Up to 80 measurements can be made in an 8-hour day by a semi-skilled operator. Grit is separated from samples (200 g) of dried fruit by sieving, and grit particles greater than 50 μm diameter are collected on a small sieve. After separation of organic matter and grit in a saturated solution of potassium carbonate the height of grit in a tube of diameter 2 mm gives an index of the grit content of the fruit. Values obtained with the gritometer show significant correlation with values obtained using a previously accepted slower method.

Introduction

Dried vine fruits often contain traces of soil on their surfaces which can impart a 'gritty taste' to the product if present in sufficient amounts. In order to control quality it is necessary to determine the grit content of large numbers of samples of dried fruit both before and after processing in Packing Houses. The methods currently available are laborious, and relatively expensive laboratory equipment is used. The small amounts of soil involved (in the order of 100 mg/100 g dried fruit) require the use of sensitive laboratory balances to weigh the soil after removal of organic matter by 'panning' and/or ignition.[1,2] A rapid method was therefore sought which would not involve costly or fragile laboratory equipment. Hawker & Grncarevic[3] briefly described laboratory trials with a prototype apparatus which satisfied the above requirements. The present paper describes an apparatus suitable for commercial use with dried sultanas.

Experimental

Materials

Dried sultanas (dried grapes of *Vitis vinifera* L. cv. Sultanina, Thompson Seedless) were obtained from Packing Houses in the Mildura irrigation district of Australia. The dried fruit delivered by the growers still contains the stalks and is referred to as unprocessed fruit. In the Packing Houses the stalks are removed, the fruit is cleaned and then packed for distribution. This dried fruit is referred to as processed fruit.

Siro gritometer

The apparatus[4] which is illustrated in Figs 1 and 2 consists essentially of two separate parts. The first (Fig. 1) was made by fitting two brass sieves loosely into a 26 gauge copper chrome-plated funnel supported over a smaller chrome-plated sieve. The top sieves had mesh apertures of 0·28 and 0·053 cm while the bottom one had a mesh aperture of 50 μm.

The second part of the apparatus (Fig. 2) consisted of four glass funnels mounted on a Perspex and wooden stand. Each funnel had an upper dia. of 10 cm, was 14 cm long and had the stem drawn out to an i.d. of 2 mm. A vinyl tube of 2 mm i.d. was attached to the funnel. A plastic turn button closed off the tube at the zero mark of a 20 cm scale which was graduated in mm. The vinyl tubes led into a stainless-steel drain.
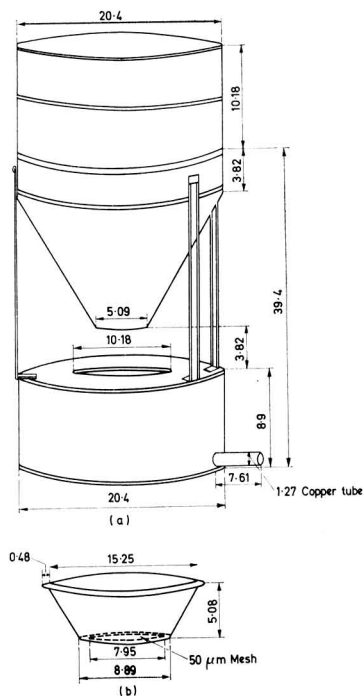

FIG. 1.   (a) *Sieves and funnel of Siro gritometer*; (b) *smaller sieve of Siro gritometer*
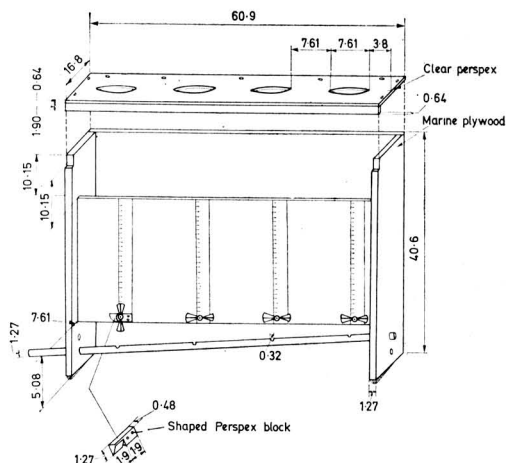All dimensions are in cm

FIG. 2.   *Separation and measurement apparatus of Siro gritometer*
All dimensions are in cm

Determination of the grit content of dried sultanas using the gritometer

Dried sultanas (200 g) together with 500 ml of tap water at 70–80° and 5 drops of liquid detergent were stirred 10 times in a container and after 10 min they were stirred again 10 times. The sultanas and washing water were then poured on to the top sieve of the gritometer and the soaking container was rinsed to ensure that all the sand was removed. The fruit on the top sieve was thoroughly sprayed with tap water to wash sand through the two upper sieves onto the smaller bottom sieve. The small sieve was removed and washed with tap water to remove any remaining particles of less than 50 μm dia. Sand particles and small pieces of organic matter retained on the sieve were transferred with the aid of a wash bottle to one of the glass funnels which was about one-quarter full of saturated potassium carbonate solution. The mixture of sand, organic matter, water and potassium carbonate solution in the upper part of the filter funnel was stirred briefly so that the stem still contained saturated solution and the funnel contained less concentrated solution. After about 10 min, when sand deposition was complete, the height of sand in the tube was measured. The funnel was emptied by opening the clamp and was then washed with water.

Determination of the grit content of dried sultanas by panning

A sample of dried fruit (100 g) was boiled in tap water. The sultanas were poured into two 8 in sieves with mesh apertures of 0·28 and 0·053 cm, arranged in series. The water and organic matter which passed through the sieves were removed by panning in white enamel basins. Panning involves swirling the soil, organic matter and water in the basins and decanting the water and organic matter. The amount of soil decanted at the same time depends on the operator. The remaining soil was washed with distilled water, dried in an oven and weighed in labelled vessels.

Partition of soil into particle sizes

Soil from dried sultanas was separated into a range of particle sizes on a series of sieves, collected, washed with distilled water, dried and weighed.

Detection of grit by consumers

Five samples of dried sultanas containing either 0, 5, 10, 20 or 35 mm of grit (gritometer values) were presented to 10 people on a taste panel on four different occasions. The fruit was scored as grit-free, slightly gritty, definitely gritty or objectionably gritty.

Commercial sampling procedure for unprocessed dried sultanas

Duplicate 200 g samples of unprocessed sultanas were obtained from each of 4 boxes each containing about 70 kg of dried fruit from loads of fruit from seven growers. Grit contents were measured using the gritometer. The coefficient of variation obtained by taking different numbers of samples was calculated.

Results

Time of soaking sultanas

Grit measurements using the Siro gritometer were made after soaking 200 g samples of sultanas for 1, 5, 10 and 20 min in water at 80° containing detergent. After 10 min the values for sultanas were 80–90% of the values obtained for fruit

soaked for 20 min. A soaking time of 10 min was adopted as the routine procedure.

Variability of grit values obtained by panning and by gritometer

The variability of the values for grit in both unprocessed and processed fruit measured either by the panning method or by the gritometer is shown in Fig. 3. Weights obtained by panning have been converted to equivalent heights for comparison with the gritometer values. There was a correlation between the coefficient of variation and the mean such that as the amount of grit per sample increased the coefficient of variation decreased. However, both methods gave results with similar variability (Fig. 3).

Relationship between height and weight of grit in gritometer tube

In one series of experiments the height of grit was compared with the weight of washed grit in the gritometer tube. A correlation coefficient of over 0·96 was obtained for 20 samples of unprocessed and 20 samples of processed fruit containing 11–140 mm grit/200 g which indicated that the grit packed evenly in the tube irrespective of the height.

Relationship between grit values obtained by gritometer and those obtained by panning method

Fig. 4 shows a comparison of grit values obtained for unprocessed fruit at three Packing Houses using the gritometer with grit values obtained by panning replicate samples in the laboratory. There was a significant correlation between the values obtained by the two methods. 1 mm of grit in the gritometer tube from 200 g of dried fruit is approximately equivalent to 2·5 mg of soil from 100 g of dried fruit measured by the panning technique.

Particle sizes of grit from sultanas

The distribution of particles of various sizes in grit removed from fruit containing varying amounts of grit is shown in Fig. 5. The fruit used was obtained from the 1967 harvest. A higher percentage of grit occurred as smaller particles in the unprocessed fruit than in the processed fruit. In the processed fruit particles of grit of diameter less than 50 μm
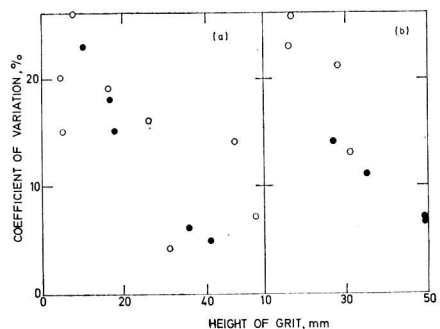

FIG. 3. *Variability of values for grit in dried sultanas measured by gritometer* (○) *or by panning and weighing* (●)

● values were converted to height for comparison with the ○ values. Each coefficient of variation was calculated from at least 10 values of grit (a) Unprocessed fruit; (b) processed fruit
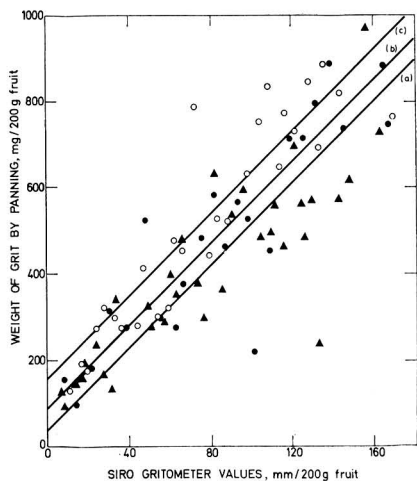
FIG. 4. *Correlation between weight of grit in unprocessed dried sultanas as measured by the panning method and height as measured by the gritometer*

The correlation coefficients for ▲ (line (a)), ● (line (b)), ○ (line (c)) are 0·86, 0·90 and 0·93 respectively

FIG. 5. *Distribution of particles in grit obtained from* (a) *unprocessed fruit and* (b) *processed fruit*
Each line represents a different sample

TABLE I

Consumer detection of grit on dried sultanas

Tasters scored 1 for no grit, 2 for slightly gritty, 3 for definitely gritty and 4 for objectionably gritty

Grit content of sultanas (gritometer values in mm/200 g)	Mean score
0	1·10
5	1·53
10	2·05
20	2·33
35	3·08
L.S.D. ($P < 0.05$)	0·35

TABLE II

Coefficients of variation (%) obtained for gritometer values by taking different numbers of samples per load of fruit

Number of boxes sampled	Number of samples per box		
	2	3	4
1	19·3	17·3	16·1
2	13·7	12·2	11·4
4	9·7	8·6	8·1
8	6·8	6·1	5·7
12	5·6	5·0	4·7

made up 10% or less of the total grit present. However, processed fruit from the 1968 harvest, during which frequent and heavy dust storms occurred, had between 30 and 60% of the total grit present as small particles. In fruit from the 1969 harvest, the values were below 20%.

Consumer detection of grit

There was a significant correlation between grit content of fruit and the mean score given by tasters (Table I). However, only fruit containing above about 10 mm of grit was consistently classified as slightly gritty (mean score value 2) which is equivalent to approximately 25 mg grit/100 g fruit obtained by the panning method. It had been shown previously that the threshold content for detection of grit in dried sultanas by consumers was 20–30 mg/100 g fruit.[5]

Sampling procedure for unprocessed dried sultanas

A trial was carried out to determine the number of samples needed per grower's load of dried fruit to obtain a reasonably accurate estimation of the mean grit content of the load. The fruit contained 20–60 mm grit/200 g fruit (gritometer values). Table II shows the coefficient of variation expected for different numbers of samples of fruit.

A reasonable procedure for sampling a load of fruit would thus be to take two 200 g samples from each of 4 boxes, mix thoroughly and carry out a grit determination on 200 g with the Siro gritometer. A value of 50 mm obtained by this procedure would represent a mean value for grit in the load of 50 ± 10 mm.

Discussion

The Siro gritometer is an inexpensive sturdy instrument which allows reliable estimates of the grit content of dried vine fruits to be made rapidly. Reliability has been demonstrated by comparison of the gritometer method with the panning technique and by taste trials by consumers. It would appear from the results (Fig. 3) that the variability

observed between measurement on the same sample of fruit is due to lack of uniformity of the grit contamination rather than to errors of measurements. This lack of uniformity would partly account for the fact that points did not all fall on the lines when comparing values obtained with the gritometer and by panning (Fig. 4). Also, the percentage of particles below 50 μm diameter in a normal season is usually small (Fig. 5), but samples can have high amounts of these particles probably as a result of wind-borne contamination. These are discarded in the gritometer but they are measured by the panning method. However the values obtained with the gritometer give a better estimate of the magnitude of the 'gritty taste' because particles below 50 μm diameter do not contribute to this defect until present at quite high concentrations.[5]

Once the small particles (< 50 μm) have been removed, organic matter is almost completely separated from grit by flotation in saturated potassium carbonate solution. Potassium carbonate was chosen because it has a convenient

density (1·4) and it is readily available within the dried fruit industry.

Owing to the simplicity of the gritometer, the cost of production and maintenance is low and operators need have no special skills. Furthermore the few steps involved in the procedure allow determinations to be carried out quickly. After a few days' experience, operators in Packing Houses can average 10 estimations per hour.

Siro gritometers are currently being used in most dried fruit Packing Houses in Austrialia.

Acknowledgments

The authors thank Miss S. Oliver and Miss J. Evans for valuable technical assistance, Mr. P. Johnston for construction work, the Australian Dried Vine Fruits Research Committee for financial support and Mr. K. M. Cellier of the C.S.I.R.O., Division of Mathematical Statistics for statistical analyses.

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SUGAR CHANGES DURING THE PREPARATION OF BURUKUTU BEER

By S. I. FAPARUSI*

In the study of sugar changes during preparation of Burukutu beer, samples analysed were obtained from a local brewer. A freshly prepared drink was analysed together with gari – a starch adjunct. The pattern of sugar changes during this 5-day malting stage was also followed. Twelve sugars were identified in the beverage while six sugars were shown to be present in the gari adjunct. Analyses of both germinated and ungerminated sorghum grains show that no new types of sugars resulted from the process of germination. In the study of amylase activity of the malts it was shown that detectable amylase activity began from the second day of germination onwards. Because of the relatively high concentrations of malto-oligosaccharides in the drink, starch amylosis should have continued during the fermentation stage.

Introduction

Burukutu is an alcoholic beverage brewed from the guinea corn grains (*Sorghum vulgare; = S. bicolor* (Moench)); in the savannah regions of Nigeria. During the preparation, sorghum grains are steeped in water overnight. The grains are then poured into a basket and the water is allowed to drain off for about 2 hours.

The grains are spread out on leaves or mats in a bed of about 3–4 in. in thickness and covered with another layer of leaves. During this malting period, the grains are watered on alternate days and occasionally turned over.

Germination, which starts within 24 h after steeping, is allowed to continue for a period of 4–5 days. The length of plumule is used in judging when germination has gone far enough. However, germination is usually complete after a period of 4 days.

After germination the malt is spread in thin layers in the sun to dry for 1 or 2 days. The dried malt is ground into powder.

In the next stage, gari (a starchy powder produced from the tuber of cassava plant, *Manihot utilissima* Pohl.) is added to a mixture of the ground malt and water and stirred with a stick. The resulting mixture (gari–malt powder–water) is roughly in the ratio 1 : 2 : 6 by vol.

The mixture is left to ferment for 2 days. At the end of fermentation, the mixture is boiled for about 4 h. The drink is then left to mature for another period of 2 days. The end product is a suspension of some particles in a creamy liquid.

Similar alcoholic beverages produced from cereal grains have been described in other parts of the world, e.g. in the Republic of South Africa, a kaffir beer is produced from *Sorghum caffrorum* Beauv.[1,2] In Nigeria little study has been made on any of the beverages produced from the cereals. Recently, a study of the microflora of Pito drink was started (Ekundayo, J. A., personal communication). Pito is a beverage which can be brewed from millet or corn or sorghum grains.

The present study was undertaken to determine the different types of sugars in the Burukutu beer. The changes which occur in the various sugar concentrations were followed during the malting stage in order to ascertain the type and amount of sugars formed during the germination of the grains.

Experimental

Preparation of sorghum malt

All the samples analysed were obtained from a local brewer, who bought the sorghum grains from the local markets. The grains were steeped in water overnight (about 16 h). The water was allowed to drain off from a basket for about 1 h.

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The grains were then spread out in a bed of up to 3 in. in thickness and covered with banana leaves and sacks. Each day samples were taken out from the germinating grains and spread out in thin layers to dry in the sun.

The remaining grains were watered and turned over every day. The dried malt was ground in a Waring blender. Germination was allowed to continue for five days.

Extraction of sugars from the samples

5·0 g of ground malt were weighed into a 100 ml conical flask. The sugars were extracted with three 50 ml portions of 80% ethanol on a boiling water bath. Each portion was extracted for 1 h. Three such extractions were shown from the preliminary experiments, to be sufficient for complete extraction of the sugars.

The solid particles in the extract were centrifuged and the supernatant concentrated *in vacuo* at a temperature of 60° to a volume of about 20 ml. The proteins were precipitated with a suspension of basic lead acetate in water. The red–brown pigment got from the coat of the grains was also adsorbed by the thick precipitate formed. After centrifuging the precipitate the lead ions were removed by two successive bubblings of H_2S gas through the solution.

H_2S was boiled off and the volume of the extract was reduced *in vacuo* at a temperature of 60° to about 3·0 ml. The total volume was made up to 5·0 ml with 20% (by vol.) aqueous isopropanol.

Sugar analysis

0·01 ml of each sugar extract was spotted in triplicate on Whatman No. 1 chromatography paper. The chromatogram was developed for 72 h in 1-butanol–acetic acid–water (4 : 1 : 1 by vol.) using a descending paper chromatographic method.

TABLE I

Analysis of sugars in gari

Sugar	Conc., % by wt.
Mannose	0·90
Galactose	0·50
Maltose	0·32
Maltotriose	0·30
Maltotetraose	0·09
Raffinose	0·45

TABLE II

Analysis of sugars in Burukutu beer
The samples were analysed immediately
after the 2-day maturing period

Sugar	Conc., % by wt.
Fructose	0·20
Mannose	0·65
Glucose	0·60
Galactose	0·36
Galacturonic acid	0·48
Sucrose	0·20
Maltose	0·42
Isomaltose	0·38
Raffinose	0·60
Maltotriose	0·56
Stachyose	0·43
Maltotetraose	0·16

After drying, one of the triplicate columns was cut off and the sugars on it were located using the method described by Dube & Nordin.[3] Then, areas containing the spots were marked out, and corresponding areas on each of the remaining duplicates were removed and eluted for 3 h with 5·0 ml distilled water.

To 2·0 ml of an aqueous eluate was added 0·05 ml of 80% (wt./vol.) aqueous phenol reagent followed by a rapid addition of 5·0 ml of conc. H_2SO_4. After the sample had stood at room temperature (25°) for 30 min, the optical density was determined at 480 nm. The concentration of each sugar was calculated from the standard curve. All samples were read against a blank containing distilled water in place of the sugar solution.[4]

Assay of amylases

100 ml of 0·2% (wt./vol.) calcium acetate at pH 4·6 were used to extract the amylases from 10 g of the ground sorghum malt for 3 hours.[3] The extract was centrifuged at 12,000 rev/min for 30 min at a temperature of between −1° and +1°.

The clear supernatant extract was assayed at 37° using a modified method of Street & Close[5] as described by Street.[6] After the preliminary experiments it was considered essential to dilute the extract 10 times. The extract was always diluted with the calcium acetate solution.

Results and Discussion

Table I shows the sugar content of the gari adjunct. The sugar content in the Burukutu beer is shown in Table II. The sugars identified in the beverage were fructose, mannose, galactose, glucose, galacturonic acid, sucrose, maltose, isomaltose, raffinose, maltotriose, stachyose and maltotetraose.

Fig. 1 shows the variation of sugars with the number of days of the germination of the sorghum grains. The pattern of variation of the sugars during this malting stage showed that the sugars could be divided into three distinct groups: those sugars which decreased in concentrations on the first day of germination and then showed a rapid increase in concentration on the second day until the third day after which there was no further substantial increase in concentration, e.g. glucose and sucrose; those sugars which showed a steady increase in their concentrations until the second day, after
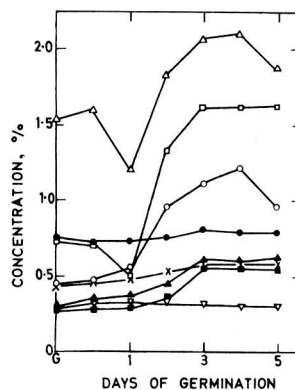

FIG. 1. *Concentration of sugar vs. days of germination*
△ Sucrose; □ glucose; ○ fructose; ● raffinose; ▲ maltose; × maltotriose; ■ isomaltose; ▽ stachyose

which there was a relatively sharp rise in the concentrations but they did not increase after the third day, e.g. fructose, maltose, isomaltose and maltotriose; and those sugars which did not show any increase in their concentrations, e.g. raffinose and stachyose.

Previous analyses of sorghum grains have shown the presence of a number of sugars. Von Holdt & Brand[7] reported that only fructose, glucose and sucrose were present in ungerminated sorghum grains. They also showed, in addition to these three sugars, that maltose, isomaltose, maltotriose and traces of higher malto-oligosaccharides were present in germinated grains. Nordin[8] reported the presence of stachyose in sorghum grains.

The purpose of the malting stage is to effect the hydrolysis of starch to fermentable sugars. It is the view of many workers that amylases are responsible for this process of hydrolysis. There have been conflicting views as to when the activity of these enzymes becomes manifested. Novellie[2] concluded that the enzymes are synthesised during the germination stage. However, Dube & Nordin[3] reported that the amylases are usually present in the insoluble condition in the grains even before germination begins. Here, there is evidence (Fig. 2) that detectable amylase activity started from the second day.

During the steeping stage the metabolic activities of the grains which were previously dormant, were resumed. Some of the sugars would be used in the metabolic processes. Sorghum grains germinate rapidly and hence will require high energy-level compounds. This could be responsible for the initial fall in the concentrations of some of the sugar. However, when the amylase activity resumes, more sugars will be produced than is required for metabolism; thus the concentrations of these sugars increase.

It appears that all these sugars were already present in the grains before germination started. Some suggestions have been made that the moulds which sometimes grow on the grains, produce amylases which would attack the starch.[9] This initial sugar production cannot be excluded because Nigeria is a humid country and moulds grow readily on food substances.

The local brewers add gari to the mash to increase the viscosity of the drink. A consumer regards a thin drink as being too dilute and is therefore considered to be of an inferior quality.

Analysis of the gari powder (Table I) showed that mannose, galactose, maltose, raffinose, maltotriose and maltotetraose were present. Thus, only galacturonic acid was not identified free in any of the ingredients used in the preparation of the drink. Galacturonic acid is usually obtained by enzymic hydrolysis of pectin in plant materials. It is expected that pectic substance in gari could have been hydrolysed by the enzymes of the microflora which ferment the drink.

From Table II it can be seen that there are relatively high concentrations of the malto-oligosaccharides, in the drink. Since the mash was not boiled the amylases of the malt remained active and a certain degree of amylosis could occur during fermentation. Also the micro-organisms responsible for this fermentation could produce amylases which could hydrolyse the starch. Novellie[10] reported such a starch amylosis during kaffir beer fermentation.

During the preparation of gari the starch should have undergone some degree of gelatinisation. This would make the breakdown of the starch rapid and thus more sugars
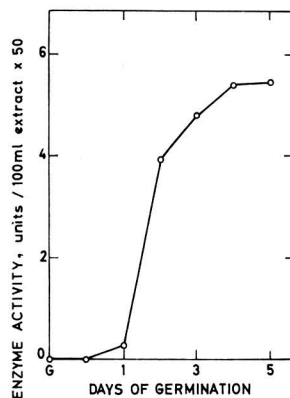
FIG. 2. *Graph of amyalse activity against days of germination*
Enzyme activity is expressed in Street–Close units

would be produced.[11] Because some of the sugars which arise from starch hydrolysis, e.g. glucose and maltose, could be used up in the fermentation process while the malto-oligosaccharides are unfermented, it is expected that these maltosugars would remain in relatively high concentrations.

Sucrose appears to be the sugar used mainly in the fermentation. This is not surprising since some of the micro-organisms which could be responsible for the fermentation of the drink usually ferment sucrose in preference to other sugars. Although these micro-organisms have not been analysed, however, it has recently been shown that *Saccharomyces cerevisiae* is one of the yeasts which ferment Pito drink (Ekundayo, J. A., personal communication). It is expected that this yeast would play a major role in this beverage. Also, from the sour taste of the drink, some lactobacilli may have played a substantial rôle in the fermentation. This question will be resolved when the study of the microflora which ferment Burukutu beer is completed.

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SUPERFICIAL SCALD, A FUNCTIONAL DISORDER OF STORED APPLES

VI.*—Evaporation of α-farnesene from the fruit

By F. E. HUELIN and I. M. COGGIOLA

During storage Granny Smith apples were ventilated with air at two flow rates differing by a factor of 10. The α-farnesene evaporated, α-farnesene retained by the fruit, and the severity of scald were determined. Increasing the ventilation increased the evaporation of α-farnesene. In two cases the amount evaporated was approximately equal to that retained. In some cases increased ventilation gave less retention of α-farnesene by the fruit and less scald, but in others the difference was negligible because increased evaporation stimulated production. The factors controlling evaporation and the concentration of α-farnesene remaining in the fruit during storage are discussed.

Introduction

The evidence already presented[1,2] for a rôle of α-farnesene in superficial scald includes the effects of variety and maturity, the transfer of α-farnesene to the oiled wrap, and the inhibition of its oxidation by diphenylamine. It remains to be considered whether the rôle of α-farnesene is the same as that of the volatile or gaseous substances postulated by Brooks *et al.*[3] as the cause of scald. Their theory was based primarily on observations that increased air movement over the fruit reduced scald. If α-farnesene is involved, air movement should increase its loss by evaporation and reduce its concentration in the fruit.

In experiments over three seasons samples of 20–25 apples were stored in drums ventilated with air at 2 or 20 1/h. The α-farnesene evaporated, α-farnesene remaining in the fruit, and the severity of scald were determined. Comparisons were made in 1965 between untreated and diphenylamine-treated apples, in 1966 between apples from two districts, and in 1967 between two storage temperatures.

Experimental

Granny Smith apples were picked and separated into comparable samples of 20–25 apples as previously described.[1] Apples from the Bathurst district of New South Wales were picked on 20 April 1965 and separated into 28 samples. Half of these were untreated and the other half were dipped before storage in a solution of 0·01M diphenylamine in 50% by vol. aqueous ethanol and dried on wire trays. Two untreated and two diphenylamine-treated samples were analysed as previously described[1] after one week at 1°. Each of the other samples was stored at 1° in a galvanised iron drum through which air was drawn at 2 or 20 1/h. Before entering the drums air at outside ambient temperature was first purified by passage through a furnace tube at 500° and then drawn into the room at 1°, where condensed water was removed in a trap. The air leaving the trap for the drums would be almost saturated with water. At the lower flow rate of 2 1/h the air leaving a drum contained less than 0·1% of carbon dioxide. The six samples of each treatment–flow rate combination were removed in pairs after 11, 21 and 31 weeks at 1°. One sample of each pair was examined

for scald and analysed immediately after removal, while the other sample was examined for scald after a further week at 20°.

During each of the 30 weeks at 1° a measurement of the rate of evaporation of α-farnesene was made on one sample for final analysis from each treatment–flow rate combination. In successive weeks measurements were made on each sample in turn. The α-farnesene and other volatile substances in the air from the drum were absorbed by drawing the air through a U-tube cooled in an ice–calcium chloride mixture followed by two spiral absorbers in series, each containing 20 ml of purified hexane and cooled in solid CO_2–ethanol. After three days the contents of the U-tube and spiral absorbers were transferred to a separating funnel, rinsing with purified hexane. The aqueous layer was separated and the hexane solution was dried with 2 g anhydrous sodium sulphate, filtered into a 100 ml volumetric flask and made up to volume. The ultra-violet absorbance and concentration of α-farnesene were measured as previously described for extracts of the apple coating and underlying cells.[1] The rate of evaporation of α-farnesene was calculated as $\mu g/cm^2/week$.

In 1966 the experimental procedure was the same as in 1965, except that all the apples were untreated and 14 samples were obtained from the Griffith district of New South Wales and 14 samples from the Huon district of Tasmania. They were picked on 24 March and 5 April respectively to ensure as far as possible comparable maturity. Measurements of evaporation were made for only 24 weeks and samples were analysed after 1, 9, 17, and 25 weeks at 1°. The furnace tube was replaced by a tube of activated charcoal, which was found to purify the air more effectively.

In 1967 28 samples from the Bathurst district were picked on 17 April 1967. All were untreated but 14 samples were stored at 5° and 14 samples at 15°. The trap at 5° still condensed an appreciable quantity of water from the entering air, hence the air leaving this trap would be approximately saturated. To make the air at 15° of comparable saturation it was drawn through a tube containing strips of wet filter paper. Owing to the rise in respiration with temperature, the concentration of carbon dioxide in air leaving a drum at 2 1/h was 0·4% at 5° and 1·0% at 15°. Measurements of evaporation were made for two days at 5° and one day at 15°, and the period of the experiment was reduced to 19 weeks at 5° and 10 weeks at 15°.

Results

Evaporation from untreated and diphenylamine-treated apples at 1°

The weekly rates of evaporation for a flow rate of 20 l/h are given in Table I. The rate of evaporation was negligible for the first 3 weeks, rose to about $1 \cdot 2$ $\mu g/cm^2/week$ at 21 weeks, and maintained this level until 26 weeks. The rates for untreated and diphenylamine-treated apples did not differ significantly. The rates for 20 l/h were mostly more than 20 times the corresponding rates for 2 l/h, which did not exceed $0 \cdot 05$ $\mu g/cm^2/week$.

The solutions, in which the evaporated α-farnesene was condensed, generally gave absorbance curves with the major peak at 232 nm and no more than a slight peak at 269 nm. The curves of the unpurified solutions after 30 weeks at 1° were exceptional in having a prominent peak at 269 nm with a shoulder at 260 nm and a subsidiary peak at 280 nm. The peak at 269 nm, which indicated conjugated triene oxidation products with the evaporated α-farnesene, was as prominent for diphenylamine-treated as for untreated apples. At this time there was no evidence of oxidation to conjugated trienes in the coating extracts of diphenylamine-treated apples, although the α-farnesene evaporated from them was accompanied by oxidation products. On account of oxidation of α-farnesene after evaporation, the measured rates of evaporation after 30 weeks at 1° for a flow rate of 20 l/h were probably too low.

The α-farnesene evaporated at 11, 21, and 31 weeks at 1°, as shown in Table II, was obtained by adding the rates of evaporation for the previous weeks. The α-farnesene retained in the fruit (coating and cells combined) is given in the next column. The total α-farnesene (evaporated and retained) is equal to the α-farnesene produced less that lost by oxidation or other chemical change. The results indicate that up to 21 weeks the higher evaporation at 20 l/h was accompanied by a lower retention in the fruit and total α-farnesene was not significantly affected. After 31 weeks retention was less affected by air flow but total α-farnesene was much greater for 20 l/h. It appears that in the last 10 weeks the increased evaporation at 20 l/h stimulated an increased production by the fruit. The diphenylamine-treated fruit retained more α-farnesene at 31 weeks than the untreated fruit, probably owing to inhibition of oxidation.

In the untreated fruit both oxidation to conjugated trienes and severity of scald were less at the higher flow rate. The reduction of scald was, however, much less than the almost

TABLE I

Rate of evaporation of α-farnesene from untreated and diphenylamine-treated apples at 1°C and 20 l/h

Weeks at 1°C	Rate of evaporation $\mu g/cm^2/week$	
	Untreated	Diphenylamine, treated
1	0·01	0·01
2	0·01	0·01
3	0·01	0·01
4	0·05	0·03
5	0·12	0·11
6	0·21	0·14
7	0·28	0·19
8	0·54	0·61
9	0·56	0·63
10	0·75	0·79
11	0·76	0·83
12	0·84	0·79
13	1·04	1·04
14		0·93
15	1·02	1·12
16	1·07	0·97
17	1·09	1·12
18	1·00	0·95
19	1·13	1·13
20	1·11	0·99
21	1·26	1·23
22	1·15	1·19
23	1·10	1·13
24	1·18	1·18
25	1·19	1·29
26	1·16	1·32
27	1·00	1·04
28	1·04	1·22
29	1·02	1·14
30	0·82	0·76

TABLE II

α-Farnesene evaporated from and retained in untreated and diphenylamine-treated apples at 1°C

Weeks at 1°C	Treatment* and flow rate, l/h	α-Farnesene, $\mu g/cm^2$			Conjugated trienes†	Scald score**	
		Evaporated	Retained	Total			
1	U		1·9	1·9	−0·13	0	(0)
	D		1·3	1·3	−0·12	0	(0)
11	U; 2	0·1	42·2	42·3	0·00	0	(0)
	U; 20	2·5	36·0	38·5	−0·17	0	(0)
	D; 2	0·1	32·0	32·1	−0·59	0	(0)
	D; 20	2·5	34·7	37·2	−0·71	0	(0)
21	U; 2	0·3	62·1	62·4	2·11	0	(3·80)
	U; 20	12·6	53·4	66·0	1·25	0·04	(2·21)
	D; 2	0·3	70·3	70·6	−0·58	0	(0·04)
	D; 20	12·4	52·9	65·3	−0·86	0	(0)
31	U; 2	0·6	32·1	32·7	3·52	2·58	(6·29)
	U; 20	23·6	38·6	62·2	2·89	1·91	(4·96)
	D; 2	0·5	64·9	65·4	−0·08	0	(0·35)
	D; 20	23·9	61·4	85·3	−0·25	0	(0·17)

* U = Untreated, D = Diphenylamine-treated
† Rise in absorbance from 262 to 269 nm for coating extract of sample in 500 ml, adjusted to surface area of 3000 cm²
** Score in brackets is for sample examined after a week at 20°C

complete control obtained in most cases by Brooks *et al.*[3] Increased air movement also reduced scald in the diphenylamine-treated fruit, although the incidence was only slight.

Evaporation from apples from different districts at 1°

Hicks & Evans (unpublished results), when comparing apples from different districts of Queensland, New South Wales, and Tasmania, found an inverse relation between scald and water loss. Apples from the Griffith district gave most scald and least water loss, while apples from the Huon district gave least scald and most water loss. The present experiment with the apples from these districts was undertaken to determine whether the lower incidence of scald in the Huon apples was also associated with increased evaporation of α-farnesene with less α-farnesene remaining in the fruit.

The weekly rates of evaporation for a flow rate of 20 l/h are given in Table III. The rate of evaporation from the Griffith apples was negligible for the first 3 weeks, rose to about 1·0 μg/cm²/week at 14 weeks, and declined a little subsequently. The rate of evaporation from the Huon apples rose more rapidly at first and continued to rise until it reached about 1·2 μg/cm²/week towards the end of storage. As in the previous experiment most of these rates were more than 20 times the corresponding rates for 2 l/h.

The α-farnesene evaporated and retained in the fruit, oxidation to conjugated trienes, and the severity of scald are shown in Table IV. After 25 weeks the α-farnesene evaporated from the Griffith apples at 20 l/h was approximately equal to that retained at 2 l/h. This evaporation without increased production would be sufficient to remove all the α-farnesene from the apples at 20 l/h, but in fact retention was little affected by flow rate. The evaporation at 20 l/h was largely balanced by increased production, and total α-farnesene was greater for the higher flow rate. In the Huon apples the higher flow rate caused an increase of production, but this was sufficient to balance the increased evaporation only up to 17 weeks. In apples from both districts increased flow rate gave a little reduction of scald.

The lower incidence of scald in the Huon apples was not due to increased loss of α-farnesene by evaporation. During storage the Huon apples evaporated about 25% more α-farnesene than the Griffith apples at 20 l/h, but retained more at both flow rates. Reduction of scald in the Huon apples appears to be due to less oxidation of α-farnesene as indicated by the level of conjugated trienes. It is suggested that these apples had more natural antioxidant.

TABLE III

Rate of evaporation of α-farnesene from Griffith and Huon apples at 1°c and 20 l/h

Weeks at 1°c	Rate of evaporation, μg/cm²/week	
	Griffith	Huon
1	0·00	0·02
2	0·00	0·02
3	0·00	0·02
4	0·03	0·06
5	0·04	0·15
6	0·05	0·30
7	0·22	0·41
8	0·25	0·52
9	0·24	0·50
10	0·75	0·87
11	0·55	0·75
12	0·85	0·94
13	0·87	
14	1·12	1·12
15	1·03	0·94
16	0·94	0·96
17	0·92	1·18
18	0·96	1·06
19	0·72	1·12
20	0·87	1·21
21	1·03	1·17
22	1·08	1·19
23	0·76	1·21
24	0·75	1·24

TABLE IV

α-Farnesene evaporated from and retained in Griffith and Huon apples at 1°c

Weeks at 1°c	District* and flow rate, l/h	α-Farnesene, μg/cm²			Conjugated trienes†	Scald score**
		Evaporated	Retained	Total		
1	G		1·1	1·1	−0·03	0 (0)
	H		1·1	1·1	−0·09	0 (0)
9	G; 2	0·0	23·2	23·2	0·42	0 (0·15)
	G; 20	0·6	25·9	26·5	0·40	0 (0·05)
	H; 2	0·0	22·0	22·0	−0·11	0 (0)
	H; 20	1·5	21·0	22·5	−0·14	0 (0)
17	G; 2	0·2	18·4	18·6	1·27	0·55 (3·60)
	G; 20	6·9	20·7	27·6	0·36	0·75 (3·20)
	H; 2	0·2	20·1	20·3	0·26	0 (0·25)
	H; 20	8·6	18·7	27·3	0·21	0 (0·21)
25	G; 2	0·4	13·8	14·2	1·91	4·25 (6·17)
	G; 20	14·0	12·1	26·1	2·07	2·90 (5·50)
	H; 2	0·4	33·7	34·1	1·28	0·50 (3·36)
	H; 20	17·9	25·6	43·5	1·25	0·50 (2·12)

* G = Griffith, H = Huon
† Rise in absorbance from 262 to 269 nm for coating extract of sample in 500 ml, adjusted to surface area of 3000 cm²
** Score in brackets is for sample examined after a week at 20°c

Evaporation from apples at 5 and 15°

In the experiments at 1° already described, the effect of flow rate on the retention of α-farnesene was variable and the effect on scald was much less than that obtained by Brooks *et al.*[3] who did much of their work at 15°. It is possible that increased evaporation at higher temperatures would cause a greater effect of air movement on the retention of α-farnesene and the severity of scald. On this account experiments were carried out in 1967 at 5° and 15°.

The weekly rates of evaporation are given in Table V. For a flow rate of 20 l/h the rate of evaporation at 5° rose to about 2·2 μg/cm²/week at 13 weeks and maintained this level until 17 weeks. The rate of evaporation at 15° rose to about 8·4 μg/cm²/week at 6 weeks. Most of these rates were more than 20 times the corresponding rates at 2 l/h.

The α-farnesene evaporated and retained in the fruit are shown in Table VI. In spite of the effect of increasing temperature on evaporation, the α-farnesene evaporated was generally less than that retained in the fruit. After 10 weeks at 15° and 20 l/h the α-farnesene evaporated was approximately equal to that retained, but in all other cases was much less. The higher temperatures gave a greater initial production of α-farnesene as well as evaporation, and the maximum concentration of α-farnesene in the fruit was reached at 5°. In all cases the increased evaporation at 20 l/h was accompanied by an increased production, and flow rate had little effect on oxidation to conjugated trienes or on scald. Included in the scald score were some small depressed areas which differed from the scald usually encountered at 1°.

Relation of evaporation to vapour pressure

The maximum rate of evaporation at 20 l/h was reached at approximately the same time as the maximum concentration of α-farnesene in the fruit. The maximum rates were 1·0–1·2, 2·2, and 8·4 μg/cm²/week at 1°, 5°, and 15° respectively. These correspond to 24, 44, and 162 μg/h for a sample of 20–25 apples. With these rates of evaporation the concentration of α-farnesene in the air leaving the fruit was 1·2, 2·2, and 8·1 μg/l. These concentrations correspond to partial pressures for α-farnesene of 0·10, 0·19, and 0·71 μm of mercury at 1°, 5°, and 15° respectively. At 2 l/h the concentrations in the air leaving the fruit were less, as the rate of evaporation was less than 5% of the rate at 20 l/h.

An approximate estimate of the vapour pressure of α-farnesene at 1–15° was attempted. The gas chromatographic retention time of α-farnesene on Apiezon L (a non-polar phase) at 140° was found to be practically identical with that of 1-pentadecene, indicating approximately equal vapour pressures. The vapour pressure of 1-pentadecene was measured from 173° to 268° by Camin & Rossini,[4] who found that their values fitted the equation $\log_{10}P = 7 \cdot 01555 - 1781 \cdot 974/(162 \cdot 582 + t)$, where P = vapour pressure in mm and t = temperature. From determinations of boiling points between 0·5 and 10 mm as part of Research Project

TABLE V

Rate of evaporation of α-farnesene from apples at 5 and 15°C

Weeks	Rate of evaporation, μg/cm²/week			
	5°C		15°C	
	2 l/h	20 l/h	2 l/h	20 l/h
1	0·05	0·09	0·09	0·36
2	0·02	0·23	0·03	0·90
3	0·05	0·75	0·10	2·92
4	0·05	1·31	0·09	5·64
5	0·03	2·20	0·23	7·56
6	0·06	1·61	0·18	8·56
7	0·03	2·00	0·33	8·42
8	0·04	1·70	0·25	7·84
9	0·03	2·28	0·07	8·80
10	0·03	1·90		
11	0·03	2·26		
12	0·03	2·02		
13	0·03	2·29		
14	0·03	2·17		
15	0·03	2·21		
16	0·03	2·25		
17	0·03	2·19		
18	0·03	2·11		

TABLE VI

α-Farnesene evaporated from and retained in apples at 5 and 15°C

Temperature, °C	Weeks	Flow rate, l/h	α-Farnesene, μg/cm²			Conjugated trienes*	Scald score†	
			Evaporated	Retained	Total			
5	1			2·6	2·6	−0·19	0	(0)
	7	2	0·3	63·0	63·3	−0·27	0	(0)
		20	6·2	64·7	70·9	−0·27	0	(0)
	13	2	0·4	116·7	117·1	0·65	0	(0·48)
		20	18·3	107·3	125·6	0·81	0·50	(0·92)
	19	2	0·6	114·3	114·9	2·34	1·25	(3·67)
		20	31·6	94·3	125·9	2·34	1·90	(2·52)
15	1			2·3	2·3	−0·15	0	(0)
	4	2	0·2	35·2	35·4	−0·35	0	(0)
		20	4·2	35·0	39·2	−0·44	0	(0)
	7	2	0·7	86·9	87·6	0·35	0	(0)
		20	25·9	78·9	104·8	0·47	0	(0·04)
	10	2	1·4	46·0	47·4	1·86	1·50	(1·50)
		20	51·0	52·0	103·0	1·55	0·53	(1·00)

* Rise in absorbance from 262 to 269 nm for coating extract of sample in 500 ml, adjusted to surface area of 3000 cm²

† Score in brackets is for sample examined after a week at 20°C

42 of the American Petroleum Institute it appears that the equation gives vapour pressures which are accurate within 1 % error down to 130°, but which are 5 % too low at 100° and 15 % too low at 80°. The equation gives the vapour pressures of 1-pentadecene at 1°, 5°, and 15° as $0 \cdot 13$, $0 \cdot 24$, and $0 \cdot 96$ μm respectively. Assuming that the true values are about double these figures and that the vapour pressures of α-farnesene and 1-pentadecene remain approximately equal down to 1°, the vapour pressure of α-farnesene at 1°, 5°, and 15° is given as $0 \cdot 26$, $0 \cdot 48$ and $1 \cdot 92$ μm.

These figures suggest that the partial pressure of α-farnesene in the air leaving the fruit at 1–15° and 20 l/h was about 38 % of the vapour pressure, which is the partial pressure in equilibrium with pure liquid α-farnesene. The partial pressure in equilibrium with α-farnesene in the apple coating would be much less than the vapour pressure, hence the α-farnesene content of the air leaving the fruit at 20 l/h may have been close to saturation. Although the α-farnesene content of the air at 2 l/h could not be determined accurately, it appeared to be less saturated than the faster air.

Discussion

In these experiments the rate of evaporation of α-farnesene from 20–25 apples at a flow rate of 20 l/h was generally more than 20 times the rate of evaporation at 2 l/h. Although the total α-farnesene evaporated at 20 l/h reached an appreciable proportion of that retained in the fruit at 2 l/h and in two cases was approximately equal to it, the effect of flow rate on the concentration of α-farnesene in the fruit was much less than expected. In some cases the higher flow rate gave less retention of α-farnesene by the fruit and less scald. In others the difference was negligible because the increased evaporation stimulated a greater production by the fruit.

Although Brooks *et al.*[3] obtained in most cases almost complete control of scald by increased air movement, other workers have obtained results more in agreement with ours. The apples used by Brooks *et al.* may have lacked the ability to produce more α-farnesene in response to increased evaporation. It is also suggested that lower humidity, which has been found to reduce scald,[5] may affect this ability. The present experiments were conducted at high humidity, and lower humidities may reduce the water content of the epidermis and hypodermis and the ability of this tissue to produce additional α-farnesene.

Conditioning the storage atmosphere with activated carbon has given little or no reduction of scald.[6,7] Huelin in an earlier paper[8] suggested that the partial pressure of the volatile agent reached only a low proportion of saturation, hence the deficit, which controlled evaporation, was little affected by absorbents. This work on the evaporation of α-farnesene does not support this explanation, if the α-farnesene content of the air leaving the fruit was close to saturation. Absorption of α-farnesene by activated carbon might promote evaporation, but this could have little or no effect on scald if it stimulated increased production.

The evidence presented in this and previous papers[1,2] is sufficient to indicate that α-farnesene occupies the rôle in superficial scald ascribed to volatile or gaseous substances by Brooks *et al.*[3] It has been shown that α-farnesene is sufficiently volatile to explain the effects of air movement and oiled wraps on the incidence of scald. Although the oxidation of α-farnesene appears to be the immediate cause of scald, the rate of oxidation is partly determined by the concentration of α-farnesene in the fruit, and this concentration can be affected by air movement or oiled wraps.

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VITAMIN B CONTENT OF COWPEAS
(*VIGNA UNGUICULATA* WALP)
II.*—Pyridoxine, pantothenic acid, biotin and folic acid

By B. K. OGUNMODEDE and V. A. OYENUGA

The microbiological estimations of pyridoxine, pantothenic acid, biotin and folic acid were carried out on thirty varieties of cowpeas. Precautions needed to obtain consistent results for each of the vitamins were recorded.

Individual values of the vitamins in the samples were recorded and statistical analyses of the results for both the varieties and the three types of cowpeas are discussed. Varietal differences in the vitamin contents were noted.

Introduction

In an earlier report[1] the thiamine, riboflavin and niacin values of thirty varieties of cowpeas (*Vigna unguiculata* Walp) and the results of the statistical analyses of the varietal differences in these vitamin contents were described.

In the present paper, assays for vitamin B_6 (pyridoxine), pantothenic acid, biotin and folic acid in the same thirty varieties of cowpeas are reported. The vitamin contents of each variety are recorded, and comparative studies of the varietal differences in these vitamins are also shown.

Experimental

Materials

The sources and varieties of the cowpeas used, their method of cultivation, fertilisation, treatment and preparation for analyses were as described previously.[1] Microbiological estimation of pyridoxine, pantothenic acid, biotin and folic acid were made. In each case, the procedure involved preparations of stock culture, inoculum, standard and sample tubes; the tubes were sterilised, inoculated and incubated in the usual way.

Procedure

Vitamin B_6 (pyridoxine)

The pyridoxine activities of the cowpea varieties were determined in weighed samples that were autoclaved at 15 lb pressure for 4 h in 80 ml of 0·055N-HCl. This length of time was chosen to ensure complete extraction of the vitamin. The protein in the extract was precipitated by adjusting the pH to 4·5 with 15% NaOH and the extract was diluted with water to make the volume up to 200 ml. The mixture was filtered through Whatman filter paper No. 44 into brown-coloured glass bottles which prevented exposure to light. In cases where the filtrates were not clear, the filtration was repeated. Measured aliquots were added to the assay and standard tubes respectively as described by Atkin *et al.*[2] The tubes were stoppered with cotton plugs and their contents were sterilised by steaming at 100° for 10 min. After the tubes had been cooled to the same temperature, the contents were inoculated aseptically with 1 ml of inoculum of *Saccharomyces carlsbergensis* 4228. The tubes were placed in the tray of the shaking machine which was set in motion for 16 h, the temperature of the tubes being kept at approxi-

mately 30°. The growth of the cells was stopped by steaming the tubes at 100° for 5 min and the density of the cell growth was determined in a colorimeter fitted with a 640 nm filter.

The concentrations of the three forms of vitamin B_6 were also determined in the varieties of cowpeas. The mixture of pyridoxamine and pyridoxal was measured by the method of Rabinowitz & Snell[3] using *Streptococcus faecalis*, while the response of *Lactobacillus casei* was used to measure pyridoxal.[4] The pyridoxamine activity was obtained by difference. Care was taken to keep constant temperatures for the assay and standard tubes, and appropriate dilution factors were used to calculate the vitamin B_6 contents of the samples.

Pantothenic acid

Known weights of cowpea samples were suspended in water, and the pH of the suspension was adjusted to 6·8; 1 ml of the suspension was digested for 4 h with 0·2 ml (100 units) of alkaline phosphatase, 0·05 ml of acetone and peroxide-free chicken liver extract washed in ether. The mixture was diluted with 4 ml of water and a 2 ml aliquot was diluted to 40 ml with water. The mixture was filtered with Whatman paper No. 540 and different volumes, ranging from 0 to 5 ml, were measured into assay tubes. Varying volumes of standard calcium pantothenate solution (0·05 ng pantothenic acid/ml) were measured into standard tubes and all the tubes were sterilised at 15 lb pressure for 10 min, Difco–Bacto pantothenate medium being the basal medium used. After sterilising the tubes, the contents were inoculated with *Lactobacillus arabinosus* 17-5, and they were incubated at 37° for 72 h.

A standard curve for the assay was drawn by plotting the volume of 0·1N-NaOH used in titrating the acid content of the standard tubes against nanogrammes of pantothenic acid per tube. The pantothenic acid contents of the cowpea samples were obtained by interpolating their titre values on the standard curve. Five determinations were made for each sample, and the means were calculated.

Biotin

The biotin contents were also measured by a microbiological method. Preliminary trials were carried out to ascertain the best normality of the acid and the length of time for the extraction of biotin from cowpeas. In the trial 6N, 4N, and 3N sulphuric acid and length of time ranging from 10 min to 3 h were tested. The assay samples were eventually

extracted by autoclaving them at 15 lb pressure for 2 h in 4N sulphuric acid. The mixture was diluted with water and 20% NaOH was used to adjust the pH to 6·8, bromothymol blue being used as an external indicator. The mixture was filtered and aliquots of the sample solutions were measured into assay tubes. Varying levels of standard biotin solution (0·2 ng/ml) were also measured into standard tubes. Difco–Bacto biotin assay medium was used as the basal medium. The tubes were sterilised and inoculated with *L. arabinosus* 17-5. Acid production was measured by titration with 0·1N sodium hydroxide and the biotin contents of the samples were calculated by reference to a standard biotin curve.

Folic acid

The assays for folic acid were designed to distinguish between the free and the total folic acid. A preliminary study was conducted using *S. faecalis* and *L. casei* respectively as the assay organisms. The use of *S. faecalis* was adopted since there was no appreciable difference between the results obtained with the two organisms and because the results of assays with *S. faecalis* were more consistent than those with *L. casei*.

The cowpea samples were mixed with 1% sodium acetate buffer and were heated for 5 min at 100° on a water bath; after cooling to room temperature, hog kidney extract was added. The extract was prepared by trimming fat off the kidney, blending the organ with water and centrifuging; the supernatant was filtered through super-cel.

The mixture in the tube was layered with toluene and incubated for 24 h at 37°. The mixture was then heated for 5 min in a bath of boiling water, and after cooling to room temperature it was neutralised to pH 6·7. The mixture was diluted to 100 ml with water and it was filtered through Whatman paper No. 40. The distilled water used was boiled until half the volume evaporated. Assay and standard tubes were set up by measuring aliquots of the sample solutions or the standard folic acid solution (2 ng/ml) respectively into the tubes. Difco–Bacto folic acid assay medium was used and the tubes were inoculated with *Streptococcus faecalis* ATCC 8043 after sterilisation for 10 min at 15 lb pressure. The tubes were incubated for 72 h at 37° and the folic acid contents of the cowpea samples were estimated by the acid production of the organism. A standard curve for folic acid was used as the reference.

Results and Discussion

Five determinations for each vitamin in the varieties studied were made; the mean and the standard deviations are shown in Tables I–V. Table I shows the folic acid contents of the cowpea varieties. A range of 0·14 mg/100 g of 64377 of the 'blue eye' type to 0·19 for 6422M2 of the 'brown' type was observed. No difference was found between the means for the brown and the 'black eye' types (0·16). Also the student's *t* test for the means of the 'blue eye' type and the 'brown' type revealed no statistical differences at either the 5% or the 1% levels of probability. The means of the three types were considered as it is possible to have varieties of the same type of cowpeas mixed together by some local farmers. However, for purposes of improving the supply of nutrients to consumers, individual varieties are best considered.

The analysis of variance shown in Table VI revealed significant differences between the folic acid contents of the varieties of cowpeas. Thus the beans with high quantities of folic acid could be selected in preference to those having lower values. This is important in rural areas where antenatal care is not readily available to pregnant women who are advised to eat cowpeas as a source of protein. It should be appreciated that individual varieties and not types have to be considered in the case of the folic acid content of cowpeas. For example, Table I shows that each type contains some varieties with relatively high values for folic acid. Thus varieties 64355 of the 'blue eye' type, 64375M1 of the 'black eye' type and 64321 of the 'brown' type contain about 0·19 mg folic acid/100 g respectively.

The procedure for the analysis of folic acid requires further comment. *S. faecalis* measures free folic acid, while *L. casei* was used to estimate the total folic acid. The negligible difference obtained between the results of the two assays showed that the folic acid contents of cowpeas are probably in the free form or possibly in loose combinations with proteins or other compounds in the beans. For the assay, *S. faecalis* was chosen as Stokstad & Hutchings[5] observed that the use of *L. casei* gave rise to less reproducible results. This observation was confirmed in a preliminary trial. Similarly, precaution was taken to ensure correct results by boiling the distilled water as Price & Gore[6] found that distilled water may sometimes contain an inhibitor of *S. faecalis*.

TABLE I

Folic acid content of locally grown cowpeas,
mg/100 g

'Black eye' type		'Blue eye' type		'Brown' type	
Accession No.	Mean	Accession No.	Mean	Accession No.	Mean
6466 M1	0·137 ± 0·005	6420 M1	0·142 ± 0·002	66201 Dugbe	0·172 ± 0·007
6467 M1	0·151 ± 0·0	6470 M2	0·175 ± 0·0	66202 Oyo	0·168 ± 0·002
64373M1	0·142 ± 0·001	64355	0·186 ± 0·007	64381 M1	0·148 ± 0·002
64375M1	0·186 ± 0·001	64140	0·149 ± 0·007	6422 M2	0·189 ± 0·001
64380M1	0·173 ± 0·0	64359	0·145 ± 0·002	64228	0·135 ± 0·002
64395M1	0·174 ± 0·005	64368	0·150 ± 0·001	64298	0·145 ± 0·0
64150	0·142 ± 0·002	64369	0·140 ± 0·001	64319	0·151 ± 0·002
64306	0·185 ± 0·001	64377	0·135 ± 0·002	64321	0·184 ± 0·007
64385	0·162 ± 0·001	64438	0·159 ± 0·001	64426	0·172 ± 0·001
64407	0·156 ± 0·002	64442	0·142 ± 0·007	64495	0·153 ± 0·003
Mean:	0·161 ± 0·013	Mean:	0·152 ± 0·02	Mean:	0·162 ± 0·017

TABLE II

Vitamin B₆ activities of locally grown cowpeas,
mg/100 g

'Black eye' type		'Blue eye' type		'Brown' type	
Accession No.	Mean	Accession No.	Mean	Accession No.	Mean
6466 M1	$0 \cdot 336 \pm 0 \cdot 003$	6420 M1	$0 \cdot 295 \pm 0 \cdot 003$	66201 Dugbe	$0 \cdot 406 \pm 0 \cdot 008$
6467 M1	$0 \cdot 353 \pm 0 \cdot 007$	6470 M2	$0 \cdot 274 \pm 0 \cdot 002$	66202 Oyo	$0 \cdot 409 \pm 0 \cdot 004$
64373M1	$0 \cdot 336 \pm 0 \cdot 006$	64355M2	$0 \cdot 274 \pm 0 \cdot 009$	64381 M1	$0 \cdot 397 \pm 0 \cdot 001$
64375M1	$0 \cdot 339 \pm 0 \cdot 007$	64140	$0 \cdot 293 \pm 0 \cdot 006$	6422 M2	$0 \cdot 412 \pm 0 \cdot 002$
64380M1	$0 \cdot 343 \pm 0 \cdot 002$	64359	$0 \cdot 309 \pm 0 \cdot 030$	64228	$0 \cdot 388 \pm 0 \cdot 013$
64395M1	$0 \cdot 351 \pm 0 \cdot 023$	64368	$0 \cdot 283 \pm 0 \cdot 004$	64298	$0 \cdot 398 \pm 0 \cdot 003$
64150	$0 \cdot 355 \pm 0 \cdot 002$	64369	$0 \cdot 298 \pm 0 \cdot 003$	64319	$0 \cdot 404 \pm 0 \cdot 004$
64306	$0 \cdot 333 \pm 0 \cdot 001$	64377	$0 \cdot 304 \pm 0 \cdot 009$	64321	$0 \cdot 417 \pm 0 \cdot 004$
64385	$0 \cdot 347 \pm 0 \cdot 002$	64438	$0 \cdot 276 \pm 0 \cdot 0$	64426	$0 \cdot 385 \pm 0 \cdot 001$
64407	$0 \cdot 346 \pm 0 \cdot 007$	6442	$0 \cdot 312 \pm 0 \cdot 005$	54495	$0 \cdot 407 \pm 0 \cdot 011$
Mean:	$0 \cdot 344 \pm 0 \cdot 011$	Mean:	$0 \cdot 292 \pm 0 \cdot 019$	Mean:	$0 \cdot 402 \pm 0 \cdot 018$

The results of vitamin B₆ activities of cowpea varieties are shown in Table II. The extraction for 4 h appeared to have liberated all the vitamin and this might have contributed to the consistent results. Furthermore, cotton plugs were used instead of metallic caps as metallic contaminations appeared to interfere with the assay. It was imperative to maintain the tubes at the same temperatures before inoculation and while placed on the shaker, since small differences in temperature, especially at the beginning of the assay, influence the growth rate of the organism more than they influence the growth over longer periods of incubation.

The segregation of the cowpea varieties into groups on the basis of type is quite distinct in this case. The mean total B₆ activity of the 'brown type' was $0 \cdot 40$ mg/100 g and the differences between this mean and the mean of $0 \cdot 34$ for the 'black eye' was significant at both 5% and 1% levels. Similarly, statistical differences exist between the means for the 'black eye' and the 'blue eye' types. The comparison embracing all 30 varieties was done by the analysis of variance, and differences between the varieties was revealed by the variance ratio.

The study of the three forms of vitamin B₆ showed the distribution of these forms in cowpeas. In this study, pyridoxine was used as a generic name for the three forms of the vitamin while pyridoxol, pyridoxal and pyridoxamine were used for the respective forms. The distribution of the three forms are shown in Table III. Most of the pyridoxine is present in cowpeas as pyridoxol which accounts for about 61% of the total. The percentage of pyridoxal present is slightly more than that of pyridoxamine. As pyridoxal and pyridoxamine are nearly equal to pyridoxol in molecular weight and in activity, the actual amounts of pyridoxine in the source was determined by the use of *Saccharomyces carlsbergensis*.

The extraction of biotin in 4N sulphuric acid was done after preliminary trials with 6N, 4N and 3N. This was necessary as there is no universal procedure for liberating biotin from natural materials. The choice of time for autoclaving the sample was made after preliminary trials. Autolysis of the samples and enzymic hydrolysis of the samples do not release as much biotin as acid hydrolysis; the observation of Thompson *et al.*[7] on the destruction of biotin by 6N-H_2SO_4 in plant materials was confirmed by this preliminary observation. It is necessary to undertake such a study of the best hydrolytic procedure before any determination of biotin is made in any natural substance.

TABLE III

Distribution of the three forms of pyridoxine in cowpeas

Accession No.	Total Vitamin B₆, mg/100 g	Pyridoxol	Pyridoxal	Pyridox-amine
6466 M1	$0 \cdot 336$	$0 \cdot 205$	$0 \cdot 075$	$0 \cdot 056$
6467 M1	$0 \cdot 353$	$0 \cdot 216$	$0 \cdot 078$	$0 \cdot 059$
64373M1	$0 \cdot 336$	$0 \cdot 206$	$0 \cdot 075$	$0 \cdot 055$
64375M1	$0 \cdot 339$	$0 \cdot 207$	$0 \cdot 075$	$0 \cdot 056$
64380M1	$0 \cdot 343$	$0 \cdot 209$	$0 \cdot 076$	$0 \cdot 057$
64395M1	$0 \cdot 351$	$0 \cdot 215$	$0 \cdot 078$	$0 \cdot 057$
64150	$0 \cdot 355$	$0 \cdot 217$	$0 \cdot 079$	$0 \cdot 058$
64306	$0 \cdot 333$	$0 \cdot 203$	$0 \cdot 074$	$0 \cdot 056$
64385	$0 \cdot 347$	$0 \cdot 212$	$0 \cdot 077$	$0 \cdot 058$
64407	$0 \cdot 346$	$0 \cdot 211$	$0 \cdot 077$	$0 \cdot 058$
6420 M1	$0 \cdot 295$	$0 \cdot 180$	$0 \cdot 066$	$0 \cdot 050$
6470 M2	$0 \cdot 274$	$0 \cdot 167$	$0 \cdot 061$	$0 \cdot 046$
64355M2	$0 \cdot 274$	$0 \cdot 168$	$0 \cdot 061$	$0 \cdot 047$
64140	$0 \cdot 293$	$0 \cdot 179$	$0 \cdot 065$	$0 \cdot 049$
64359	$0 \cdot 309$	$0 \cdot 189$	$0 \cdot 069$	$0 \cdot 051$
64368	$0 \cdot 283$	$0 \cdot 173$	$0 \cdot 063$	$0 \cdot 047$
64369	$0 \cdot 298$	$0 \cdot 182$	$0 \cdot 066$	$0 \cdot 050$
64377	$0 \cdot 304$	$0 \cdot 186$	$0 \cdot 068$	$0 \cdot 050$
64438	$0 \cdot 276$	$0 \cdot 169$	$0 \cdot 061$	$0 \cdot 047$
64442	$0 \cdot 312$	$0 \cdot 190$	$0 \cdot 069$	$0 \cdot 052$
66201 Dugbe	$0 \cdot 406$	$0 \cdot 248$	$0 \cdot 090$	$0 \cdot 068$
66202 Oyo	$0 \cdot 409$	$0 \cdot 250$	$0 \cdot 091$	$0 \cdot 068$
64381M1	$0 \cdot 397$	$0 \cdot 242$	$0 \cdot 088$	$0 \cdot 066$
6422 M2	$0 \cdot 412$	$0 \cdot 251$	$0 \cdot 091$	$0 \cdot 069$
64228	$0 \cdot 388$	$0 \cdot 230$	$0 \cdot 084$	$0 \cdot 075$
64298	$0 \cdot 398$	$0 \cdot 250$	$0 \cdot 080$	$0 \cdot 072$
64319	$0 \cdot 404$	$0 \cdot 269$	$0 \cdot 071$	$0 \cdot 064$
64321	$0 \cdot 417$	$0 \cdot 275$	$0 \cdot 076$	$0 \cdot 066$
64426	$0 \cdot 385$	$0 \cdot 226$	$0 \cdot 083$	$0 \cdot 076$
66495	$0 \cdot 407$	$0 \cdot 268$	$0 \cdot 076$	$0 \cdot 067$

After extraction of biotin, the solution was diluted with water. This was necessary as the high sensitivity of the assay may be interfered with by compounds such as lipids that stimulate the growth of *L. arabinosus*. For example, Williams *et al.*[8] showed that oleic acid and related compounds are growth factors for lactic acid bacteria. The dilution reduced the efficacy of these substances and ensured a greater accuracy of the assay, while filtration removed more of the impurities.

The values of the biotin contents of locally grown cowpeas are shown in Table IV. Varieties of the 'blue eye' type have

TABLE IV

Biotin content of locally grown cowpeas,
μg/100 g

'Black eye' type		'Blue eye' type		'Brown' type	
Accession No.	Mean	Accession No.	Mean	Accession No.	Mean
6466 M1	18·5 ± 0·2	6420 M1	24·4 ± 0·4	66201 Dugbe	21·3 ± 0·3
6467 M1	18·2 ± 0·05	6470 M2	24·3 ± 0·3	66202 Oyo	21·8 ± 0·2
64373M1	18·6 ± 0·2	64355M2	26·1 ± 0·5	64381 M1	19·4 ± 0·3
64375M1	18·3 ± 0·2	64140	25·1 ± 0·3	6422 M2	20·0 ± 0·2
64380M1	18·8 ± 0·3	64359	25·1 ± 0·2	64228	22·3 ± 0·3
64395M1	18·2 ± 0·3	64368	26·0 ± 0·1	64298	20·1 ± 0·1
64150	18·2 ± 0·2	64369	24·9 ± 0·0	64319	21·4 ± 0·3
64306	18·1 ± 0·1	64377	25·3 ± 0·3	64321	21·5 ± 0·2
64385	18·7 ± 0·3	64438	24·2 ± 0·3	64426	22·2 ± 0·3
64407	18·3 ± 0·3	64442	26·7 ± 0·4	64495	21·8 ± 0·3
Mean:	18·4 ± 0·2	Mean:	25·2 ± 0·8	Mean:	21·2 ± 1·0

TABLE V

Pantothenic acid content of cowpeas,
mg/100 g

'Black eye' type		'Blue eye' type		'Brown' type	
Accession No.	Mean	Accession No.	Mean	Accession No.	Mean
6466 M1	2·03 ± 0·05	6420 M1	2·23 ± 0·10	66201 Dugbe	1·82 ± 0·0
6467 M1	1·98 ± 0·05	6470 M2	2·22 ± 0·20	66202 Oyo	1·84 ± 0·05
64373M1	2·04 ± 0·10	64355M2	2·18 ± 0·05	64381 M1	1·72 ± 0·0
64375M1	2·04 ± 0·0	64140	2·16 ± 0·05	6422 M2	1·98 ± 0·01
64380M1	1·95 ± 0·01	64359	2·24 ± 0·0	64228	1·80 ± 0·0
54395M1	1·98 ± 0·0	64368	2·17 ± 0·07	64298	1·90 ± 0·02
64150	1·99 ± 0·02	64369	2·19 ± 0·07	64319	1·82 ± 0·01
64306	2·01 ± 0·07	64377	2·23 ± 0·0	64321	1·82 ± 0·05
64385	2·05 ± 0·10	64438	2·13 ± 0·01	64426	1·73 ± 0·03
64407	2·00 ± 0·0	64442	2·01 ± 0·05	64495	1·81 ± 0·05
Mean:	2·01 ± 0·033	Mean:	2·18 ± 0·071	Mean:	1·82 ± 0·073

TABLE VI

Analysis of variance for some vitamins in cowpeas

		Folic acid	Pyridoxine	Biotin	Pantothenic acid
Number of observations		150	150	150	150
Total amount of vitamins		18·81	518·96	3,147·7	298·36
Correction factor		2·36	1796	66,053	593·5
Sum of squares about the mean		1·433	31·59	5,066·36	5·5
Sum of squares	varieties	1·404	29·60	3,078·83	4·2
	residual	0·0209	1·99	1,987·5	1·3
Degree of	varieties	29	29	29	29
freedom	residual	120	120	120	120
Mean square	varieties	0·0484	1·021	106·19	0·145
	residual	2·42 × 10⁻⁴	0·0166	16·56	0·013
Variance ratio	varieties	200·3	61·54	6·42	11·14
	residual				

The 5% point corresponding to 29 and 120 degrees of freedom in the variance ratio distribution is 1·55 while the 1% point is 1·86

the highest mean biotin content, and the mean of $25 \cdot 2$ $\mu g/$ 100 g is significantly greater than the mean of $21 \cdot 2$ for the brown type. Also, significant differences were observed between the 'brown' and the 'black eye' types. The analysis of variance of the biotin contents, shown in Table VI, revealed a variance ratio of $6 \cdot 4$, thereby confirming significant differences in the biotin contents of the cow pea varieties.

In the assay for pantothenic acid, alkaline phosphatase and chicken liver extracts were used as Mylase P has been shown[9] to be ineffective with many samples. Different levels of the test solutions were used as the procedure permitted evaluation of the validity of the assay within the limits of the standard curve. Furthermore, the standard calcium pantothenate solutions were prepared fresh for the assay as the solutions did not appear to keep.

The mean pantothenic acid contents of cowpeas are shown in Table V. As was the case for the biotin contents, varieties of the 'blue eye' type have the highest mean pantothenate content. The mean of $2 \cdot 2$ mg/100 g was significantly greater than the $2 \cdot 0$ for the 'black eye' type. Also, unlike the case for the other B vitamins, varieties of the 'brown' type contained the lowest amount of pantothenic acid. The analysis of variance for the pantothenate is reported in Table VI. The variance ratio of $11 \cdot 4$ showed that real differences exist between the pantothenic acid contents of the cowpea varieties.

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CHEMICAL COMPOSITION AND AMINO ACID CONTENT OF CHICKPEA SEEDS AT DIFFERENT STAGES OF DEVELOPMENT

By S. ABU-SHAKRA, S. MIRZA and R. TANNOUS

Chickpea seeds at five stages of seed development were analysed for contents of nitrogen, ether extract, ash, crude fibre, β-carotene, amino acid, and several minerals. Moisture, β-carotene and ash content on a dry weight basis decreased steadily during maturation, but other major components did not change appreciably. All the essential amino acids except lysine, cystine and tryptophan decreased at 28 or 35 days after flowering, then increased at maturity. Lysine was relatively high at 14 days after flowering (immature stage) but dropped sharply thereafter to a nearly constant value. An overall decrease in the mineral content of the seed was observed as the seeds matured.

Introduction

Chickpeas (*Cicer arietinum* L.) are grown and consumed as a seed legume in the Middle East, and Asian and African countries; for example the average daily *per capita* consumption in India and Pakistan ranges from 65 to 71 g.[1] Chickpeas provide an inexpensive source of dietary protein, the protein content of those grown in the Lebanon ranging from 17 to 25%, a level about double that of cereals. Nearly 8% of the protein and 15–30% of the protein calories consumed in the world are derived from legumes in which chickpeas contribute an appreciable proportion.[1]

Chickpeas, broadbeans, and lentils constitute the three major legume seeds consumed by inhabitants of the Middle East. Of these, chickpeas were found the most suitable for the development of protein-rich food mixtures for feeding infants and children in the Middle East.[2] In the Middle East, chickpeas are consumed as a green vegetable at various stages of maturity when the seeds are still green and tender. These immature seeds contain carotene and ascorbic acid which are almost absent in the dry seeds.

The purpose of the present study was to determine the changes in the chemical composition of the chickpea seed during different stages of development in an effort to evaluate the nutritive value of the developing seed for human consumption. In addition to proximate composition, carotene and mineral content, the complete amino acid pattern of the seeds was also determined.

Experimental

Growing and harvesting

Seeds of a local variety of chickpea (*Cicer arietinum* L.) were planted in a field in the Beqa's Plain and in sand culture in a glasshouse at the American University of Beirut.

Normal plant growth in the field was obtained by spacing plants 50×50 cm and fertilising the soil with 200 kg P_2O_5 and 120 kg of N/ha. The plants were grown under furrow irrigation.

In the glasshouse the seeds were sown in 15 cm dia. pots filled with washed white mountain sand (pH 7). Hoagland's complete nutrient solution was applied to the pots 2 weeks

after seed germination; subsequent watering with the nutrient solution was done every week throughout the experimental period. De-ionised water was supplied when necessary.

Open flowers on the plants grown in the field and in the glasshouse were labelled at the peak of flowering for a period of 5 days. Seed samples were harvested periodically from pods set on labelled flowers only. The first seed harvest was made 2 weeks after labelling and 4 subsequent harvests were made at 1 week intervals. In order to eliminate plant variation, a few pods of the same age were picked from many plants at each harvest. Approximately 500 pods from the field plants and 100 pods from the glasshouse were harvested at each stage. Immediately after harvest, the seeds were shelled, weighed, and stored below 0° until chemical analyses were carried out. Seeds harvested from the sand culture were used for mineral determination and those collected from the field were used for proximate analyses, and for determining carotene and amino acid content.

Chemical analyses

Moisture, nitrogen, ether extract, ash and fibre contents were determined according to the methods adopted by the Association of Official Agricultural Chemists (A.O.A.C.).[3] Protein was calculated by multiplying the nitrogen content by 6·25; nitrogen-free extract (NFE) was determined by difference.

For the determination of β-carotene, the A.O.A.C. chromatographic procedure was used. Carotene was measured spectrophotometrically at 440 μm.

For the determination of amino acids, the seed samples were freeze-dried and ground to a fine powder. Samples were then hydrolysed with 6 N-HCl.[4] Suitable aliquots of the hydrolysates were analysed on an automatic amino acid analyser (Phoenix Model K-8000) based on the method of Spackman et al.[5] The basic amino acids were separated on a short (15 cm) column, while the acidic and neutral amino acids were separated on a long (150 cm) column. The peak areas were compared with those obtained with standard quantities of pure L-amino acids.

Tryptophan was determined chemically because of its destruction during acid hydrolysis. The chemical method used was based on that of Lombard & Delange.[6]

Minerals were determined in digested samples using an atomic absorption flame photometer (Perkin-Elmer, Model 303). The method of wet digestion, as described by Jackson,[7] was used for preparation of the samples. 1 g portions of the freeze-dried ground samples were digested overnight with 17 ml concentrated nitric acid. Standards for each element were prepared. Mineral determinations included calcium, magnesium, sodium, potassium, iron, manganese, zinc, and copper.

Results and Discussion

The changes in the chemical composition of the developing chickpea seed are shown in Table I. The values are expressed on both wet and dry weight bases. The moisture content decreased steadily from 69·8% at 14 days after flowering to 12·5% at the 42nd day, averaging a decrease of 2·0% per day. The percentage (wet weight basis) of total nitrogen, ether extract, crude fibre, ash, and NFE increased steadily with the development of the seed. These results, due partly to the steady decrease in moisture content of the maturing seed, are in agreement with the findings of Verma et al.[8] on Bengal gram seed (synonym of chickpeas) harvested at four stages of development. However, the fibre content of Bengal gram which these authors investigated was approximately double that of chickpeas used in the present study. When the chemical composition values (Table I) were expressed on dry weight basis, the results became different. Total nitrogen and NFE showed no appreciable change during the five stages of development. The ether extract content increased rapidly from the 14th to the 21st day after flowering but subsequently remained constant. The fibre content increased till the 28th day and then decreased, while the ash content decreased till the 28th day and then became rather constant.

The level of β-carotene in chickpea seeds decreased continuously with maturity (Table I). At 14 days after flowering the seed contained on a wet weight basis 1·23 mg/100 g of β-carotene compared to 0·33 mg/100 g at 42 days after flowering. Similar results were observed when the values were expressed on a dry weight basis. These results are in agreement with those of Naik & Narayana.[9] The consideration of results expressed on wet weight basis is of nutritional value when the maturing green seed is consumed at these stages. It may be concluded from these results that the nutritive value of the seed with respect to total nitrogen and fat content increased with maturity but decreased with respect to pro-vitamin A.

The amino acid composition of chickpeas at the five stages of seed development is shown in Table II. The amount of lysine was relatively high at the 14th day after flowering but decreased sharply thereafter to a nearly constant value.

TABLE I

Changes in chemical composition of the developing chickpea seed

Days after flowering	Moisture, %	Total Nitrogen, %	Ether extract, %	Crude fibre, %	Ash, %	N-free extract, %	β-carotene, mg/100 g
Wet weight basis:							
14	69·8	1·1	0·9	1·3	1·5	19·5	1·23
21	61·1	1·2	2·7	1·8	1·3	25·5	0·71
28	46·1	1·6	3·9	2·7	1·7	35·4	0·68
35	23·7	2·5	5·0	3·6	2·2	39·2	0·38
42	12·5	2·8	5·7	3·1	2·8	58·4	0·33
Dry weight basis:							
14		3·7	3·0	4·3	5·0	64·6	5·24
21		3·1	6·9	4·6	4·5	65·6	2·99
28		3·0	7·2	5·0	3·1	65·7	2·89
35		3·2	6·6	4·7	2·9	65·4	1·61
42		3·2	6·5	3·5	3·2	66·9	1·38

TABLE II

Amino acid content of chickpea at different stages of seed development, mg/g N

Amino acid	Days after flowering				
	14	21	28	35	42
Lysine	496	254	256	253	252
Histidine	15	71	89	103	192
Arginine	406	474	298	308	558
Aspartic acid	445	387	355	378	206
Threonine	202	150	115	117	195
Serine	232	191	157	169	286
Glutamic acid	482	629	578	577	289
Proline	322	225	181	140	364
Glycine	170	167	133	138	228
Alanine	353	213	148	140	232
Cystine	19	38	40	39	65
Valine	303	212	165	159	265
Methionine	77	67	64	49	85
Isoleucine	189	182	134	158	242
Leucine	298	308	233	265	466
Tyrosine	135	102	92	120	199
Phenylalanine	189	242	167	204	369
Tryptophan	60	69	68	71	61

On the other hand, histidine and cystine were very low at the early stages of development but afterwards increased steadily until maturity. Arginine, threonine, serine, proline, glycine, isoleucine, leucine, tyrosine, and phenylalanine values were high at 14 and 21 days after flowering but decreased at the 28th day and then increased again at maturity. Similarly, alanine, valine, and methionine values decreased but at the 35th day after flowering. Aspartic acid and glutamic acid values were high 14 days after flowering and remained so until the 35th day, then decreased as the seeds became completely mature. Tryptophan was low and remained so throughout the developmental stages of the seeds.

All the macro- and micro-elements determined in chickpea seeds during development decreased in percentage during ripening (Table III). The apparent decrease in mineral content is relative to the rapid increase of dry matter. The pattern of decrease varied with most elements. However, the divalent elements calcium and magnesium showed similar fluctuation; a decrease from the first to second stages of development, an increase during the third stage, followed by a decrease again. This similarity in the fluctuations of calcium and magnesium content may be explained by their comparable functions in biological reactions. The results for calcium and ion are similar to those reported by Naik & Narayana.[9]

TABLE III

Changes in mineral content of the developing chickpea seed
mg/100g dry matter

Mineral	Days after flowering			
	14	21	28	42
Calcium	182·69	150·00	170·85	140·86
Magnesium	158·34	144·20	150·00	140·86
Sodium	65·84	50·00	40·86	40·86
Potassium	196·28	135·86	118·34	125·85
Copper	1·00	0·88	0·74	0·90
Manganese	3·68	3·12	3·08	2·40
Zinc	3·96	3·20	2·74	2·12
Iron	9·56	7·60	6·32	7·12

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CONTENT OF *p*–HYDROXYBENZYLGLUCOSINOLATE IN SEED MEALS OF *SINAPIS ALBA* AS AFFECTED BY HEREDITY, ENVIRONMENT AND SEED PART

By E. JOSEFSSON

Seed meals of different cultivars and breeding lines of white mustard (*Sinapis alba*) grown at different localities and in different years were analysed for content of *p*-hydroxybenzylglucosinolate. The environmentally determined variation generally amounted to $\pm 12\%$. Cultivar differences were not significant in the material available but a much larger variation was found among individual plants. A statistical treatment of parent plant material and corresponding progenies gave a correlation coefficient of $+0\cdot68$ and a highly significant t^2 value. This indicates that the glucosinolate content of white mustard seed is mainly genetically determined.

Analysis of samples from field experiments with fertilisers containing different amounts of sulphate revealed that the glucosinolate content was not influenced by a reduction in the amount of sulphate applied to heavy soils.

The content of glucosinolate, while similar in cotyledons and hypocotyls, was, however, much lower in the seed coat.

Introduction

Seeds of present Swedish cultivars of white mustard (*Sinapis alba* L.) contain about 35% oil calculated on a dry matter basis.[1,2] After extraction of oil a meal remains with a protein content of 40–45% in the dry matter.[3] Analysis has shown that the amino acid composition of meal is fairly well balanced.[3] The use of the meal as animal feed is, however, limited by its content of *p*-hydroxybenzylglucosinolate, which on enzymic hydrolysis yields glucose, hydrogen sulphate and *p*-hydroxybenzyl isothiocyanate,[4,5] the latter compound and its split products being harmful to animals (see for instance Barothy[6] and references cited therein). Studies of the genetically and environmentally dependent variation in glucosinolate content were initiated in order to investigate the possibility of reducing this content by plant breeding. The glucosinolate content in different parts of the seed was also studied to determine the possibility of modifying the glucosinolate content by technical treatment such as removing the seed coat.

Experimental

Seed material was obtained from the Oil Crops Division of the Swedish Seed Association, Svalöf.

The fertiliser experiments were performed by the Institute of Soil Management and Fertility at The Royal Agricultural College at Ultuna, Uppsala, which also supplied the seed for analysis.

Reagents were of analytical reagent grade.

Myrosinase was prepared using the method outlined by Wrede.[7,8]

The seed was de-fatted according to the method of Troëng[9] and glucosinolate analysis of the meal was performed according to the method of Josefsson.[10] All analyses were made in duplicate. Since often only five seeds were available for analysis of inbred plants the de-fatting process had to be modified in order to avoid losses of glucosinolates during the procedure. The de-fatting was performed in steel tubes as described by Troëng[9] using, however, only 5 ml benzene and only one steel pellet. The homogenate was washed on a Hirsch funnel (W. Haldenwanger, Berlin). As a control five-seed samples were analysed from a sample which contained 9·09% *p*-hydroxybenzylglucosinolate in the dry matter of the meal. 15 five-seed samples, de-fatted and analysed

separately, gave an average value of 8·97% *p*-hydroxybenzyl-glucosinolate in the dry matter of the meal with a standard deviation of $\pm 0\cdot36$. Since few inbred seeds were available for methodological studies from the highly cross-fertilising white mustard plants, the analysed seeds were derived from a population. Some of the variation found therefore refers to sample differences.

In order to study the glucosinolate content in different morphological seed parts, the seeds were steeped in water and kept at room temperature for 24 h. They were then separated into three fractions: seed coats, cotyledons and 'hypocotyls' (i.e. the section remaining after removal of the seed coat and the laminas of the cotyledons).[1]

p-Hydroxybenzylglucosinolate has been isolated from white mustard meal as the sinapine salt.[11] Since it is not known whether the sinapine ion and the *p*-hydroxybenzylglucosinolate ion are present in equivalent quantities in the meal, the glucosinolate content has been expressed as *p*-hydroxybenzyl-glucosinolate ion in this paper.

Protein content was determined by the Kjeldahl method and oil content by the method of Troëng.[9]

Results and Discussion

Cultivars of *Sinapis alba* grown under various conditions

Table I shows the content of *p*-hydroxybenzylglucosinolate in seed meals of Svalöf cultivars and breeding lines. The samples were taken from isolated multiplications grown in different years and localities. Cultivar differences in gluco-sinolate content were not significant. The environmentally conditioned variation was $\pm 12\%$, which is of the same order

TABLE I

p-Hydroxybenzylglucosinolate content in seed meals of Svalöf cultivars or selections of white mustard, grown at various localities in Sweden, % in dry matter

Cultivar or selection	Number of samples	Mean	Range
Sv Primex	1	9·04	—
Sv Seco	10	9·15	7·91–10·12
Sv Trico	5	8·84	8·27– 9·30
Sv 0405	4	8·66	8·37– 8·94
Sv 54/122	3	8·48	7·91– 9·15

of magnitude as that found in rape and turnip rape.[12] Such a variation was also found among samples of one cultivar within one locality (Table II).

Sv 54/122 was continuously selected from Primex with the the aim of obtaining a higher oil content. Simultaneously a selection was also made for low oil content. The selection for low oil content may be of special interest since such a material will probably have a high protein content.[13] The content of oil, protein and p-hydroxybenzylglucosinolate in the two selections is shown in Table III. The protein content of the de-fatted seed meal, calculated on a dry matter basis, was $42 \cdot 59\%$ in the selection for high oil content and $50 \cdot 85\%$ in the selection for low oil content. The first figure is in accordance with the average for white mustard seed meal while the other is much higher than average. There was no such significant difference between glucosinolate content in the meal of the two selections, although the value for the low oil content selection was the higher one. Since there was a larger meal proportion in the selection for low oil content, this selection produced more protein and glucosinolates than the high oil content selection. However, the proportion of glucosinolate to protein was most favourable in the former selection.

According to Lööf[1] the oil content per unit dry matter of the seed coat is only about one-quarter of that of the cotyledons. Consequently, most of the seed coat was included in the meal fraction at the de-fatting process. This meant that a larger part of the meal was derived from the seed coat in plants with a high oil content than in plants with a low oil content. As shown in Table V protein content as well as glucosinolate content was lower in the seed coat than in the

TABLE II

Content of p-hydroxybenzylglucosinolate in seed meals of the white mustard cultivar Seco, cultivated under varying conditions

Growing conditions		% in dry matter
Cultivated at the same locality (Svalöf) in different years		
1963		$10 \cdot 12$
1964		$8 \cdot 86$
1965		$8 \cdot 97$
	Mean	$9 \cdot 32$
Cultivated at different localities in 1964		
Svalöf		$8 \cdot 86$
Flädie		$9 \cdot 20$
Klockrike		$8 \cdot 97$
	Mean	$9 \cdot 01$

TABLE III

Content of oil and crude protein in seed and content of p-hydroxybenzylglucosinolate in seed meals of white mustard lines, selected for high and low oil content respectively, and grown in the same field

Material	% in dry matter of the seed		p-Hydroxybenzylglucosinolate, % in dry matter of de-fatted meal
	Oil	Crude protein	
Sv 67/1541. Selection from Sv 54/122. High oil content	$38 \cdot 42$	$26 \cdot 23$	$8 \cdot 55$
Sv 67/1542. Low oil content	$22 \cdot 17$	$39 \cdot 58$	$9 \cdot 17$

embryo. Therefore the content of protein and glucosinolates should be higher in the material with a low oil content. In this case, however, glucosinolate content had increased relatively little.

The data presented in Table I are derived from all the cultivars and breeding lines of white mustard in Sweden from which several multiplications were available. Seco is a cross between 'Rumanian white mustard' and Primex. Trico and Sv 0405 are selections from Sv 54/122, which in turn is a selection from Primex. Table I therefore represents only a small part of the total variation in white mustard.

Since the cultivar differences in glucosinolate content in Swedish cultivars and breeding lines appeared to be small, analysis was made of seed meals from material which was collected from south-east Europe and India. Twenty samples showed a range in p-hydroxybenzylglucosinolate content of only $8 \cdot 30$–$9 \cdot 61\%$ in the dry matter.

In an analysis of six samples of white mustard seed Scharrer et al.[14] found a range in content of p-hydroxybenzylglucosinolate from $7 \cdot 40$ to $9 \cdot 45\%$ in the de-fatted meal. Although this variation was larger than that found in the present study, none of the samples was sufficiently low in glucosinolate content to form the basis for a breeding programme. Furthermore, it was not known whether the variation found was genetically determined. According to the present data it is necessary to start breeding for low glucosinolate content in white mustard with single plant selections, since no significant differences have been found between cultivars.

A p-hydroxybenzylglucosinolate content in dry matter of meal of $8 \cdot 90\%$, which is the mean value of the samples in Table I, corresponds to 210 μmole/g. This is a higher value than has been obtained in other cruciferous crops.[8] However, in feeding experiments with cows white mustard seed meal has given results comparable to rapeseed meal.[15] It is still an open question whether these results are valid only for ruminants or whether the specific toxicity of p-hydroxybenzyl isothiocyanate is lower than that of the split products of glucosinolates in rapeseed meal.

Variation in content of p-hydroxybenzylglucosinolate in seed meals of individual plants

As only small differences were found in the glucosinolate content of seed meals between cultivars and breeding lines of white mustard, individual plants were analysed. It seems likely that the genes responsible for a low amount of glucosinolates are recessive.[16] Since white mustard is an obligate cross-fertiliser the effect of these genes is suppressed to a large extent by corresponding dominant genes. The search for low glucosinolate genotypes therefore would have been greatly facilitated if carried out on inbred lines, although unfortunately such homozygous lines were not available since white mustard is particularly sensitive to inbreeding.

Fig. 1 shows the distribution of p-hydroxybenzylglucosinolate content in seed meals from individual F_1 plants, i.e. the result of inbreeding one generation. In this material the highest content was approximately twice that of the lowest content. If this variation is mainly genetically determined it should be possible to reduce the glucosinolate content of white mustard seed by plant selection.

In order to determine whether the variation in glucosinolate content among individual plants is genetically determined, the following experiment was performed. Seed meals were analysed from 53 individual plants. The rest of the seed from each plant was sown separately and the progenies of the individual plants were analysed. The correlations between

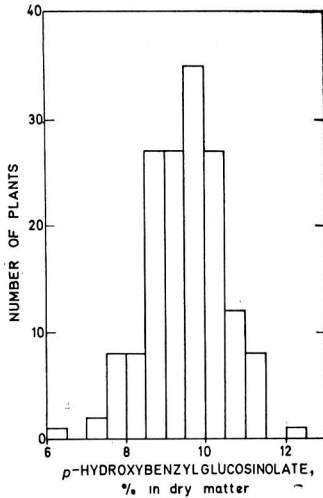FIG. 1. *Variation in p-hydroxybenzylglucosinolate content in seed meals of 156 F₁ progenies of white mustard (Sv 67/1591)*

FIG. 2. *Parent–progeny correlation in content of p-hydroxybenzyl-glucosinolate in seed meals of white mustard*

% in dry matter

the contents of *p*-hydroxybenzylglucosinolate in seed meals of these two generations are shown in Fig. 2. The statistical treatment of the data gave an r value of $+0 \cdot 68$ and a t^2 value of $57 \cdot 8731$, which is highly significant ($P < 0 \cdot 001$).

White mustard is a typical cross-fertiliser with a very poor seedset when self-fertilised. For this reason inbred plants were not used and the plants analysed were strongly hetero-zygous. In spite of this, a relatively high correlation coefficient was found between parents and offspring indicating that the variation in glucosinolate content between plants is largely genetically determined.

In a similar study of the winter form of *Brassica napus* L. 39 parent plants were compared with their progenies obtained after self-fertilisation. The r value was $+0 \cdot 41$ and the t^2 value $7 \cdot 4955$ ($P < 0 \cdot 01$) (Josefsson, E., unpublished results).

Ellerström & Josefsson[17] analysed thiocyanate-yielding glucosinolates in the vegetative parts of individual plants of fodder rape and the progenies from these plants after self-fertilisation, obtaining an r value $+0 \cdot 67$ and t^2 value $34 \cdot 09$ ($P < 0 \cdot 001$). Considering that the white mustard plants in the present study were not inbred the values found for r and t^2 seem relatively high. The higher precision of the analytical method used in the present study may partly explain this closer correlation between parents and offspring with regard to glucosinolate content. Another reason could be that white mustard seed, contrary to rape seed, contains only one glucosinolate of quantitative importance.

Fertiliser experiments

Studies of summer rape grown in soil-free cultures with additions of varying quantities of sulphur fertilisers have shown that the glucosinolate content of the seed is low at low levels of sulphur fertiliser.[12] It has also been found that the glucosinolate content in seed from crops produced on light soils could be significantly lowered if no sulphur fertiliser was given for a number of years.[12] Recent studies with rape and turnip rape clearly indicate, however, that the sulphur
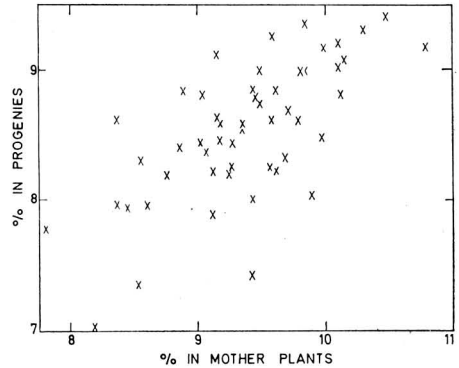
content in heavy soils will not be sufficiently low for produc-tion of seed low in glucosinolates, even if no sulphur fertiliser has been given for several years.[18]

In order to determine whether variation in amount of sulphur given to the soil could cause significant differences in glucosinolate content of white mustard seed, samples from three fertiliser experiments were analysed in 1968. In each experiment the effect of different types and amounts of feriliser had been studied. The experiments had been running for 2, 10 and 13 years, respectively. Each single plot had been given the same type of fertiliser each year, the soil being a clay loam in all three experiments. Variation in type and amount of fertiliser had very little effect on glucosinolate content in the seed harvested as shown for one of the experiments in Table IV. The figures clearly indicate that no useful reduc-tion in the glucosinolate content of white mustard seed can be expected through reduction in the amount of sulphur in the fertilisers, particularly on the heavy soils upon which most Swedish oil crops are grown. Furthermore, there were no significant differences in content of crude protein of the seed meal derived from plots given different amounts of sulphur fertilisers.

Content of glucosinolate in different parts of white mustard seeds

Table V shows that the glucosinolate content was similar in cotyledons and hypocotyls while the seed coat contained only small amounts. Therefore removal of the seed coat would not reduce the glucosinolate content of the meal. These results are wholly in accordance with those found for glucosinolate distribution in seed of rape and turnip rape (Josefsson, E., unpublished results).

Conclusions

Although the effects of environmental factors on the gluco-sinolate content of white mustard seed meals have not been extensively investigated, the present study shows that this content can probably not be reduced by changes in fertiliser application. These findings agree with results obtained for rapeseed meal. Furthermore, white mustard seed meal and rapeseed meal show a similar distribution of glucosinolates in different parts of the seed.

TABLE IV

Effect of different levels of sulphur and phosphorus applications for 10 years on *p*-hydroxybenzylglucosinolate content in seed of white mustard, cv. Seco, grown in the field in 1968

The figures for amounts of sulphur and phosphorus applied refer to cereals and forage crops; in 1959 and 1968 when oil crops were grown on the field, the amounts were doubled.   In plots Nos 2–4 the sulphur was applied as superphosphate, in plots Nos 5, 6 as gypsum

Plot No.	kg S/ha/year	kg P/ha/year	*p*-Hydroxybenzyl-glucosinolate, % in dry matter
1	0	0	9·53
2	1·22	20	9·27
3	12·64	20	9·09
4	30·68	20	9·50
5	12·64	0	9·74
6	30·68	0	9·63

The glucosinolate content of white mustard seed meal is higher than that of rape, turnip rape, and crambe seed meals, both when calculated as percentage of dry matter and when expressed as μmole/g meal.   Contrary to rape, no cultivar of white mustard has been found with a significantly low glucosinolate content. The variability in glucosinolate content among individual plants is relatively large, however, and since this variation seems to be mainly genetically determined it may be possible to obtained cultivars with a lower glucosinolate content by repeated selection.

TABLE V

Content of *p*-hydroxybenzylglucosinolate and crude protein in cotyledon, hypocotyl and seed coat of white mustard, cv. Seco

Mean values of three replications, each consisting of 100 seeds, ± S.E.

Seed part	% of total seed weight	% in oil-free dry matter	
		p-Hydroxybenzyl-glucosinolate	Crude protein
Cotyledon	68·14 ± 0·31	11·64 ± 0·08	47·43 ± 0·16
Hypocotyl	12·84 ± 0·26	10·71 ± 0·05	56·10 ± 0·32
Seed coat	19·02 ± 0·32	0·77 ± 0·24	17·91 ± 0·31

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GLUCOSINOLATE CONTENT AND AMINO ACID COMPOSITION OF RAPESEED *(BRASSICA NAPUS)* MEAL AS AFFECTED BY SULPHUR AND NITROGEN NUTRITION

By E. JOSEFSSON

Rapeseed plants have been grown in soil-free culture with varying amounts of nitrogen fertiliser applied as nitrate, and with 50% of the nitrate-N exchanged for ammonium. The experiment was performed at two levels of sulphate fertiliser. Yield, protein content, glucosinolate content and amino acid composition were studied. The glucosinolate content was lower and the protein content was higher at a high level of nitrogen fertiliser. Exchange of 50% of the nitrate-N for ammonium caused no significant change in glucosinolate or protein content. The amount of glucosinolate was higher at the high sulphate level. There were no sulphur–nitrogen interactions. Content of total aspartic acid increased with nitrogen fertilisation, while total content of other amino acids did not change significantly with fertilisation.

The effect of a wide variety of applications of sulphate fertiliser on glucosinolate and total amino acid content have been studied in a separate experiment. Although both protein content and methionine content were reduced at a low sulphate level, glucosinolate content was reduced considerably more.

Experiments in the field revealed that although a reduction in glucosinolate content of rapeseed may be obtained from using fertilisers low in sulphur on sandy soils this does not seem possible on heavy soils.

Introduction

Seed meals of rape (*Brassica napus* L.) and turnip rape (*Brassica campestris* L.) contain 40–45% protein in the dry matter with a well balanced amino acid composition.[1] According to Miller *et al.*[1] the limiting amino acids from a nutritional point of view are isoleucine and methionine. Since methionine very often is limiting in proteins of vegetable origin, it is important to obtain as high a content of this amino acid as possible. A factor that is still more limiting for the use of these meals, however, is the content of gluco-sinolates. These substances can be hydrolysed by the enzymic system myrosinase, yielding sulphate, glucose and isothiocyanates, the last-mentioned compound and its secondary products being toxic to animals, causing for example goitre in non-ruminants.[2] Other hydrolysis products, for example nitriles, have also been found.[3] Studies of glucosinolate content in seed of rape and turnip rape as affected by variety and environment were made by Josefsson & Appelqvist.[4] By fertiliser experiments in plastic boxes (containers), they showed that low applications of sulphate fertiliser resulted in a low content of glucosinolates in the seed, although the protein production approached normal quantities. Fields with light soils where the sulphur fertilisa-tion had been kept on a low level gave samples with a gluco-sinolate content that was significantly lower than in samples from other fields.[4]

In the studies of Josefsson & Appelqvist, no significant variation in glucosinolate content was found between different levels of nitrogen fertiliser. Trzebny[5] found, however, in a study of field-grown material that a high level of nitrogen fertiliser caused a significantly lowered glucosinolate content. Similar results were reported by Eaton[6,7] for vegetative parts of *Brassica nigra* L. Allcroft & Salt[8] reported that the goitrogenic effects of thousand-headed kale, grown in the field, increased when sodium nitrate was added to the soil, while no similar increase occurred when ammonium sulphate was used as the fertiliser. In a study on cabbage goitro-genicity, Sedlák *et al.*[9] obtained similar results. Their interpretation of these results implied that sulphate utilisation by the higher plants requires the presence of nitrates in the nutrient medium. The present study was undertaken in order to elucidate this problem with regard to the glucosinolates.

Studies by Chisholm & Wetter[10,11] and by Serif & Schmotzer[12] have shown that methionine is a precuror for the biosynthesis of the glucosinolates that are most prominent in *Brassica* seed. It seems possible, therefore, that a low content of glucosinolates in the seed subsequent to a certain fertilisation may be due to a low content of methionine available for the biosynthesis of glucosinolates. The results of Josefsson & Appelqvist[4] showing that the glucosinolate content could be markedly lowered by a low application of sulphate fertiliser, although the protein approached normal values, may indicate that plants that are deficient in sulphur use this primarily for protein biosynthesis and secondly for glucosinolate production. Analysis of protein content and of amino acid composition, therefore, was made in the present study.

Experimental

Materials

Seeds for the fertiliser experiments were obtained from the Oil Crops Division, Swedish Seed Association, Svalöf. Samples from field experiments were obtained from the Institute of Soil Management and Fertility at The Royal Agricultural College at Ultuna, Uppsala.

Reagents were of analytical reagent grade.

Analytical methods

The seed was de-fatted and the oil content was determined according to the method of Troëng.[13]

Glucosinolate analysis was performed according to the method of Appelqvist & Josefsson.[14] Glucosinolates pro-ducing volatile isothiocyanates were calculated as gluconapin (potassium 3-butenylglucosinolate); and those producing oxazolidinethiones, as progoitrin (potassium 2-hydroxy-3-butenylglucosinolate).

Crude protein (N × 6·25) was determined by the Kjeldahl method.

The amino acid analyses were made at the Institute of Biochemistry, University of Uppsala, Uppsala. Since the content of sulphur-containing amino acids was of special interest in this study, the samples were oxidised with per-formic acid, which converts total cystine and cysteine into

cysteic acid, and methionine into methionine sulphone. After hydrolysis for 24 h, the amino acids were determined as described by Eaker[15] using a 55 cm column of Beckman AA-15 resin. As histidine is destroyed extensively by the oxidation procedure, basic amino acids were determined separately from an unoxidised sample using an 8 cm column of Beckman AA-27 resin.[15] Tryptophan and tyrosine were not determined.

Fertiliser experiments

Sulphur–nitrogen experiment

Plants of summer rape (cv. Regina II) were grown in soil-free culture at two levels of sulphate, three levels of calcium nitrate and one combination in which the same quantity of nitrogen as of the medium level of calcium nitrate was applied as ammonium nitrate. The rape was sown in the spring in 25 l rectangular plastic boxes containing 2 kg of Perlite (Deutsche Perlite A.-G., Dortmund). The following chemicals were added to all the boxes: 15 g $CaCO_3$, 17 g K_2HPO_4, 17 g $Ca(H_2PO_4)_2$, 375 mg Fe-Na_2-EDTA, 10 mg $MnCl_2 \cdot 4H_2O$, 7·5 mg H_3BO_3, 0·75 mg $Cu(NO_3) \cdot 3H_2O$, 0·5 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0·5 mg $(CH_3COO)_2Zn \cdot 2H_2O$. The nutrients that were varied were added to each box in the following quantities. Sulphur: low level = 0·67 g S as Na_2SO_4; high level = 1·33 g S as Na_2SO_4. Nitrogen as calcium and magnesium nitrate: low level = 2·0 g N as $Ca(NO_3)_2$; medium level = 6·0 g N as $Ca(NO_3)_2$ and 1·75 as $Mg(NO_3)_2 \cdot 6H_2O$; high level = 12·0 g N as $Ca(NO_3)_2$ and 1·75 as $Mg(NO_3)_2 \cdot 6H_2O$. Nitrogen as ammonium nitrate: medium level = 7·75 g N. To the combinations without magnesium nitrate, Mg was added as $MgCl_2 \cdot 6H_2O$ in an equivalent quantity.

Calcium carbonate was added in solid form at sowing. The other chemicals were dissolved in de-ionised water. One-fourth of these chemicals were added at sowing; and equal amounts, three times at 20-day intervals.

The pH of the solutions with calcium nitrate was 6·3 and that of the ammonium nitrate solution, 6·6.

There were eight combinations in the experiment, replicated three times. The boxes were kept in a glasshouse and the plants were watered with de-ionised water whenever it was considered necessary.

Individual boxes were analysed separately with regard to the yield per box, and the content of oil, protein and gluco-sinolates in the seed. Equal weights of seed meals from each replicate were mixed to form an average sample for the analysis of amino acid composition of the meal.

Sulphate experiment

Summer rape (cv. Regina II) was grown in Perlite with the amounts of sulphate nutrient varied. Five different applications of sulphate were tested: 0, 0·17, 0·33, 0·67 and 2·67 g S per box. A more detailed report of the performance of this experiment was given by Josefsson & Appelqvist,[4] where the results of yield and of analysis for protein and glucosinolate contents were also reported. Sampling for the analysis of amino acid composition of the meal was made as described above.

Fertiliser experiments in the field

Analysis of glucosinolate and protein contents was made of samples from field experiments in which the same fertiliser had been applied to the plots for 2–30 years. The sulphur was supplied as sulphate, which is a constituent in the phosphate fertiliser, or as gypsum. These experiments included not only *Brassica napus* but also *B. campestris*.

Results

Sulphur–nitrogen experiment

Results of the sulphur–nitrogen experiment are given in Table I.

Mean seed yields in Table I are highest at the higher nitrogen levels. The increase in mean value of yield obtained with exchange of calcium nitrate fertiliser for ammonium nitrate at the low sulphur level was not statistically significant.

The oil content of the seed was not changed significantly by the different quantities of nutrients.

Statistical analysis of the material in Table I showed that the exchange of 50% nitrate-N for ammonium-N did not change the total glucosinolate content or protein content significantly. When the combinations with ammonium nitrate were omitted from the analysis glucosinolate content was found to be significantly higher ($P < 0.0005$) at a high level of the sulphate fertiliser and significantly lower ($P < 0.001$) at a high level of nitrogen fertilisers. No significance was found for the interaction of sulphur–nitrogen fertilisations.

TABLE I

Seed yield and content of oil, protein and glucosinolates of the seed of *Brassica napus* cv. Regina II summer rape supplied with different levels of sulphur and nitrogen fertilisers

Mean values ± S.E.

Nitrogen nutrients supplied and sulphur/nitrogen levels	Seed yield, g/box	Oil content, % in dry matter of seed	Content of crude protein, % in dry matter of oil-free meal	Glucosinolate content, % in dry matter of oil-free meal		
				Gluconapin	Progoitrin	Gluconapin + progoitrin
$Ca(NO_3)_2$ Low/low	13·3 ± 3·3	36·8 ± 0·3	46·4 ± 0·5	1·35 ± 0·05	4·07 ± 0·13	5·42 ± 0·14
$Ca(NO_3)_2$ Low/medium	16·3 ± 0·4	35·1 ± 0·1	47·6 ± 0·5	1·36 ± 0·21	3·47 ± 0·15	4·83 ± 0·25
$Ca(NO_3)_2$ Low/high	23·0 ± 2·6	36·5 ± 0·2	50·2 ± 1·0	0·87 ± 0·26	3·38 ± 0·22	4·25 ± 0·25
NH_4NO_3 Low/medium	21·0 ± 3·0	34·7 ± 0·5	48·5 ± 0·6	1·35 ± 0·14	4·04 ± 0·27	5·39 ± 0·41
$Ca(NO_3)_2$ High/low	17·7 ± 1·4	34·0 ± 1·1	46·5 ± 0·6	1·78 ± 0·34	4·60 ± 0·33	6·38 ± 0·32
$Ca(NO_3)_2$ High/medium	20·8 ± 3·4	34·0 ± 0·2	48·1 ± 0·3	1·42 ± 0·01	4·47 ± 0·25	5·89 ± 0·25
$Ca(NO_3)_2$ High/high	24·4 ± 2·0	34·4 ± 0·5	48·3 ± 0·1	1·38 ± 0·02	4·07 ± 0·12	5·45 ± 0·11
NH_4NO_3 High/medium	19·3 ± 5·5	34·1 ± 1·0	47·9 ± 0·3	1·38 ± 0·20	4·20 ± 0·16	5·58 ± 0·34

TABLE II

Amino acid composition of seed meals of *Brassica napus* cv. Regina II as affected by sulphur and nitrogen fertilisations

Nitrogen nutrients supplied and sulphur/nitrogen levels	Amino acid, g/16 g N															
	Lys	His	Arg	Cys	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Phe
Ca(NO₃)₂ Low/low	6·23	2·83	6·23	3·03	7·39	2·84	4·51	4·66	20·22	6·46	5·12	4·45	5·37	4·10	7·38	4·08
Ca(NO₃)₂ Low/medium	6·62	3·14	6·87	2·91	7·70	2·88	4·53	4·76	20·57	6·60	5·24	4·53	5·40	4·13	7·50	4·30
Ca(NO₃)₂ Low/high	6·39	2·90	6·14	2·88	8·04	2·80	4·51	4·90	20·45	6·58	5·39	4·60	5·62	4·45	7·55	4·29
NH₄NO₃ Low/medium	6·42	3·00	6·92	2·34	6·58	2·43	3·95	4·06	16·07	5·39	4·43	3·86	4·62	3·62	6·39	3·72
Ca(NO₃)₂ High/low	6·29	2·79	5·98	3·14	7·05	2·87	4·49	4·87	20·14	6·94	5·13	4·42	5·07	3·90	6·95	4·20
Ca(NO₃)₂ High/medium	6·41	2·89	6·52	3·07	7·30	2·97	4·45	4·87	20·77	6·89	5·19	4·54	5·37	4·22	7·25	4·03
Ca(NO₃)₂ High/high	6·24	2·86	6·46	3·07	7·32	2·80	4·45	4·77	20·34	6·55	5·06	4·40	5·06	3·89	7·28	4·08
NH₄NO₃ High/medium	6·13	2·78	6·45	2·83	7·25	2·67	4·33	4·63	19·24	6·34	4·90	4·29	4·91	3·84	7·10	3·84

TABLE III

Methionine content estimated after acid hydrolysis and the amount of methionine used for the biosynthesis of glucosinolates as affected by sulphur and nitrogen fertilisation

Nitrogen nutrients supplied and sulphur/nitrogen levels	Methionine content after hydrolysis, g/100 g meal (I)	Methionine used for biosynthesis of glucosinolates, g/100 g meal (II)	Ratio II/I
Ca(NO₃)₂ Low/low	1·21	1·91	1·58
Ca(NO₃)₂ Low/medium	1·23	1·70	1·38
Ca(NO₃)₂ Low/high	1·27	1·50	1·18
NH₄(NO₃) Low/medium	1·07	1·90	1·80
Ca(NO₃)₂ High/low	1·21	2·26	1·86
Ca(NO₃)₂ High/medium	1·30	2·08	1·60
Ca(NO₃)₂ High/high	1·23	1·92	1·56
NH₄(NO₃) High/medium	1·30	1·97	1·52

TABLE IV

Amino acid composition of seed meals of *Brassicus napus* cv. Regina II, as affected by sulphur nutrition

g amino acid/16 g N

Amino acid	S, g/box				
	0	0·12	0·33	0·67	2·67
Lysine	5·77	5·27	5·85	5·75	6·00
Histidine	2·88	2·56	2·79	2·72	2·76
Arginine	10·17	7·20	6·39	5·60	6·25
Cystine	1·54	1·87	2·55	2·88	3·18
Aspartic acid	13·78	10·59	9·47	7·77	8·36
Methionine	2·29	2·34	2·63	2·64	2·99
Threonine	5·30	4·77	4·82	4·70	4·77
Serine	5·62	4·88	5·26	4·70	5·05
Glutamic acid	21·23	18·43	20·11	19·36	21·39
Proline	5·90	5·48	6·44	6·59	7·09
Glycine	5·96	5·34	5·57	5·04	5·45
Alanine	5·21	4·70	4·89	4·46	4·81
Valine	6·16	5·50	5·75	5·15	5·63
Isoleucine	4·84	4·34	4·58	4·08	4·35
Leucine	8·17	7·47	7·72	6·95	7·49
Phenylalanine	3·35	3·36	4·20	2·98	3·21

A high level of nitrogen fertiliser resulted in a significantly higher protein content of the seed meal. The two levels of sulphur fertilisation used in this experiment did not show any differential effects on protein content. Nor in this case was any significance found for the interaction of sulphur–nitrogen fertilisations.

The results of the amino acid analyses of samples from the sulphur–nitrogen experiment are presented in Table II. Since the carbon chain of methionine can be derived from aspartic acid or threonine, the content of these amino acids are of special interest besides the sulphur-containing ones. As a rule, the differences in amino acid composition between various fertilisations were not very large. Aspartic acid (which includes asparagine) increased with nitrogen fertilisation. This may reflect that more of the acids from the tricarboxylic acid cycle are converted into amino acids via aspartic acid and relatively lesser amounts are converted into carbohydrates at high levels of nitrogen fertilisation. Cystine, representing cystine and cysteine, and methionine and threonine did not seem to vary appreciably with nitrogen fertilisation.

The glucosinolates of rapeseed seem to be biosynthesised from methionine[10-12] with the exception of phenylethyl-glucosinolate for which phenylalanine is a precursor.[16] Phenylethylglucosinolate content, however, amounts to only ~ 3% of the total glucosinolate content of the seed.[17] A calculation of the quantities of methionine used in rapeseed for biosynthesis of proteins and glucosinolates shows that generally more is used for synthesis of the latter than of the former. The ratios of the quantities of total methionine

estimated after acid hydrolysis and methionine used for synthesis of glucosinolates in the different fertilisations in this experiment are shown in Table III. As methionine content did not vary much with fertilisation, relatively less was used for the synthesis of glucosinolates at the highest level of nitrogen fertiliser.

Sulphate experiment

Results of the sulphate experiment on glucosinolate content, protein content and seed yield have been given in an earlier paper.[4] The amino acid composition of the seed is shown in Table IV. The differences in amino acid composition between various levels of sulphur fertilisation were larger than the differences between nutrient combinations in the experiment discussed above. This was to be expected since more extreme applications of fertilisers were used in the sulphate experiment. The content of sulphur-containing amino acids increased considerably with sulphur fertilisation, cystine more than methionine. At the lowest sulphur level, arginine and aspartic acid contents were very high. Table V shows that the plants used a very small part of their methionine for glucosinolate synthesis when cultivated at a low sulphur level.

Fertiliser experiments in the field

The fertiliser experiments in the field in this study were exclusively on relatively heavy, clay soils – soils on which most of the oil crops in Sweden are grown. In no case was the glucosinolate content so low as had been found earlier in samples grown on light soil, and the variation found did not always correlate with the sulphate fertilisation. Therefore, detailed figures are not given for all the individual experiments. Data showing the range in sulphate fertilisation, glucosinolate content and protein content are presented in Table VI. Although the differences in the amounts of sulphate fertilisers were very large, the variation in glucosinolate content was not larger than that normally found between different years and localities.[4] In some experiments the glucosinolate content was higher at the higher sulphate fertilisations, while in others no tendency could be observed. In one experiment there was a slightly higher glucosinolate content at the lower sulphate fertilisations. The differences found were never great, however. Variation in protein content was still less.

More detailed figures are given in Table VII for the experiment in which field plots had been receiving the same annual application of fertiliser for the longest time. The higher glucosinolate contents were obtained from plots that received the highest sulphate fertilisations. Although the differences in sulphate applications were very large and the experiment had been in progress for 30 years, the variation in glucosinolate content was very small.

Discussion

The influence of sulphur fertilisation on glucosinolate content found was in accordance with earlier results.[4,6,7,9] A lower glucosinolate content in the seed meal as a consequence of a high level of nitrogen fertilisers agrees well

TABLE V

Methionine content estimated after acid hydrolysis and the amount of methionine used for the biosynthesis of glucosinolates as affected by sulphate experiment

S, g/box	Methionine content after hydrolysis, g/100 g meal (I)	Methionine used for biosynthesis of glucosinolates, g/100 g meal (II)	Ratio II/I
0·0	0·80	0·04	0·05
0·17	1·03	0·22	0·21
0·33	1·19	0·90	0·76
0·67	1·23	1·70	1·38
2·67	1·35	1·74	1·29

with the results of Trzebny[5] and Eaton.[6,7] Since nitrogen is a constituent of the glucosinolate molecule, it might seem probable that a high nitrogen fertilisation would cause a higher glucosinolate content. Eaton,[6,7] however, explains the lower values found as a result of 'dilution'; at a high nitrogen fertilisation the production of other substances of the plant will rise more than the glucosinolate production. Since nitrogen fertilisation presumably results in a more extensive use of the products of the tricarboxylic acid cycle for production of protein and relatively less of carbohydrates, glucose might be a limiting factor for glucosinolate synthesis at high levels of nitrogen fertilisation.

The results of Allcroft & Salt[8] and of Sedlák *et al.*[9] showing a lower goitrogenic effect when ammonium was used as the fertiliser instead of nitrate were not supported by the analyses made in the present study. It should be noticed, however, that these workers[8,9] made their studies on *Brassica oleracea* L., grown in the field, and on the vegetative parts of the plant, which also contain other glucosinolates.

The results in Table I indicate that the glucosinolate content of rapeseed meal may be modified to some extent by the fertilising increments. A high rate of nitrogen fertilisers, however, had a relatively small effect on the glucosinolate content. In a study by Josefsson & Appelqvist,[4] in which more extreme combinations of sulphur fertilisation were used than in the present experiment, it was possible to reach very low values of glucosinolates. A content of glucosinolates below 40% of the normal value has been found in rapeseed from a field cultivation on a light soil supplied with phosphate fertilisers low in sulphate for several years.[4] Although such a reduction in glucosinolate content would be of value, it would not be sufficient to eliminate the effects of glucosinolates on the nutritional value of the seed meal. The results in this study show that use of fertilisers low in sulphur did not lower the glucosinolate content significantly on heavy soils. This may be due to the fact that sulphate does not leach out quickly on such soils and that sulphur is transported to the soil from the atmosphere.[18]

The results reported in Table II, showing that methionine did not vary significantly with nitrogen fertilisation, should be compared with the results of Schuphan,[19] who found that methionine content in leaves of *Valerianella olitoria* L. and *Spinacia oleracea* L. was lower at a high level of nitrogen fertiliser. This was especially the case in the older leaves. On the other hand, Wenzel & Michael[20] found that, although a high level of nitrogen fertiliser increased protein-N in leaves of spinach, it did not change the amino acid composition of the protein. Non-protein-N was increased and especially the content of nitrate, amides and arginine. Although no amino acid analyses were made on leaf material in the present

TABLE VI

Glucosinolate and protein content of some *Brassica* crops grown on clay soils at different levels of sulphate fertilisers

Locality and year of harvest	Material	kg of S/ha/year (range)	Number of years the fertilisers were applied	Glucosinolate content, % in dry matter (range)	Protein content, % in dry matter (range)
Lund 1967	*Brassica napus* (winter type) cv. Argus	1·2–31	10	6·00–6·28	—
Malmö 1967	*Brassica napus* (winter type) cv. Argus	1·2–31	10	4·86–5·95	—
Malmö 1968	*Brassica napus* (winter type) cv. Argus	1·2–31	7	5·64–6·39	37·34–39·19
Mjöhult 1968	*Brassica napus* (winter type) cv. Victor	1·4–62	11	6·24–6·85	36·17–38·16
Edsvalla 1968	*Brassica campestris* (summer type) cv. Bele	0·8–39	2	3·03–4·05	37·75–40·63
Kil 1968	*Brassica campestris* (summer type) cv. Bele	0·8–39	2	3·09–4·13	36·44–39·51

TABLE VII

Glucosinolate content of seed meals of *Brassica napus* cv. Victor, harvested in 1967 in a field that has been divided into plots receiving different fertilisations since 1937

Sulphur applied as a constituent of the phosphate fertiliser.
Soil: medium clay

Fertilisation	Glucosinolate content, % in dry matter		
	Gluco-napin	Progo-itrin	Gluconapin + progoitrin
None	1·85	4·27	6·12
40 kg P_2O_5 and 26 kg S each year	1·88	4·05	5·93
240 kg P_2O_5 and 156 kg S every sixth year*	1·90	4·21	6·11
40 kg P_2O_5 and 0·4 kg S each year	1·80	4·03	5·83
240 kg P_2O_5 and 2·4 kg S every sixth year*	1·85	4·03	5·88

*Last application made in 1963

experiments, a decrease in methionine content in the vegetative parts of the plant might be a reason for the lower glucosinolate content of the seed at a high level of nitrogen fertiliser.

Kirby[21] reported that when 75% of the nitrogen fertiliser was supplied as ammonium to white mustard the free proline content of the leaves increased significantly. No data were given, however, with regard to the total proline content or the proline content of the seed.

Mulder & Bakema[22] found that a high level of nitrogen fertiliser in potatoes increased the non-protein-N fraction of the tubers, while the relative content of free aspartic and glutamic acids decreased; however, the corresponding amides increased.

Coïc et al.[23] reported that the percentage of aspartic acid (including asparagine) in the leaf proteins of barley increased 3–4 times when the plants were grown at a low sulphur level. There also was an increase of this amino acid in the seed proteins, but not so large as in the leaves. They found a small increase of glutamic acid (including glutamine) and a large decrease in cystine in the seed proteins. The proportion of methionine did not decrease more than the proportions of most other amino acids. The nitrogen content of (−)-S seed was higher than in the (+)-S ones. Much of this nitrogen, however, was accumulated as free asparagine.

Coleman[24] found that free arginine increased appreciably in green parts of sulphur-deficient plants of *Desmodium uncinatum* DC., white clover, tomato and flax, and that

amides accumulated. He also reported an increase in glycine and serine at a low level of sulphur supply. Mertz et al.[25] found that the content of free aspartic acid (probably mostly present as asparagine) increased in S-deficient lucerne leaves.

It has frequently been reported that protein level can be influenced by fertilisation but that the composition of the proteins is only slightly affected.[26,27] On the other hand, the non-protein amino acids can be strongly influenced by mineral nutrition.[20–26] In some material, however, fertilisation may cause a change in the ratio between different protein fractions resulting in a changed composition of protein amino acids.[27]

Conclusions

As was demonstrated by Josefsson & Appelqvist,[4] the glucosinolate content of rapeseed meal could be lowered significantly when the plants are grown in boxes at a low level of sulphur fertiliser. At the same time, there was some reduction in crude protein content and in total sulphur-containing amino acids. However, these reductions were not so large as the reduction of the glucosinolate content.

A high level of nitrogen fertiliser decreased the glucosinolate content and increased the protein content. The reduction of glucosinolates is not large enough to solve the practical problems that are caused by the presence of glucosinolates in seed meals.

Although glucosinolate content of rapeseed can be reduced on sandy soils by using fertilisers low in sulphur over an extended period, it does not seem possible to bring about the same effect on heavy soils.

From a practical point of view, it seems important to use the wide genetic variation for glucosinolate content that has been found[4,28] to breed varieties with a low glucosinolate content. These varieties should then receive proper fertilisation in order to give a high yield of high quality protein.

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AVAILABILITY OF LYSINE IN PROTEIN CONCENTRATES AND DIETS USING CARPENTER'S METHOD AND A MODIFIED SILCOCK METHOD*

By H. OSTROWSKI,† A. S. JONES and A. CADENHEAD

A comparison was made of methods for the evaluation of the available lysine in protein concentrates and diets. Some modifications of the Silcock method, developed by Roach *et al.* for lysine determination are suggested, and the optimum range of lysine concentration in samples has been evaluated.

With animal protein concentrates, the Silcock method for measuring the total and residual lysine, after treatment with 1-fluoro-2,4-dinitrobenzene, gave results in close agreement with those for available lysine obtained using Carpenter's method. The short-column system appeared to be free from carbohydrate interference which with Carpenter's method was probably responsible for higher values for available lysine especially for vegetable protein concentrates and cereal diets. The differences between the methods in values obtained for the available lysine in vegetable protein concentrates were significant.

The modified short-column technique improved the separation of ornithine and lysine. The optimum range of lysine concentration that is recommended for application to a column is between 25 and 75 μg in 1 ml. By the use of the technique suggested it is possible to carry out a large number of analyses without the use of the automatic analyser system as used by Roach *et al.*

Introduction

It has been reported by several workers that lysine is the first limiting amino acid in many foods of plant origin, especially in cereal grain.[1-3] It is thought that the nutritive value of proteins may be directly related to the proportion of lysine in the protein molecule which has free ε-amino groups. Carpenter has called this form available lysine.

Several methods have been developed for measuring the availability of lysine in different protein sources. These may be grouped as chemical,[4-10] enzymic,[11,12] microbiological,[13,14] and as biological methods based on either the growth of animals[15-18] or the 'digestibility' of lysine.[19,20]

At present Carpenter's method is commonly used in many laboratories for the routine determination of available lysine in animal and vegetable protein sources. However, it has been reported in a number of papers[5,7,9] that Carpenter's method is subject to interference by carbohydrate and this interference is especially high in vegetable protein concentrates. Interference results in a low and variable recovery of

lysine as ε-2,4-DNP L-lysine.[10] The effect is probably due to the destruction of ε-DNP lysine by carbohydrates during acid hydrolysis.

Roach *et al.*[9] working in the laboratories of Messrs Silcocks have developed the Silcock method, for the estimation of total and residual lysine, from which available lysine is obtained by difference. They have also compared the methods of Carpenter[5] and Rao *et al.*[7] with the Silcock method for the estimation of the available lysine in fishmeal and groundnut meal, and have shown that the Silcock method is not subject to interference by carbohydrate. Compared with Carpenter's method, the Silcock method gave lower values for available lysine.

In this paper a comparison has been made of the Carpenter and Silcock methods, and some modifications to the Silcock method are proposed which allow the rapid routine determination of total and available lysine in different protein concentrates and in diets, without an automatic analyser.

Experimental

Four protein concentrates, meat and bone meal, white fishmeal, soyabean meal and groundnut meal, were analysed. Twelve samples of each type of protein concentrate were collected from 100–250 kg commercial lots, and a number of single samples of poor and high quality meals were also taken.

Some of the protein concentrates were then used in the preparation of four diets for growing pigs, each calculated from analyses of the ingredients (Carpenter's method) to provide 0·65% available lysine. Twelve samples from 700 kg of each diet were analysed for available lysine, by the two methods.

The proximate analyses of the four protein concentrates and the diets used are given in Table I.

The 1-fluoro-2,4-dinitrobenzene method described by Carpenter[5] and the Silcock method described by Roach *et al.*[5] were used to determine the amount of available lysine in the protein concentrates and the diets. The following slight modifications to the Silcock method were made. The chromatographic columns were prepared from 20 cm × 10 mm bore glass tubes by adding Zeo-Karb 225 resin, with diameter of particles 13–17 μm, suspended in buffer until the length of settled resin was 8 cm. The columns were washed with 0·5 N sodium hydroxide and regenerated to the acid form with buffer, pH 5·28, until free from alkali. One ml of a hydrolysate was applied to a column, and the sample was passed into the resin by applying air pressure to the top. The sample was washed with 1 ml of pH 5·28 buffer, which was passed on to the top of the resin and then the space above the resin was filled with buffer.

Instead of using an automatic analyser, two columns were connected to containers filled with buffer, and the eluate was collected in 2 ml aliquots using a fraction collector. A constant rate of flow of eluate (40–45 ml/h) was obtained by adjustment of the height of the buffer containers. Fractions from the columns were collected in two rows of tubes with drops being counted from one column only.

The standard ninhydrin–hydrindantin reagent used was prepared once every 7–8 days and stored in a dark flask in an atmosphere saturated with oxygen-free N_2.

Optical density was measured at 570 nm with a Unicam SP600 spectrophotometer using an SP625 Autocell accessory. The area of the lysine peak obtained was compared with the areas obtained from 25 and 75 μg/ml standard lysine solutions, which were run each day. This procedure was carried out for total and residual lysine from which the available lysine was calculated. At the start of the work, three columns were used in conjunction with one fraction collector, but later 12 columns were used with two collectors.

Ornithine and lysine separation

To ensure adequate separation of ornithine from lysine, a modified buffer was used. 72 g sodium hydroxide and 122·25 g of citric acid were each dissolved in 500 ml of de-ionised water; the two solutions were then mixed and 34·5 ml of concentrated hydrochloric acid (sp. gr. 1·18), 21·88 g NaCl and 5 g phenol were added. After cooling, 30 ml of 50% wt./vol. Brij 35 solution were added, the volume was made up to 5 l with de-ionised water and the pH was then adjusted to 5·28.

Recovery of L-lysine

The recovery of L-lysine was checked, and an estimation was made of the optimum load of lysine for the column. Four standards, with concentrations of 25, 75, 125 and 250 μg L-lysine in 1 ml solution were analysed on each of three columns. Subsequently, 1 ml of hydrolysed samples was put through the columns. There were further runs using firstly 1 ml samples together with 1 ml of the 25 μg standard followed by 1 ml sample and 1 ml of the 75 μg standard and so on. All estimates were made in quadruplicate.

Results and Discussion

Comparison of the two methods

Table II shows the available lysine values obtained for the various protein concentrates. The results show that with animal protein concentrates the available lysine values obtained using Carpenter's and Silcock's methods were not significantly different, but that with vegetable protein concentrates Carpenter's method gave significantly higher values (P < 0·01). Roach *et al.*,[9] who compared Carpenter's and Silcock's methods for available lysine in fishmeal and groundnut meal, obtained similar results, although the magnitudes of the differences between the two methods reported here are rather lower. The greater differences between the methods were associated with lower recovery of ε-DNP lysine, particularly with vegetable protein concentrates. Matheson,[10] who compared a chromatographic method of isolating ε-DNP lysine with Carpenter's method for available lysine determination in feedingstuffs, showed that when Carpenter's method was applied to a vegetable protein concentrate (groundnut

TABLE I

Composition of the protein concentrates and the diets used

Specification	Moisture, %	Crude protein, % as received	Ether extract % as received	Ash, % as received
Protein concentrates:				
Meat and bone meal	5·3	56·3	13·3	26·1
White fishmeal	9·6	65·8	6·4	19·8
Soyabean meal	12·0	46·9	1·9	5·3
Groundnut meal	9·2	45·9	8·3	5·6
Diets containing:*				
17·6% meat and bone meal	10·4	16·6	4·1	7·2
9·7% white fishmeal	12·2	11·1	2·4	4·6
20·3% soyabean meal	11·8	15·1	2·1	4·6
31·4% groundnut meal	11·9	19·8	4·2	5·0

* Other components: 58·6% barley meal, 10% weatings. Cassava meal and maize starch were used to enable the available lysine and total digestible nutrients to be equated between diets. Minerals and vitamins were added so that diets covered the A.R.C. (1967) recommendations.

TABLE II

Available lysine in four protein concentrates, %

Protein concentrate	No. of samples	Lysine				Silcock		Carpenter				Significance of difference between the methods[c]	S.E. of difference between the methods
		Total		Residual		Total-residual		Corrected[a]		Recovery,[b] %			
		$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.		
Meat and bone meal	12	2·86	± 0·254[d] ± 0·008[e]	0·69	± 0·063 ± 0·006	2·17	± 0·193 ± 0·010	2·16	± 0·186 ± 0·011	98·93	± 4·80 ± 0·06	n.s.	± 0·008
White fishmeal	12	4·56	± 0·402 ± 0·011	0·50	± 0·041 ± 0·005	4·06	± 0·361 ± 0·009	4·08	± 0·347 ± 0·011	86·75	± 3·71 ± 0·26	n.s.	± 0·017
Soyabean meal	12	2·06	± 0·188 ± 0·009	0·10	± 0·009 ± 0·003	1·96	± 0·074 ± 0·009	2·19	± 0·086 ± 0·011	81·70	± 5·27 ± 0·41	**	± 0·023
Groundnut meal	12	1·39	± 0·126 ± 0·007	0·11	± 0·010 ± 0·002	1·28	± 0·079 ± 0·006	1·44	± 0·081 ± 0·008	79·18	± 6·07 ± 0·61	**	± 0·025

[a] Corrected for loss of ε-DNP lysine during acid hydrolysis
[b] Recovery of mono-ε-DNP lysine
[c] n.s. not significant; ** P < 0·001
[d] Standard error of mean based on variation between 12 samples
[e] Standard error of mean based on variation between replicates of the same hydrolysate

TABLE III

Available lysine in four cereal diets, %

Diets containing	No. of samples	Lysine				Silcock		Carpenter				Significance of difference between the methods[c]	S.E. of difference between the methods
		Total		Residual		Total-residual		Corrected[a]		recovery,[b] %			
		$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.		
17·6% meat and bone meal	10	0·74	± 0·046[d] ± 0·004[e]	0·07	± 0·004 ± 0·002	0·67	± 0·029 ± 0·004	0·74	± 0·021 ± 0·003	82·25	± 6·33 ± 0·02	**	± 0·019
9·7% white fishmeal	10	0·70	± 0·040 ± 0·004	0·06	± 0·004 ± 0·003	0·64	± 0·026 ± 0·003	0·75	± 0·027 ± 0·004	82·03	± 5·15 ± 0·21	**	± 0·025
20·3% soyabean meal	10	0·75	± 0·037 ± 0·004	0·07	± 0·005 ± 0·002	0·68	± 0·031 ± 0·003	0·77	± 0·029 ± 0·005	82·18	± 7·26 ± 0·44	**	± 0·027
31·4% groundnut meal	10	0·71	± 0·043 ± 0·003	0·08	± 0·006 ± 0·002	0·63	± 0·028 ± 0·003	0·75	± 0·021 ± 0·005	81·38	± 7·09 ± 0·38	**	± 0·029

[a] Corrected for loss of ε-DNP lysine during acid hydrolysis
[b] Recovery of mono-ε-DNP lysine
[c] ** P < 0·01
[d] Standard error of mean based on variation between 10 samples
[e] Standard error of mean based on variation between replicates of the same hydrolysate

meal) it always gave higher values than the chromatographic method. He showed also that when the carbohydrate concentration in an analysed sample increased, the recovery of ε-DNP lysine decreased. With the addition of pure starch to groundnut meal, the amounts of ε-DNP lysine detected by using Carpenter's method could be reduced to less than 70% of that found by his chromatographic method. These low recoveries of ε-DNP lysine with the Carpenter method for vegetable proteins have also been ascribed to carbohydrate interference in a number of reports.[7,9,21,22] The results of the experiment in Table II show that even when the differences in the values obtained by the two methods were highly significant, as was the case with soyabean meal and groundnut meal, both methods gave good reproducibility. The values

obtained for the available lysine using the two methods were the same for animal protein concentrates. It is noteworthy that the available lysine content in soyabean meal determined by the Carpenter method was found to be higher than the total lysine in soyabean meal found using the Silcock method. Roach et al.[9] obtained similar results relating to groundnut meal.

When, however, the available lysine values for individual feedstuffs obtained using Carpenter's method were used as the basis for mixing ingredients to form four diets each containing 0·65% available lysine, the available lysine values for the mixed diets as measured by direct analysis using Carpenter's method were higher than the values computed from the analyses of the ingredients (Table III). Analysis of these

same mixed diets by the Silcock method gave results in better agreement with expectations based on the analyses of the separate ingredients. The recovery value for ε-DNP lysine was similar for all diets, although the percentage of the protein concentrate in the diets differed from 9·7 to 31·4%.

These results suggest that the Silcock method may be suitable for the available lysine estimation in cereal-based diets, since the results obtained were closer to the predicted values. Carpenter[21] has reported the difficulty in applying his procedure to vegetable materials where the lysine content is low in relation to the level of carbohydrate. The values for available lysine obtained using the Silcock method appeared to be independent of concentration of carbohydrate in samples, and the loss of ε-DNP lysine during acid hydrolysis. Another advantage of using Silcock's method is that total and available lysine are determined successively, which is especially attractive to a number of laboratories interested in lysine problems.

In the original Silcock method the separation of ornithine and lysine was poor, thus causing some error in the estimation of lysine. The present authors found that ornithine gave a small shoulder on the lysine peak (Fig. 1). Williams[22] reported that this could be totally resolved by lengthening the column, although a higher pressure would then be necessary to maintain a sufficiently rapid rate of flow. In cases where the ornithine peak is small and not clearly separated from the lysine peak, but is clearly distinguishable as part of it, it is possible to compute the true area due to lysine. Sometimes, however, the ornithine level is high in comparison to the area of the residual lysine peak, and so there could be a considerable error in the estimation of the true available lysine of the analysed samples.

In this study, by the use of Zeo-Karb 225 bead resin having a particle diameter size in the range 13–17 μm, and with an increase in the concentration of sodium ions in the buffer to 1% without changing the pH, ornithine and lysine separation was obtained without lengthening the column (Fig. 2). This separation allows the short column method to be used with higher precision for the evaluation of true lysine without correction for ornithine.

The recovery of L-lysine added to the column is shown in Table IV. The results indicated that the maximum permissible lysine concentration in samples for application to the 8 cm column was 125 μg lysine/ml. Up to that concentration recovery was satisfactory. Above 125 μg lysine/ml, recovery of lysine was low, and for solutions of 250 μg lysine/ml recovery was only 86%. The recommended concentration of lysine in samples applied to the short column is 25–75 μg/ml.

It is generally accepted that errors are introduced when Carpenter's method is applied to vegetable protein concentrates. In this study, no differences were obtained between the two methods for the values of available lysine in animal protein concentrates. For vegetable protein concentrates, the differences between the values were significant ($P < 0.01$), but the magnitude of the differences are of interest. In a diet for pigs, containing for example 15% soyabean meal and cereals, the values computed for the available lysine content of the whole diet would only differ by 0·02% if the values obtained by Carpenter's method for soyabean meal were used instead of those obtained by Silcock's method; such a small difference is insignificant in terms of practical pig feeding. In the case of the analysis of complete diets, however, the magnitude of the differences would be significant in practical terms.
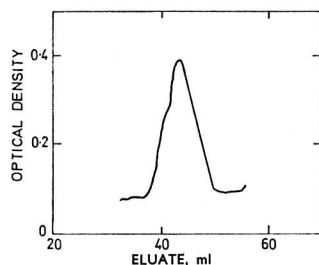
Acknowledgments

FIG. 1. *Chromatographic separation of ornithine and lysine standard mixture on the column Zeo-Karb 225, fraction 20–24 μm, buffer pH 5·28 with 0·83% sodium concentration*
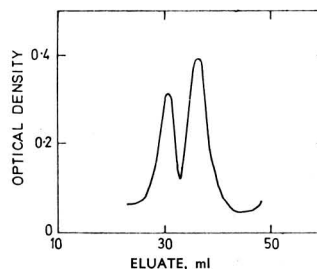

FIG. 2. *Chromatographic separation of ornithine and lysine standard mixture on the column Zeo-Karb 225, fraction 13–17 μm, buffer pH 5·28 with 1·0% sodium concentration*

TABLE IV

Recovery of L-lysine from columns

Estimates	L-lysine concentration in standard (μg/ml) added to hydrolysate							
	25		75		125		250	
	$\bar{x}$	S.E.†	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
Sample + standard, mg	58·4	± 0·4	107·6	± 0·6	155·0	± 0·9	241·6	± 5·8
Recovery of standard,* mg	25·5	± 0·2	74·8	± 0·4	122·2	± 0·8	212·2	± 5·4
% recovery of L-lysine	102·1	± 1·2	99·7	± 1·4	97·6	± 2·1	84·9	± 5·3

     * By calculation: (sample + standard) − sample
     † Based on four runs

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FLUORIDE CONTENT OF
FISH PROTEIN CONCENTRATE AND RAW FISH

By P. J. KE, H. E. POWER and L. W. REGIER

The fluoride content of fish protein concentrate prepared by the Halifax isopropanol process and also of several possible raw materials was determined by distillation without fusion. The concentration of fluoride in this dry material ranged from 20 to 760 ppm and was dependent upon the raw materials. The major source of the fluoride was the bones which were incorporated in the product to a variable extent. The levels in fresh fillets ranged between 2·3 and 5·4 ppm while whole eviscerated fish contained 3·6–57 ppm fluoride.

Introduction

Much interest has developed recently in fish protein concentrate (*FPC*) as a high quality protein for incorporation in human diets.[1,2] *FPC* is a dry almost flavourless powder made from fish by solvent extraction with isopropanol. The nutritional value and the proximate chemical composition of *FPC* made from several raw materials have been the subject of several investigations.[3-7]

The fluoride content of *FPC* has been a point of concern, especially to those regulatory agencies which have responsibility for approval of products such as this for human consumption.[8] The total intake of fluorides in the diet must be limited[9] and it is thus quite important to know the content of this element in any potentially significant source. No detailed data on the fluoride content of fish products have been available.

An improved 'non-fusion' method[10] for the determination of fluoride which is based upon the use of a fluoride selective solid-membrane electrode[11] was developed. This method gives results which are correlated closely with the longer A.O.A.C. official method, and it was used in the analyses of several samples of raw fish materials and *FPC* made from many of these materials.

Experimental

Apparatus

An Orion model 401 specific ion meter equipped with an Orion model 94–09 fluoride solid-membrane electrode and a standard calomel reference electrode was used for direct measurements of fluoride concentrations in solutions.

Reagents

A standard stock fluoride solution (100 ppm) was prepared from Merck reagent quality sodium fluoride after purification.[12] This was stored in a polyethylene bottle. A total ionic strength adjusted buffer (TISAB-1) was prepared as described previously.[10] All other reagents were prepared from A.C.S. grade chemicals. Double-distilled water was used for all reagents.

Samples

The *FPC* was produced by the Halifax isopropanol process[13] on a pilot-plant scale in the Halifax Laboratory of the Fisheries Research Board of Canada. The products are 200 mesh sized powders which have a white to slightly grey colour depending upon the raw material. From each batch, about 1 kg was used as the basic sample. From this, 50 g were removed by piling and quartering and were stored in a polyethylene bottle.

The raw fish samples consisted of portions from at least four freshly killed fish. In the case of whole fish, the viscera were removed, the body was cut into several small pieces and the whole was ground in a Waring Blendor to a homogeneous mass. The fillet and viscera samples were individually ground in the blendor. The fish frames, which are the residue of the fish after the removal of the fillets, were obtained from the production lines of the National Sea Products Ltd. (Lunenburg, Nova Scotia), and were ground in the blendor with no other treatment. All the fresh samples were stored in polyethylene bottles in a freezer until needed.

To obtain flesh-free bones, pieces of fish were steamed in polyethylene bags and then separated by hand. The bones were ashed in nickel crucibles at 600° for about 15 h. The fine bone ash was kept in polypropylene vials.

Methods

Distillation without fusion was used for the determination of fluoride in the samples of *FPC* and ground raw fish.

The fluoride content of the fish bones was determined by the direct method of Singer & Armstrong[14] with some modifications. 50–100 mg of bone ash were weighed into a 25 ml polyethylene beaker and dissolved by the addition of 5 ml 4N nitric acid. The excess acid was partly neutralised by 3 ml of 4N sodium hydroxide. After transferring to a 50 ml volumetric flask, 10 ml of TISAB-1 was added and the sample was diluted to volume. The fluoride concentration in this solution was measured directly with the calibrated Orion specific ion meter.

Results and Discussion

The fluoride content of the several batches of *FPC* are shown in Table I. It should be noted that only the *FPC* made from cod fillets and from whole capelin had fluoride contents below 100 ppm. These results also show that a fairly wide range of concentrations might be expected in the product. *FPC* from whole eviscerated cod had values from 140 to about 240 ppm, while *FPC* from eviscerated herring gave values of 123 and 189 ppm. It may be noted that the level was nearly the same for the samples of *FPC* made from whole cod and those from cod frames. The product made from dogfish had over twice the fluoride level of any of the other samples.

Table II summarises the analytical results obtained on the raw materials. In each sample which contained bones, except for the capelin, the fluoride content was quite high. The low level in capelin seems unusual but may be due to a relatively low content of calcified bone in the animals.

TABLE I

Fluoride contents in *FPC* determined by distillation without fusion and use of a membrane electrode

Sample no.	Source of raw materials	No. of times analysed	F content, ppm ± S.D.
A-111	whole cod fish	3	143·3 ± 0·40
A-112*	,,	3	205·6 ± 0·35
A-113	,,	5	239·9 ± 0·67
A-121	cod fillets	3	21·3 ± 0·07
A-122*	,,	4	25·9 ± 0·09
A-131*	cod frames	3	191·3 ± 0·51
A-231*	haddock frames	4	307·1 ± 0·48
A-232	,,	2	290·1 ± 0·30
A-311	whole herring	3	189·0 ± 0·40
A-312	,,	3	123·2 ± 0·29
A-411	whole capelin	4	56·7 ± 0·13
A-511	whole dogfish	3	761·0 ± 0·10
A-611	whole skate	3	372·0 ± 0·14

* Samples were taken from a mixture of several batches

TABLE II

Fluoride content in several fish and portions of fish determined by distillation without fusion and use of a membrane electrode

Sample no.	Description	No. of samples analysed	F content, ppm ± S.D.
B-111	eviscerated cod	2	57·0 ± 0·10
B-122	cod fillet	2	2·70 ± 0·02
B-123	,,	4	2·34 ± 0·03
B-131	cod frames	2	89·4 ± 0·25
B-141	cod viscera	3	5·15 ± 0·04
B-221	haddock fillet	3	5·06 ± 0·03
B-231	haddock frame	2	49·8 ± 0·20
B-241	haddock viscera	3	9·25 ± 0·04
B-311	eviscerated herring	4	28·5 ± 0·11
B-321	herring fillet	3	5·36 ± 0·04
B-341	herring viscera	2	8·63 ± 0·05
B-411	whole capelin	3	3·55 ± 0·04
B-521	dogfish fillet	3	5·26 ± 0·04
B-541	dogfish viscera	2	3·65 ± 0·03

TABLE III

Fluoride content in ashed fish bones determined by two membrane electrode methods

Sample no.	Fish	F content, ppm ± R.S.D.%	
		By direct method[14]	By distillation method[10]
C-101	cod	1410 ± 0·20	1410 ± 0·09
C-201	haddock	558 ± 0·40	561 ± 0·05
C-202	,,	640 ± 0·30	645 ± 0·08
C-301	herring	3650 ± 0·40	3651 ± 0·11
C-401	capelin	764 ± 0·40	767 ± 0·10
C-501	dogfish	6820 ± 0·50	6819 ± 0·10

In the cod, haddock and herring, the concentration in the viscera was higher than the concentration in the fillets. The opposite relationship was observed in the case of the dogfish samples. No reason for this is apparent. The fluoride content of the haddock frame *FPC* was much higher than in the cod frame *FPC* while just the opposite relationship was observed in the samples of the fresh material. The dry and fresh samples were not from the same batches of materials and, thus, cannot be compared directly. These differences might arise from age, seasonal or sampling variations. The levels found in the fillet samples here are in general agreement with those which reported for canned fish.[15]

The fluoride content of the bone ash from several fish species are presented in Table III. The levels found are comparable to those found in ash of bones from other sources.[14,16] The wide range of values observed here probably can form the basis for an explanation of some of the variations seen in the *FPC* and in the raw materials. The low level of fluoride in the bone ash of capelin is reflected in the low level in the *FPC* made from whole fish. On the other hand, the high level of fluoride in the dogfish ash indicates that the bone must be the source of the large amount found in *FPC* prepared from whole dogfish.

Although these results do not represent a systematic sampling over a period of time and from several areas of production, they show that there will be significant variations in the fluoride level of *FPC*. Although the species of fish used as the raw material probably is the most important factor in determining the fluoride level, large variations may be expected within each species.

Studies are underway to establish the sources and fate of the fluoride in the *FPC* process as well as to devise a means to decrease the level of fluoride in this product.

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ANTIBACTERIAL ACTION OF VEGETABLE EXTRACTS ON THE GROWTH OF PATHOGENIC BACTERIA

By K. S. AL-DELAIMY and S. H. ALI

Under controlled conditions, extracts of garlic, onion, turnip, green peppers and radishes were used to inhibit *Escherichia coli*, *Salmonella typhosa*, *Shigella dysentriae* and *Staphylococcus aureus*, which are all pathogenic bacteria. It was found that 1–4% by vol. of garlic extract completely inhibited the growth of all the bacteria used. 4% by vol. of onion extract completely inhibited the growth of both *Shigella dysentriae* and *Staphylococcus aureus* at 10^{-6} dilution. *Salmonella typhosa* and *E. coli* were not completely inhibited; the inhibition was 48.3% for *E. coli* and 95.3% for *Salmonella typhosa*. At 10^{-4} bacterial dilution, onion extract decreased the colony number substantially in all four bacteria. 4% extracts from turnip, green peppers and radishes did not show a definite antibacterial action against any bacterium at the given dilutions. On the contrary some growth stimulating activity of these extracts on some bacteria was observed.

Introduction

In addition to their nutritional value, some vegetables have been found to demonstrate bactericidal and/or bacteriostatic action *in vitro* which may have the same action *in vivo*.

Antibacterial activity has been reported in onion and garlic[1-4] and in onion.[5-9] Pederson & Fisher[10] who conducted their research mainly on the bactericidal action of cabbage juice, found bactericidal activity in onion and garlic juices as well as in the juice of cabbage. They found less action or no bactericidal action in other vegetable juices, such as the juices of turnip, tomatoes, carrots, cucumber, broccoli, cauliflower, celery and Chinese cabbage. Sherman & Hodge[11] on the other hand, found bactericidal activity in turnip juice as well as in that of cabbage. Bergamot, orange and lemon were shown to exhibit antimicrobial activity owing to their essential oils.[12-14]

The present work was undertaken to study the effect of the extracts of onion, garlic, turnip, radishes and green pepper on the growth of *Escherichia coli*, *Shigella dysentriae*, *Salmonella typhosa* and *Staphylococcus aureus* which are all pathogenic bacteria. It is of value to establish this type of data *in vitro*, before any attempt is made to conduct similar experiments *in vivo*.

Experimental

Preparation of the vegetable extracts

Onion and garlic scales, and stems of turnip, white radishes and green peppers were extracted, and the extracts were used in this experiment. The vegetables were grown at the College of Agriculture Experiment Station at Abu-Ghraib in Iraq.

Turnips (*Brassica rapa*), white radishes (*Raphanus sativum* var. *longinnatus*) and green peppers (*Capsicum frutescens*) were cleaned with tap water and detergent to remove any adhering soil on their surface, and then rinsed several times with sterilised distilled water. The outer skins of garlic (*Allium sativum*) and onion (*Allium cepa*) were removed. 100 g of each of the vegetables were cut into small pieces using a sterilised knife and were blended with 100 ml sterilised distilled water separately in a previously sterilised Waring blender for 5 min at medium speed. The macerates were filtered using a sterilised Buchner funnel and a No. 2 Whatman filter paper with the aid of slight suction. The filtrates were

refiltered through a sterilised Seitz filter. The first 50 ml of the filtrates were collected and immediately stored in a refrigerator for experimentation. This final filtrate had twice the volume of the natural vegetable juice. 1 ml of each of the final extracts (4% by vol. of the total Petri dish content), ½ ml of each bacterial dilution and 11 ml of a nutrient agar medium were placed in a Petri dish to make a final volume of 12.5 ml. The ingredients were then mixed well, solidified, turned upside down and incubated at 37° for 24 h before the colonies were counted. The results were expressed as the percentage inhibition from the average number of colonies per Petri dish per dilution of triplicate samples.

Bacterial cultures and dilutions

Pure cultures of *Escherichia coli*, *Salmonella typhosa*, *Shigella dysentriae* and *Staphylococcus aureus* which were obtained from the bacteriology laboratory of the College of Agriculture, University of Baghdad, were used in this study. 9 ml of a nutrient broth were inoculated with 1 ml of the stock culture of each bacterium and incubated at 37° for 24 h. From this new culture, dilutions of 10^{-4} and 10^{-6} were made. ½ ml of each dilution from each bacterium culture was used and found to be the most suitable combination to result in colony numbers of 'too numerous to count' (t.n.c.) for the 10^{-4} dilution and less than 300 per Petri dish for the 10^{-6} dilution. Control samples of the media alone, media plus the extracts, and the media plus the bacterium were also conducted in triplicate.

Results and Discussion

The effect of the extracts of garlic, onions, turnips, radishes and green peppers upon the growth of *E. coli*, *Salmonella typhosa*, *Shigella dysentriae* and *Staphylococcus aureus* are shown in Table I. It was found that garlic extract completely inhibited the growth (100% inhibition) of all the bacteria under investigation.

At a level of 4% by vol. garlic extract inhibited the growth of the bacteria cultures diluted to 10^{-4} and to 10^{-6}. A similar inhibition of the bacterial growth was also demonstrated by an addition of as little as 1% by volume of garlic extract.

Treatment with onion extract on the other hand did not completely inhibit the growth of all bacteria at their two

TABLE I

Antibacterial action of five Iraqi vegetable juices on the growth of four pathogenic bacteria

Results are expressed as the % inhibition of the average no. of colonies per Petri dish
per dilution of triplicate samples

Bacterial treatment	Control (no extract)	Treatment with 4% vegetable extracts				
		Garlic	Onion	Turnip	Green pepper	Radish
Escherichia coli						
Control (no bacteria)	0	0	0	0	0	0
No. of colonies at 10^{-4}	t.n.c.	0	314	t.n.c.	t.n.c.	t.n.c.
% inhibition	0	100	—	—	—	—
No. of colonies at 10^{-6}	236	0	122	197	141	196
% inhibition	0	100	48·3	16·5	40·3	16·4
Salmonella typhosa						
Control (no bacteria)	0	0	0	0	0	0
No. of colonies at 10^{-4}	t.n.c.	0	152	t.n.c.	t.n.c.	t.n.c.
% inhibition	0	100	—	—	—	—
No. of colonies at 10^{-6}	64	0	3	t.n.c.	83	85
% inhibition	0	100	95·3	—	(29·7)	(30·3)
Shigella dysentriae						
Control (no bacteria)	0	0	0	0	0	0
No. of colonies at 10^{-4}	t.n.c.	0	127	t.n.c.	t.n.c.	t.n.c.
% inhibition	0	100	—	—	—	—
No. of colonies at 10^{-6}	110	0	0	189	95	88
% inhibition	0	100	100	(70·2)	8·6	8·0
Staphylococcus aureus						
Control (no bacteria)	0	0	0	0	0	0
No. of colonies at 10^{-4}	t.n.c.	0	33	t.n.c.	t.n.c.	t.n.c.
% inhibition	0	100	—	—	—	—
No. of colonies at 10^{-6}	106	0	0	120	138	62
% inhibition	0	100	100	(13·2)	30·2	41·5

t.n.c. = too numerous to count
Parentheses indicate stimulation of growth, %

different dilutions. At 10^{-6} bacterial dilution, the addition of 4% by volume of onion extract completely inhibited the growth of both *Shigella dysentriae* and *Staphylococcus aureus*. This amount of extract had only reduced the number of colonies of *E. coli* and of *Salmonella typhosa* from 236 and 64 colonies in the non-treated plates to 122 colonies (or 48·3% inhibition) and 3 colonies (or 95·3% inhibition) in the treated plates respectively. At a dilution of 10^{-4} the onion extract of 4% had reduced the number of colonies from t.n.c. in the untreated plates of all bacteria under experiment to 314 for *E. coli*, 152 for *Salmonella typhosa*, 127 for *Shigella dysentriae* and 33 colonies for *Staphylococcus aureus*.

The growth-inhibiting substances present in garlic and in onion are due to their essential oils[2] or to their contents of acrolein and crotonaldehyde.[3,4] Virtanen & Mattikala claimed[8] that the antimicrobial substances of the onion homogenates were due to both *S*-methylcysteine sulphoxide and *S*-n-propylcysteine sulphoxide from which the corresponding thiosulphinates are formed enzymically. Nieman,[15] on the other hand, indicated that the antibacterial substances in plant tissue in general were due to their fatty acids.

Extracts of turnips, green peppers and radishes at a level of 4% by vol. did not show any definite antibacterial activity at the given bacterial dilution. The results of turnips are in agreement with those found by Pederson & Fisher,[10] but did not agree with the results obtained by Sherman & Hodge[11] who reported the presence of bactericidal activity, which was reported to be due to their flavonoid and related compounds.[16]

At a bacterial dilution of 10^{-6} the growth of *Salmonella typhosa* was found to be stimulated up to 29·7% with green peppers and to 30·3% with the radishes. Turnip extract was also found to stimulate the growth of *Shigella dysentriae* up to 70·2% and *Staphylococcus aureus* to 13·2%. This stimulation of growth was probably due to the presence of some growth-stimulating substances for certain bacteria.

It seems that many vegetable extracts contain bactericidal and/or bacteriostatic substances some of which are being lost during the extraction procedure or storage or other interaction. The method and the time of preparing the extracts, the time and temperature of their storage, and the concentration of the extracts used are among the main factors to influence the effectiveness of any antibacterial activity of any vegetable juice.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

FEBRUARY, 1970

1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

No abstracts

Physical Properties of Soils

Relationship of soil texture to sulphur oxidation. G. W. REHM and A. C. CALDWELL (*Agron. J.*, 1969, **61** (2), 333–334. 6 ref.).—When 52 soils with texture ranging from loamy sand to clay loam were incubated (30° and 50% field capacity moisture) for 30–90 days after addition of 100 ppm of S (< 100 mesh), the rate of oxidation of S to SO_4^{2-} was not significantly affected by texture, although on average there was a general trend for rate of oxidation to decrease with increasing fineness of texture. This was due to the wide range in extent of oxidation of S within each textural group.
A. H. Cornfield.

Sequential changes in nitrate content of a soil being prepared for a wheat crop. A. J. RIXON and M. MELVILLE (*J. Aust. Inst. agric. Sci.*, 1969, **25** (2), 119–122. 10 ref.).—In a red-brown earth contg. 12% of clay, nitrates increased after irrigation but were unaffected by subsequent cultivations.
E. G. Brickell.

Biological Aspects, Available Nutrients, Soil Analysis

Accumulation of soil organic matter under pasture and its effect on soil properties. N. J. BARROW (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (39), 437–444. 37 ref.).—11 pairs of pasture samples were collected from Coolup sand in the Swan Coastal Plain, Western Australia. In each pair, one was chem. analysed, and the other incubated in sealed bottles at 25° for up to 63 days, to measure the build-up of org. matter. For soil-N, and C, N, and S contents of org. matter, results confirmed previous work, but the org. P content was lower. Water-holding, cation-exchange capacities, etc., were also detd., and compared with those for virgin sand.
M. T. Rawnsley.

Fate of 2-amino-4-chloro-6-methylpyrimidine (nitrification inhibitor) in soils. C. C. WEIR (*Trop. Agric. Trin.*, 1969, **46** (3), 233–237. Engl., 3 ref.).—The amt. of inhibitor recovered from soils by acidified KCl was a logarithmic function of time, and also a function of clay content, cation exchange capacity, and surface area of the soils. Moisture content also had some influence. Adsorption by soil colloids seems to be the predominant process by which the inhibitor is immobilised.
E. G. Brickell.

Mobilisation and fixation of iron and trace elements by aerobically decomposing plant matter. C. BLOOMFIELD (*Chemy Ind.*, 1969, (45), 1633–1634. 3 ref.).—Results are discussed for (*i*) fixation of Fe, Mn, Cu, Ni, Co, Zn and Pb by lucerne incubated aerobically for ~9 months with excess of the metal carbonates or oxides at 25°, (*ii*) contents of these metals in lucerne residues before and after extraction with 0·1 N-HCl, (*iii*) uptake of Mn by pea plants grown in acid-washed quartz sand and the uptake from aerobic and anaerobic lucerne decompn. soln. In all instances, unlike the corresponding anaerobic system, there was relatively little Fe and Mn fixation by plant matter.
W. J. Baker.

Water resources and plant nutrient reserves. F. DIXEY (*Chemy Ind.*, 1969, (44), 1580–1584. 30 ref.).—Availability of surface and ground waters can be the main factor affecting plant growth, and so planned use of fertiliser nutrients is discussed in conjunction with planned use of water resources. The chem. quality of these waters, their hydrogeology, and the presence of plant nutrients and toxicants are considered. Measures to ensure the conservation and proper use of water are reviewed, esp. by restriction of run-off to the sea, by artificial recharge of ground water and by conservation of the natural vegetation and soil cover. Hydrological methods for appraisal of water resources are outlined.
W. J. Baker.

Indices of availability of potassium, magnesium, calcium, and phosphorus in Connecticut soils. D. E. PEASLER (*Agron. J.*, 1969, **61** (2), 330–331. 12 ref.).—Uptake of K, Mg and Ca by tomato plants in pot tests with six acid soils was highly correlated with levels of the three nutrients extracted from soils by 0·73N-NaOAc–0·53N-AcOH. Uptake of K and Mg was highly correlated with K and Mg extracted from the soils by a cation exchange resin procedure. Uptake of P by the plant was poorly correlated with P extracted either by the chemical extractant or by an anion exchange resin procedure. However, P uptake was highly correlated with an index of soil P derived from a regression equation involving both chemically extractable P and total soil org. P.
A. H. Cornfield.

Bray and Morgan soil extractants modified for testing acid soils from different parent materials. J. L. MCINTOSH (*Agron. J.*, 1969, **61** (2), 259–265. 25 ref.).—Replacing the Na⁺ in the Morgan reagent (10% NaOAc–4% AcOH, pH 4·8) with NH_4^+ improved the reagent with respect to measurements of the P, K, Ca and Mg status of soils and reactive forms of Mn, Fe and Al. 1·25N-NH₄-OAc–0·03N-NH₄F (pH 4·8) (modified Bray reagent) is considered to be useful for measuring reserve P in acid soils, and is also suitable for measuring the status of soils with respect to exchangeable bases.
A. H. Cornfield.

Pattern of sulphur responses shown by dryland lucerne on some brown-grey earths. L. C. BLAKEMORE, T. E. LUDECKE, M. L. LEAMY and A. J. METSON (*N.Z. Jl agric. Res.*, 1969, **12** (2), 333–351. 19 ref.).—Soil analyses, following field trials in the Maniototo basin, Central Otago, showed the presence of SO_4^{2-} in the deeper layers (2–4 ft) of the non-responsive soils but no such accumulations in responsive soils. S concn. in lucerne was normal (⩾ 0·19%) for non-responsive soils but only 0·15–0·17% for responsive soils.
E. G. Brickell.

Measurement of soil fertility. E. G. HALLSWORTH (*J. Aust. Inst. agric. Sci.*, 1969, **35** (2), 78–89. 25 ref.).—Aims of the National Soil Fertility Project are described, in particular the analysis of yield, the relationship of yield to the various controlling factors, the effect of inter-relationships between the intrinsic factors, the climatic factor, and the use of multi-factorial analysis to identify the soil factors which limit yield.
E. G. Brickell.

Nitrate determination in soil extracts with the nitrate electrode. A. ØIEN and A. R. SELMER-OLSEN (*Analyst, Lond.*, 1969, **94** (1123), 888–894. 23 ref.).—Reliable results were rapidly obtained for soil extracts or suspensions by use of the Orion NO_3^--specific electrode. The extractants used were 2N-KCl, 0·02N-CuSO₄ and distilled water; with a NO_3^- concn. ≮ 2 ppm, the coeff. of variation was 2% (suspensions) or 2·7% (filtered extracts). There were no serious interferences and results were in reasonable agreement with those obtained by the xylenol and AutoAnalyzer methods. Stability of the extracts, ratio of ml of extractant to wt. of soil, shaking time, effect of ionic strength, etc., are discussed on basis of listed data. As little as 9 lb of NO_3^--N per acre can be detd.
W. J. Baker.

Automated distillation procedure for determination of nitrogen [in soils and plant material]. J. KEAY and P. M. A. MENAGE (*Analyst, Lond.*, 1969, **94** (1123), 895–899. 7 ref.).—A distillation unit, located in the const. temp. bath, was incorporated into the automated indophenol method for detn. of NH_3; this eliminated both interference from heavy metal ions and the need for dialysis of turbid soln. The method can be extended to digests of soil, vegetation, animal faeces, etc., without the prepn. of standards in separate soln. to match the digesting conditions. Two manifolds provide for ranges 0–200 and 0–30 mg of N/l. For 5 mg of N/l, the standard deviation was ~1% (8 detn.).
W. J. Baker.

Determination of sulphur in soils by X-ray fluorescence analysis. G. BROWN and R. KANARIS-SOTIRIOU (*Analyst, Lond.*, 1969, **94** (1122), 782–786. 7 ref.).—The method, used for survey and fertility studies, permits rapid detn. (< 4 min/sample) of total S with av. error of ± 7%. Because of variations (10–90%) in org. matter content, a simple correction, based on wt.-loss at 450°, is made. In the absence of such sources of error, the graph of $K\alpha$ count-rate vs. S concn. is rectilinear. The sensitivity is < 10 ppm.
W. J. Baker.

Application of the electron-probe microanalyser to the study of soils. D. A. JENKINS (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 146. 1 ref.).— The technique is used to study the compn. of relatively undisturbed soil samples at the μg level, e.g., distribution of P in selected soil profiles. Samples are prep. as diamond-polished thin sections of resin-impregnated soil, vac. coated with C and Cu, and examined with a JEOL.JXA.3A, which has twin spectrometers with take-off angles of 20°.
S. S. Chissick.

Fertilisers

Recovery of exchangeable calcium and magnesium added to soil acidoids. H. J. RAJANI (*J. Instn Chem. India*, 1969, **41** (3), 102–105. Engl., 7 ref.).—Ca could be recovered fully from alluvial soil but black, red and laterite soils fixed 9·89, 6·10 and 6·05%, resp., of the added Ca. Mg was fixed by all four soils to the extent of 21·99 in black, 9·20 in red, 12·26 in laterite and 4·60% in alluvial.
E. G. Brickell.

Release of phosphorus from basic slag in acid soil. H. SINHA, R. S. SINGH and S. C. MANDAL (*J. Instn Chem. India*, 1969, **41** (3), 90–93. Engl., 12 ref.).—Incubation expt. with an acid soil of Ranchi are reported. No direct relation between soil pH and available P was found, though max. release tended to occur at pH 7·5. The beneficial effect of org. matter on P release was almost independent of variations in basic slag applications. Moisture was only a significant factor when > 75% of field capacity.
E. G. Brickell.

Phosphorus changes during the leaching and decomposition of hayed-off pasture plants. O. L. JONES and S. M. BROMFIELD (*Aust. J. agric. Res.*, 1969, **20** (4), 653–663. 12 ref.).—Inorg. P was readily leached from lab. samples when microbes were inhibited. Intermittent drying increased the amt. of P leached from decomp. plants though the % leached varied with the type of material. The conversion of small addn. of sol. and plant PO_4^{3-} to org. forms was often extensive but that of superphosphate was not.
E. G. Brickell.

Loss of nitrogen by ammonia volatilisation in saline and alkali soils of W. Pakistan. MUKHTAR AHMAD SINDHU and A. H. CORNFIELD (*W. Pakistan J. Agric. Res.*, 1969, **7** (1) [(5)?], 94–102. Engl., 14 ref.).—Studies in saline and alkali soils before and after reclamation are reported. Losses of NH_3 from added $(NH_4)_2SO_4$ (I) were studied at different moisture levels and were compared with losses from added urea (II). Virtually all the N from I and II was lost by NH_3 volatilisation; different losses were accounted for by different % of exchangeable Na rather than by different pH of the soils.
E. G. Brickell.

Reduction of nitrogen losses through denitrification from paddy soil by the application of pesticides. MUHAMMAD IBRAHIM AYAD and ABDUL AZIZ KHAN (*W. Pakistan J. Agric. Res.*, 1968, **6** (4), 128–133. Engl., 6 ref.).—Applications of endrin and Dimicron [phosphamidon?], at the rate of 25 ppm at 14-day intervals, considerably reduced N losses due to denitrification.
E. G. Brickell.

Effect of sodium molybdate mixed in the lime seed pellet on nodulation, nitrogen content and growth of subterranean clover. J. W. GARTRELL (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (39), 432–436. 10 ref.).—Mo is usually applied as Na_2MoO_4 (I) mixed with superphosphate. The cost can, however, be reduced by putting Mo in the lime used for pelleting seed. Tests on Norpa sand at Merredin, Western Australia, showed that I, mixed with ground limestone and pelleted on to seed inoculated with peat culture, reduced clover nodulation and growth; N/acre in the tops was proportional to the no. of nodulated plants/acre. Mixed with superphosphate, and pelleted with ground limestone, nodulation was not affected, but dry matter increased by 70%.
M. T. Rawnsley.

Iron pyrites as a sulphur fertiliser. C. L. BANATH (*Aust. J. agric. Res.*, 1969, **20** (4), 697–708. 25 ref.).—After grinding, FeS_2 was highly effective as a S fertiliser for *Trifolium subterraneum* L. and

even at very high rates of application (1360 and 680 lb/acre) of the smallest mean particle size, was not toxic to the plants. In warm, humid climates, smaller applications or larger mean particle sizes should suffice to alleviate a similar degree of S deficiency.
E. G. Brickell.

Determination of trace elements in fertilisers. J. M. SKINNER (*Proc. Soc. Analyt. Chem.*, 1969, **6** (8), 147–148. 1 ref.).—15 micro-nutrients including Al, As, Ba, B, Mg and Zn were detd. simultaneously by igniting the fertiliser sample at 500°, mixing the residue with graphite, pressing the mixture into an electrode and incorporating into a solid source mass spectrograph (G.E.C.–A.E.I. MS 702). The spectra were recorded on photographic plates and examined on a recording microphotometer.
S. S. Chissick.

Mulching of soil. E. I. DU PONT DE NEMOURS & CO. (Inventors: J. M. IWASYK and B. C. LAWES) (Br. Pat. 1,166,540, 29.12.67).— A fluid aq. foam (vol. ≪ 0·1 ft³/lb, viscosity ≪ 30 cP) containing at least a partly hydrolysed polyvinyl alcohol (I) and emulsified asphalt or wax (II) is applied to the soil and gelled. The foam is prep., e.g., by disintegrating bubbles of air within a liquid aq. soln. containing I and II plus an emulsifier. Soil treated with the foam yields more crops/acre than does untreated soil.
S. S. Chissick.

Forming a solid plant nutrient from humate-bearing ore. CONCHO PETROLEUM CO. (Br. Pat. 1,161,290, 23.1.68. U.S., 20.10.67).— The process comprises: (*a*) wet grinding the ore (leonardite) with the min. amount of water, (*b*) admixing the slurry obtained (water content ≯ 65%) with excess H_3PO_4, (*c*) adding NH_3 and dry granules (final pH 7–10), (*d*) drying the granulated product to a residual moisture content of 5–10% and (*e*) recycling a portion of the granules to step (*c*). An apparatus for carrying out the process is described.
S. S. Chissick.

Preparation of fertilisers from poultry droppings, etc. CHEMISCHE FABRIK FRANKENTHAL H. SCHMIDT KG. (Br. Pat. 1,164,958, 29.7.68. Ger., 29.7.67).—Poultry droppings, coagulated blood, liquid manure, etc., alone or mixed, are comminuted with a wateradsorbent plastic foam in the presence of air and then fermented for several days under aerobic conditions.
S. S. Chissick.

Soil-treating method. SUMITOMO CHEMICAL CO. LTD. (Br. Pat. 1,162,727, 6.3.68. Jap., 16.3.67).—A method of treating soil to suppress nitrification of NH_3-N comprises adding to the soil a compound $M(O_2C·R^1·CONHR^2)_n$, where R^1 is a 1-8C saturated alkylene, or C_6H_4, or a direct link; R^2 is Ph or pyridyl, optionally substituted with up to 5 substituents (halogen, NO_2, NH_2, Me or CCl_3); *n* is 1–3 and if *n* is 1, M is H, Na, K or NH_4; if *n* is 2, M is Mg, Ca, Cu, Zn, or Fe(II); if *n* is 3, M is Fe(III) or Al, e.g., *N*-(2,5-dichlorophenyl)succinamic acid or its salts. Fertiliser compositions containing the compd. are claimed. Results of soil tests, with nitrification suppression ratios, are tabulated.
S. S. Chissick.

Anhydrous liquid ammonia fertilisers. DIRECT NITROGEN LTD. (Inventors: E. BARNES and K. H. NANCE) (Br. Pat. 1,159,762, 28.6.67).—Anhyd. liquid NH_3 mixed with 25–150% of a solid compound which has N in a form acceptable to plant life (e.g., NH_4NO_3) is injected below the soil surface to fertilise the soil; a further plant nutrient (e.g. a P sulphide) and a pesticide may be included.
E. Enos Jones.

Fertiliser composition. ESSO RESEARCH & ENGNG CO. (Br. Pat. 1,151,813, 28.3.67. U.S., 14.12.66).—Volatilisation of NH_3 from a urea-based fertiliser (caused by microbiological activity in the soil) is minimised by dispersing in or coating on to the urea pellet a mixture of inorg. urease inhibitor (e.g., borax, 0·5–10) and a hydrophobic substance (e.g., octadecylamine, 0·1–2 wt.-% of the urea).
J. A. Sugden.

Fertiliser product. FISONS BASIC SLAG LTD. (Inventors: G. G. BROWN and D. C. HARPER) (Br. Pat. 1,160,125, 25.8.66).—The P content of basic slag is increased by adding ≯ 50% of Ca–Al-type phosphate rock to the molten slag at 1400–1800 (1550–1650)°.
J. A. Sugden.

Fertiliser production. A/S DANSK SVOVLSYRE-OG SUPERHOSPHAT-FABRIK (Inventor: K. C. B. KNUDSEN) (Br. Pat. 1,166,930, 23.6.67). —A P- and K-containing fertiliser is produced by dissolving phosphate rock (I) in mineral acid and treating the soln. with a cation exchanger charged with K^+. In an example, Morocco I is dissolved in aq. HNO_3 and sulphonated styrene–divinylbenzene copolymer charged with K^+ is added. The polymer is removed, NH_3 added to the soln. and the product granulated and dried to

yield a fertiliser containing $14 \cdot 7\%$ each of N, P_2O_5 and K_2O.
S. S. Chissick.

Fertilisers. J. W. CHAFER LTD. (Inventors: D. A. PALGRAVE and R. CLARK) (Br. Pat. 1,165,257, 10.5.67).—The liquid fertiliser is prep. by dissolving homogenised phosphate rock in excess HNO_3 (and $H_3PO_4 + H_2SO_4$) and adding aq. NH_3 (to give a pH $\not> 1$) and KCl.
S. D. Huggins.

Defluorination of phosphatic materials. HOOKER CHEMICAL COMP. (Br. Pat. 1,164,626, 30.9.66. U.S., 6.10.65).—The material is made suitable for plant fertiliser or animal feed purposes by (*i*) preparing a moist (8–14%) mixture of finely ground rock and H_3PO_4 which has the mole ratio $[P_2O_5 \text{ (acid)} + P_2O_5 \text{ (rock)} - \frac{1}{3}(CaO - \frac{1}{2} F)] : F > 0 \cdot 16$ ($0 \cdot 24$–$0 \cdot 38$), (*ii*) granulating and drying the material to 5% of H_2O and (*iii*) heating the granules to 980–$1315°$ in a bed fluidised by the combustion gases. The F content is reduced from, e.g., $8 \cdot 6$ to $< 0 \cdot 1$ wt.-%.
J. A. Sugden.

Production of calcined phosphate fertilisers. KALI-CHEMIE A.-G. (Inventors: U. HAUSCHILD, J. MASSONE and H. W. SCHMIDT) (Br. Pat. 1,159,650, 25.10.67. Ger., 9.11.66 and 12.4.67).—A granular mixture of (*i*) natural Ca phosphate, (*ii*) aq. soln. containing 40–75 wt.-% of alkali hydroxide, (*iii*) SiO_2, and (*iv*) 10–70 wt.-% of recycled material, is calcined at 950–1300°. The proportions in the mixture are such that the product has the molar ratio P_2O_5 : alkali oxide $= 1 : 0 \cdot 6 - 1 : 1 \cdot 5$, and $P_2O_5 : SiO_2 = 1 : 0 \cdot 1 - 1 : 0 \cdot 9$.
J. A. Sugden.

Production of calcined alkali phosphate fertilisers. KALI-CHEMIE A.-G. (Inventors: U. HAUSCHILD, R. HOLST, H.-H. KASPERS and W. DAHME) (Br. Pat. 1,159,660, 2.4.68. Ger., 18.4.67).—A mixture of Ca phosphate rock, alkali-providing material, e.g., carbonate or hydroxide, and, if necessary, SiO_2 is calcined (e.g., in a rotary furnace with a basic lining) at 1000–1300°. The raw mix has molar ratio P_2O_5: alkali oxide $= 1 : 1 \cdot 2 - 1 : 1 \cdot 5$, and a SiO_2 content such that the amount of CaO in excess of the ratio 2 CaO/ P_2O_5 will be bound as CaO, $CaSiO_3$; 25–85% of the alkali ide-providing material is solid carbonate and the remainder is an aq. soln. containing 40–70% of hydroxide.
J. A. Sugden.

Production of calcined phosphate fertilisers. KALI-CHEMIE A.-G. (Inventors: U. HAUSCHILD and R. HOLST) (Br. Pat. 1,165,111, 2.4.68. Ger., 17.4.67. Addn. to Br. Pat. 1,149,672).—A calcined phosphate fertiliser is produced in a rotary kiln by carbonating and concentrating an aq. soln. of KOH and/or NaOH 30–60 (45–55) wt.-%, by treatment with the hot waste gases from the kiln to form a mash containing alkali carbonate 5–50, alkali hydroxide 95–50 and total alkali, as alkali oxide, 60–70 wt.-%, all calc. on the dry wt. of the mash. This is then mixed and granulated with a phosphate rock and any SiO_2 that may be needed to provide, in the calcined fertiliser, a mole ratio of $P_2O_5 : SiO_2$ of $1 : 0 \cdot 1 - 0 \cdot 9$ and to bind all the CaO exceeding the mole ratio $2CaO : P_2O_5$ as Ca silicate. Thereafter, the granular product is calcined in the kiln at 1000–1300°.
J. M. Jacobs.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Effects of light intensity, temperature and root gaseous environment on growth of *Nicotiana tabacum* L. R. E. WILLIAMSON and W. E. SPLINTER (*Agron. J.*, 1969, **61** (2), 285–288. 6 ref.).—Studies in growth chambers showed that the extent of injury sustained by tobacco plants whose roots had been exposed for 24 h to a low-O_2 (0–1%) atm. and then returned to a normal atm. increased with temp. ($18 \cdot 3$–$29 \cdot 4°$) and light intensity (1000–4000 ft-candles).
A. H. Cornfield.

Solar ultra-violet radiation as an ecological factor for alpine plants. M. M. CALDWELL (*Ecol. Monogr.*, 1968, **38** (3), 243–268. 62 ref.).—A review.
C. V.

Photosynthesis of wheat under field conditions. II. Effect of defoliation on the carbon dioxide uptake of the community. D. W. PUCKRIDGE (*Aust. J. Agric. Res.*, 1969, **20** (4), 623–634). 22 ref.).—At anthesis and 10 days later only the top three leaves were effective in photosynthesis. Removal of the two leaves below the flag leaf reduced photosynthesis of the community by 25–28%, and further removal of the flag leaf reduced photosynthesis by an additional 24–30%. After removal of the leaf laminae and ears, the stems and leaf sheaths assimilated CO_2 at 44% of the rate of the whole community.
E. G. Brickell.

Plant moisture stress: a portable freezing-point meter compared with the psychrometer. J. W. CARY and H. D. FISHER (*Agron. J.*, 1969, **61** (2), 302–305. 11 ref.).—A portable unit is described for determining the f.p. of leaf tissue. Moisture stress values, derived from f.p. depression, of tissue from a number of vegetable species correlated well with those obtained with v.p. psychrometer measurements.
A. H. Cornfield.

Comparison of indices relating plant response to soil moisture status. E. RAWITZ and D. I. HILLEL (*Agron. J.*, 1969, **61** (2), 231–235. 16 ref.).—Studies in pot tests relating dry matter yields with four soil moisture indices showed that the max. capillary suction index (obtained by averaging the suction values, taken from the desorption curve, of the min. moisture content of each irrigation cycle) was the most satisfactory for use in controlling irrigation regimes. The utility of the method was confirmed in field tests.
A. H. Cornfield.

Evaluation of techniques for measuring drought avoidance in cereal seedlings. M. H. SALIM, G. W. TODD and C. A. STUTTE (*Agron. J.*, 1969, **61** (2), 182–185. 12 ref.).—There were significant differences among cultivars of wheat, barley and oats in the water content of seedlings dried for various periods over $CaCl_2$. Water retention by 'drought-hardened' wheat and barley seedlings was greater than that by non-treated seedlings. Water retention by detached leaves was so variable as to give poor differentiation among cultivars. At a particular seedling moisture content, survival decreased in the order wheat, barley, oats.
A. H. Cornfield.

pH effect on root growth and water uptake by plants. J. T. THORUP (*Agron. J.*, 1969, **61** (2), 225–227. 9 ref.).—When tomato plants were transferred from a nutrient of low pH ($\sim 5 \cdot 5$) to nutrients of higher pH, wilting and root damage occurred rapidly in nutrient of pH $\geqslant 8 \cdot 8$. The crit. pH for young developing roots was $8 \cdot 2$, and new growth occurred when pH was decreased below $8 \cdot 0$. High pH is probably the primary cause of plant failure in sodic soils.
A. H. Cornfield.

Plant Nutrition and Metabolism

Simulation with mathematical models of ion uptake by growing roots. R. S. ANDERSSEN, R. P. HALE and J. R. M. RADOK (*Pl. Soil*, 1969, **30** (2), 271–289. 10 ref.).—Methods of formulation of the models are presented.
A. H. Cornfield.

Theoretical study of the distribution of substances around roots resulting from simultaneous diffusion and mass flow. P. H. NYE and F. H. C. MARRIOTT (*Pl. Soil*, 1969, **30** (2), 459–472. 14 ref.).—The change in concn. of a solute moving in soil near the surface of a root by mass flow and diffusion was calculated using a computer. The effects of changes in plant-controlled variables (solvent flux at the root surface and root absorbing power) and soil variables (buffer power and diffusion coeff.) are described.
A. H. Cornfield.

Relationship between maize yields and leaf levels of ten elements. T. R. PECK, W. M. WALKER and L. V. BOONE (*Agron. J.*, 1969, **61** (2), 299–301. 18 ref.).—Regression analysis of maize yields (from four locations with plots receiving various rates and combinations of N, P and K) with leaf levels (6th leaf at early tassel) of N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn showed several significant effects and interactions. The significant interactions indicate that the critical level of some nutrients varied with the leaf levels of other nutrients.
A. H. Cornfield.

Effects of nitrogen–potassium levels on the growth and chemical composition of Kentucky bluegrass. C. A. MONROE, G. D. COORTS and C. R. SKOGLEY (*Agron. J.*, 1969, **61** (2), 294–296. 15 ref.).—The effects of 65 and 130 ppm of N and 0, 100, 200 and 400 ppm of K in all factorial combinations on wt. of tops and underground parts, vigour scores, tiller counts, blade widths, rhizome lengths and N, P, K, and org. C contents of leaf tissue were studied in sand culture.
A. H. Cornfield.

Bibliography on the effect of sodium on barley. ANON. (*Chilean Nitrate agric. Serv. Inf.*, 1969, (109), 18 pp. Engl., 51 abstr.).
P. C. W.

Rhythm of uptake and inclusion of ^{35}S into maize root protein. M. G. ABUTALYBOV, A. A. MARDANOV and P. M. SAFARALIEV (*Dokl. Akad. Nauk SSSR*, 1969, **185** (4), 954–956. Russ., 16 ref.).—Samples of maize sprouts of 5–15 days growth were analysed to determine the uptake of ^{35}S from a soln. of labelled Na_2SO_4. Adsorption into the roots and inclusion by the root protein was of a cyclic nature, having 4 or 5 maxima and minima with greater

amplitudes by day than by night. The 15-day cycles shown by older plants were more clearly defined than those of younger plants, evidently due to the emergence of a rhythm of separate physiological processes and, in particular, protein synthesis connected with the growth and onset of different functional activities of the organism.   M. E. Traxton.

Effect of phosphorus on the growth and chemical composition of some tropical pasture legumes. I. Growth and critical percentages of phosphorus. II. Nitrogen, calcium, magnesium, potassium and sodium contents. C. S. ANDREW and M. F. ROBINS (*Aust. J. agric. Res.*, 1969, **20** (4), 665–674, 25 ref.; 675–685. 23 ref.).—I. Pot trials are discussed. The quantity of P per pot, accumulated in the tops of the plants, was greatest for *Stylosanthes humilis* and *Lotononis bainesii* at all treatment levels. Critical % of P in the tops of *Phaseolus lathyroides, P. atropurpureus, S. humilis, Centrosema pubescens, Glycine javanica, L. bainesii, Medicago sativa, Desmodium uncinatum, D. intortum* and *Vigna luteola*, sampled at the immediate pre-flowering stage of growth, were 0·20, 0·24, 0·17, 0·16, 0·23, 0·17, 0·24, 0·23, 0·22 and 0·25, resp. II. N concn. in the plant tops were increased by P supply. When NaH₂PO₄ was used, the Na concn. in *V. luteola, M. sativa* and *L. bainesii* was increased but other species were not affected. Increasing PO₄³⁻ supply as Ca(H₂PO₄)₂ had little effect on plant Ca concn. but brought about an increase of Mg in *P. lathyroides* and *P. atropurpureus*. Concn. of the cations in the plants reflected the soil exchangeable cation compn. *P. atropurpureus* and *P. lathyroides* were relatively high in Mg, *L. bainesii* and *D. intortum* in K, *V. luteola, L. bainesii, M. sativa* and *P. lathyroides* in Na, and *C. pubescens* and *S. humilis* in Ca.   E. G. Brickell.

Differential aluminium tolerance of winter barley varieties and selections in associated glasshouse and field experiments. D. A. REID, G. D. JONES, W. H. ARMIGER, *et al.* (*Agron. J.*, 1969, **61** (2), 218–222. 17 ref.).—Both root and top dry matter yields of winter barley (30 cultivars and strains) grown for 7 weeks in the glasshouse in a slightly limed Al-toxic silt loam, were highly correlated with grain yields in unlimed soil in the field. There was poor correlation between glasshouse and field yields when high-limed soil was used. The cultivars and strains varied considerably in Al tolerance. Lines derived from backcrossing to Al-tolerant parents were also tolerant.   A. H. Cornfield.

Boron nutrition of sugar-beet, cotton and soyabean. J. J. OERTLI and J. A. ROTH (*Agron. J.*, 1969, **61** (2), 191–195. 6 ref.).—In nutrient soln. with B concn. of 0·01–40 ppm, max. fresh wt. yields after 8 weeks growth occurred with 2 ppm of B for soyabean, 10 ppm for cotton and 25 ppm for sugar-beet. In spite of the differences in B requirement and B tolerance, tissue levels of B at which toxicity symptoms appeared were similar for the three species. In a glasshouse sand culture test, beet yields and sugar-% were little different with nutrient B concn. ranging from 0·5 to 40 ppm.   A. H. Cornfield.

Nitrogen–sulphur relationships in wheat, maize and beans. B. A. STEWART and L. K. PORTER (*Agron. J.*, 1969, **61** (2), 267–271. 13 ref.).—When S became limiting to wheat, maize and beans, additional N did not affect yield or protein content of the plant but increased the % of NO₃⁻, amides and amino acids in the plant. There was a close relationship between the amounts of N and S metabolised in the plants. One pt. of S was required for every 12–15 pt. of N to ensure max. production of dry matter and protein.   A. H. Cornfield.

Effects of nitrogen, phosphorus and potassium and their interactions on nitrogen metabolism of vegetative barley tissue and on the chemical composition of grain in hydroponic culture. L. B. MACLEOD and R. B. CARSONS (*Agron. J.*, 1969, **61** (2), 275–278. 12 ref.).—The % of all N fractions, except NO₃⁻ in mature grain, increased with N level (10–200 ppm) in the nutrient. With increasing supply of P (10–100 ppm) and K (10–200 ppm), dry matter yield increased, but % of all N fractions decreased, particularly at the early and mid-vegetative stages. Although % protein and non-protein N in mature grain was lower with high than with low P and K supply, total production of both fractions per plant was greater with the higher rates of P and K, due to increased dry matter yield. N–K interactions on N fractions were more significant than were N–P or P–K interactions.   A. H. Cornfield.

Influence of foliar nutrient sprays on the root exudation pattern in four crop plants. A. BALASUBRAMANIAN and G. RANGASWAMI (*Pl. Soil*, 1969, **30** (2), 210–220. 17 ref.).—17 amino acids and four sugars were exuded from the roots of sorghum, tomato, millet and sunnhemp (*Crotalaria juncea*). The no. and nature

of the exuded materials varied with species and age of plant. Foliar applications of NaNO₃ resulted in a general increase in the total concn. of amino acids and a decrease in sugars in the root exudates, whilst the reverse effects occurred with foliar application of Na₂HPO₄. There were differences due to species in the responses to foliar applications of N and P.   A. H. Cornfield.

Population differentiation within *Agrostis tenuis* L. in response to colliery spoil substrate factors. M. J. CHADWICK and J. K. SALT (*Nature, Lond.*, 1969, **224**, (5215), 186. 14 ref.).—In reciprocal transplant tests, unburnt spoil (pH 2·5; H₂O-sol. Al 2·4 ppm) decreased the total growth scores of all three populations of *A. tenuis* growing on three substrates, but the population from the unburnt spoil had higher growth scores than those from burnt spoil (pH 3·9; Al 0·75 ppm) and from placeland (pH 4·2; Al 0·15 ppm). Results of rooting tests in complete nutrient soln. showed that max. Al concn. (2·7 ppm) greatly decreased root growth in all three populations, but although an increase from 0 to 0·3 ppm Al decreased root growth in the placeland population, increasing concn. of Al did not appreciably decrease root growth in the burnt population or stimulate it in the unburnt population. Differential tolerance to Al may be due to a nutrient factor, e.g., internal P pptn., correlating with Al concn.   W. J. Baker.

Effects of light and darkness on polyphenol distribution in the tea plant (*Camellia sinensis* L.). G. I. FORREST (*Biochem. J.*, 1969, **113** (5), 773–781. 9 ref.).—Polyphenol synthesis in young shoots and seedlings of the tea plant occurred in darkness at a decreased rate. The initial effect of darkness was to inhibit synthesis of the flavonoid A ring, or its linkage to the phenylpropane moiety. Later the hydroxylation of the flavanols was affected, and the ratio of simpler : more complex leucoanthocyanin monomers increased. Esterification of catechins with gallic acid was less affected, and the ratio of catechin gallates : simple catechins increased. The flavylogen content of stems (esp. seedlings), was much less decreased than that of leaves; exposure to light resulted in increased polymerisation.   J. N. Ashley.

Distribution of polyphenols in the tea plant (*Camellia sinensis* L.). G. I. FORREST and D. S. BENDALL (*Biochem. J.*, 1969, **113** (5) 741–755. 36 ref.).—Methods are described, for the separation and detn. of polyphenolic components of the tea plant by t.l.c. and colorimetric reactions. High concn. of catechins, flavonols (I) and depsides were found only in young vegetative and floral shoots; leucoanthocyanins or flavylogens (II) were present in the bulkier axial tissues. In young shoots, cell growth was correlated with increased hydroxylation of the flavonoid B ring. Max. flavylogen concn. occurred in the outer protective layers of the stem and seed coat. Mature leaves contained deriv. of apigenin and luteolin. A steady increase in polyphenol complexity occurred in developing seedlings; II were conc. at shoot and root apices and accumulated at the stem base. I which can co-polymerise, were possibly used by the plant for protection of wood and bark against decay and infection.   J. N. Ashley.

Polyphenol metabolism of tissue cultures derived from the tea plant (*Camellia sinensis* L.). G. I. FORREST (*Biochem. J.*, 1969, **113** (5), 765–772. 27 ref.).—Growth characteristics on various media, of callus tissue (I) from tea, were investigated. Synthesis of chlorophyll and anthocyanin occurred in the light. The inability of I and of root apices to synthesise complex catechins suggests that their formation is associated with cell vacuolation. Synthesis of anthocyanins, flavonols, and polyphenol oxidase was generally inversely correlated with growth rate of the cultures. Polyphenol oxidase activity was comparable with that of the apical regions of the plant.   J. N. Ashley.

Separation and distribution of simple and condensed leucoanthocyanins of the tea plant (*Camellia sinensis* L.). G. I. FORREST and D. S. BENDALL (*Biochem. J.*, 1969, **113** (5), 757–763. 19 ref.).—Leucoanthocyanin monomers of high mobilities in aq. solvents on t.l.c., were characteristic of mature bulky tissues, whilst products of lower mobility occurred only in young vegetative and floral tissues. Flavylogens (I) were separated by gel filtration on Sephadex columns into mono-, oligo-, and poly-meric fractions. Leaves had low concn. of I (monomers only), while stem tissues were rich in I (esp. polymers). Max. concn. of I occurred in phloem and cambium from mature stems. A very high polymer/monomer ratio was present in periderm tissue and seed coat. Root tissues contained high concn. of monomers.   J. N. Ashley.

Frenching of tobacco in Australian soils and in soil leachates. M. MANDRYK (*Aust. J. agric. Res.*, 1969, **20** (4), 709–717. 16 ref.).—Under glasshouse conditions, frenching occurred in 'active

soils' (Molonglo sand, Katherine, Manjimup and Shepparton soils, and a mixture of Katherine soil and vermiculite) at a wide range of temp. (18–45°); high soil temp. increased the severity of and decreased the time to appearance of symptoms. Heat and chem. sterilisation of active media eliminated their ability to induce frenching in plants, suggesting a biol. origin for the disorder.
E. G. Brickell.

Corking disorders of apples. II. Chemical composition of affected tissues. M. FAUST, C. B. SHEAR and C. B. SMITH (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 82–88. 20 ref.).—Corky apple tissue was higher in mineral elements, particularly Mg, Ca and B, than was normal tissue. Solubility of mineral elements in various extractants also differed between normal and corky tissue. The pectin content of corky tissue was higher than that of normal tissue, but the viscosity of the corky pectin was less than that of normal pectin. Changes in mineral elements and pectin due to corkiness are considered to be the result, rather than the cause, of development of corky tissue.
A. H. Cornfield.

Plant protein biosynthesis. H. J. CAMERON and G. R. JULIAN (*Baker's Dig.*, 1969, **43** (4), 22–27. 23 ref.).—The chem. nature of nucleic acids and the replication of DNA are reviewed together with the code, plant systems, plastids and control mechanisms. No coherent picture of control of plant protein synthesis has yet emerged.
I. Dickinson.

Biosynthesis of rubber from β-hydroxy-β-methylglutaryl-coenzyme A in *Hevea brasiliensis* latex. C. M. HEPPER and B. G. AUDLEY (*Biochem. J.*, 1969, **114** (2), 379–386. 38 ref.).—β-Hydroxy-β-methyl-[3-^{14}C] glutaryl-CoA (I) was incorporated into rubber when it was incubated with latex; the ^{14}C was located in the isoprene chains. The incorporation was decreased in the presence of unlabelled mevalonate, but was stimulated by NADP$^+$ and NADPH, and less so by NAD and NADH. ATP stimulated the incorporation slightly, whilst CoA inhibited it. I-reductase, which reduces I to mevalonate, was present in the particulate fraction when the latex was centrifuged; formation of NADPH occurred in the latex serum. Incorporation of I into rubber is seasonal but incorporation of mevalonate is independent of season. Conversion of I into mevalonate is of importance in the regulation of rubber synthesis. Significant degradation of I occurs in the latex, probably by the action of I-lyase.
J. N. Ashley.

Root porosity and growth responses of rice and maize to oxygen supply. R. J. LUXMOORE and L. H. STOLZY (*Agron. J.*, 1969, **61** (2), 202–204. 19 ref.).—Porosity of adventitious roots of rice and maize grown in solution culture was higher than that of primary roots. Root porosity was the same in soln. with O_2 concn. of 0·7 and 7·5 ppm. For rice, total and root dry wt. were higher at the lower O_2 concn., whilst the reverse was true for maize.
A. H. Cornfield.

Relation of hydrocyanic acid potential of leaf samples to that of whole plants of sorghum. J. A. BENSON, E. GRAY and H. A. FRIBOURG (*Agron. J.*, 1969, **61** (2), 223–224. 7 ref.).—The HCN content (fresh basis) of the first and third leaves of two varieties of sorghum cut when growth had reached 50 cm, 75 cm or early bloom, was usually significantly correlated with the HCN content of the whole plant. Although leaf analysis was suitable for ranking varieties for HCN content, the variation in the ratios of leaf to whole-plant HCN would not permit accurate estimation of whole-plant HCN from leaf analysis.
A. H. Cornfield.

Physical environment and symbiotic nitrogen fixation. VI. Nitrogen retention within the nodules of *Trifolium subterraneum* L. VII. Effect of fluctuating root temperature on nitrogen fixation. A. H. GIBSON (*Aust. J. biol. Sci.*, 1969, **22** (4) 829–838. 14 ref.; 839–846. 7 ref.).—VI. The root systems of plants nodulated by *Rhizobium trifolii* strain NA30 possessed a higher %N than those nodulated by the fully effective strain TA1, the difference being due to a greater wt. of nodule tissue on the NA30 nodulated plants and a higher % of non-protein N. At a root temp. of 8°, a higher proportion of the fixed N was retained in the nodule system compared with that for plants grown at 15 or 22°. The best *Rhizobium* symbionts are those strains which not only maintain high rates of fixation per unit dry wt. of nodule tissue but also release the highest proportion of fixed N for use in general plant growth.
VII. The rate of N fixation during normal dark periods was sometimes as high as that during periods of illumination at 30°. Daily exposure to 10° root temp. markedly reduced the overall rate of N fixation, the magnitude being influenced by short temp. and illumination treatment during exposure. The effect was as

great on plants receiving mineral N as on those dependent on symbiotic N fixation.
E. G. Brickell.

Fixation of atmospheric nitrogen by fractions obtained from lupin nodules. A. V. MANORIK and E. P. STARCHENKOV (*Dokl. Akad. Nauk. SSSR.*, 1969, **186** (4), 975–977. Russ., 5 ref.).—Lupin (fastgrowing Kiev) and soya (Amursk yellow 41) were grown under hothouse and normal conditions. Before sowing, the seeds were inoculated with active *Rhizobium* strains. Details are given for processing and fractionating extracts from the roots with attached nodules. Test slurries were 'washed' with Ar and finally incubated in an atm. of Ar 70, N_2 25 (at ^{15}N 75–81), O_2 5% at 23–24° for 40 min. The NH_3 in the incubation mass was finally distilled off and the entrained ^{15}N determined by mass spectrometry. In lupin, the quantity of bound N in the filtrate was 0·27 μg, the bacterioid fraction 0·11, the membrane fraction 0·08 and the sol. fraction zero. For soya, these values were 0·33, 3·05, 0·07 and 0·04, resp. The N-fixing complex of lupin nodules was less sensitive to O_2 than was the soya nodule homogenate.
L. A. Haddock.

Use of surface-active agents in the isolation of enzymes from plant tissues and in their assay. P. S. KRISHNAN, P. N. VISWANATHAN and R. L. MATTOO (*J. scient. ind. Res.*, 1969, **28** (5), 181–189. 122 ref.).—The review covers, (*i*) the action of detergents (*D*) on enzyme proteins and substrate mol., (*ii*) non-ionic and ionic *D*, (*iii*) application of *D* in the solubilisation of bound enzymes and their use in enzyme studies, and (*iv*) analytical aspects arising from the use of surface-active agents, including the interference of *D* in enzyme assay systems. The possible biol. significance of enzyme latency, as established *in vitro* by the use of *D*, is mentioned.
J. N. Ashley.

Germination, Growth Regulation, Senescence

Seed drying and viability in dallis grass, *Paspalum dilatatum*. H. W. BENNETT and W. W. MARCHBANKS (*Agron. J.*, 1969, **61** (2), 175–177. 8 ref.).—Viability of dallis grass seed harvested 21–28 days after the peak of flowering was high irrespective of temp. (38–60°) or time (8–72 h) of drying. Low germination of seed harvested 14 days after peak flowering was due to the presence of immature embryos. Viability of seed dried at 60° was equal to or better than that of seed dried at 38°.
A. H. Cornfield.

Effects of high salinity on germination and emergence of barley, *Hordeum vulgare*. T. J. DONOVAN and A. D. DAY (*Agron. J.*, 1969, **61** (2), 236–238. 9 ref.).—There were considerable differences in the germination of 39 varieties and lines of barley subjected to various salinity levels in both nutrient and soil cultures. Emergence in salinised soil followed the same pattern as germination in salinised soln. Even the relatively highly salt-tolerant types showed delayed emergence at high salinity.
A. H. Cornfield.

Influence of temperature and seed leaching treatment on germination of desert saltbush, *Atriplex polycarpa*. D. R. CORNELIUS and L. O. HYLTON (*Agron. J.*, 1969, **61** (2), 209–211. 3 ref.).—Over the range 5–35°, the highest germination of desert saltbush occurred at 15–20°. Soaking (48 h) and leaching before testing increased germination somewhat. Application of seed leachate to seed during testing decreased germination considerably.
A. H. Cornfield.

Seasonal dormancy in tea (*Camellia sinensis* L.). D. N. BARUA (*Nature, Lond.*, 1969, **224** (5218), 514. 5 ref.).—Evidence indicates that winter dormancy (complete at latitudes beyond ∼ 16°) is the result of short day length, acting through internal plant growth regulators; it cannot be broken by controlled methods of commercial management. Growth under short day conditions, e.g., < 11 h 15 min for at least 6 weeks, was induced by artificial illumination or by treatment with gibberellic acid (10–40 ppm). Increasing the day to 13 h during winter, by weak supplementary lighting during morning and evening, increased shoot growth, accelerated bud break and inhibited flowering.
W. J. Baker.

Other Aspects

Microanalytical methods in some aspects of horticultural chemistry. J. T. MARTIN (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 143–144).
S. S. Chissick.

Relationship between number of independent variables and number of observations in plant analysis calibration studies. W. M. WALKER, S. G. CARMER and T. R. PECK (*Agron. J.*, 1969, **61** (2), 322–324).—Data are presented which support empirically, in terms of poly-

nomial regression and the calibration of plant composition with yield, the suggestion that the no. of data points should be 5–10 times greater than the no. of potential variables in the regression model.   A. H. Cornfield.

Microanalytical methods in the study of the plant surface. P. J. HOLLOWAY (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 144–146. 8 ref.).—Applications of chromatographic techniques to the analysis of plant surface lipids and their deriv. are discussed. T.l.c., g.l.c. and g.c.–mass spectrometry are of particular value.
                                                                                 S. S. Chissick.

Interpretation of chemical plant analyses and control of nutrient status of growing plants exemplified by the tomato plant. B. FRIIS-NIELSEN (*Pl. Soil.*, 1969, **30** (2), 183–209. 25 ref.).—Yields and chemical compositions of various parts of the vegetative tissue and fruit of tomato plants at various growth stages in relation to varying applications of water, N and K were studied.   A. H. Cornfield.

Determination of iron, manganese, zinc and copper in plant material by paper chromatography and reflectance densitometry. R. A. WEBB, D. G. HALLAS and H. M. STEVENS (*Analyst, Lond.*, 1969, **94** (1122), 794–800. 16 ref.).—After destruction of org. matter by dry ashing of a 1-g sample, PO_4^{3-}, K, Ca and Mg were removed in aq. HCl–acetone by retention on a column of Dowex 1×8. The Cu, Fe, Mn and Zn were then eluted with H_2O and the soln. evaporated to dryness. They were separated by descending paper chromatography at $20°$ with BuOH–H_2O–HCl $(100 : 17 : 23)$ as solvent system. The spots were located by prediction from known R_F values or by comparison with standard strip sprayed with the appropriate reagents. After treatment, the spots were submitted to spectrophotometry (element concn. 5–40 μg) or to reflectometry (concn. $\not> 2 \mu g$). Size and shape of spots were generally not critical.   W. J. Baker.

Identification of cholesterol and progesterone in apple seeds. A. M. GAWIENOWSKI and C. C. GIBBS (*Steroids*, 1968, **12** (4), 545–550. 22 ref.).—T.l.c. and g.c. were used and both compd., together with their deriv., were identified. The analysis of 100 g of apple seeds gave 385 μg of cholesterol and 50·0 μg of progesterone.   C. V.

Rapid polarographic determination of chlorine, bromine and iodine in algae. C. CALZOLARI, L. FAVRETTO GABRIELLI and G. PERTOLDI MARLETTA (*Analyst, Lond.*, 1969, **94** (1122), 774–779. 15 ref.).—The dried, pre-treated sample was submitted to combustion at $470°$–$500°$ for $\sim 2\frac{1}{2}$ h, and the residue dissolved in boiling H_2O. The solution was filtered and the filtrate made up to 50 ml. The anodic waves of Cl^- and I^- were then detd., in a 35 ml aliquot, over the range $-0·25$ to $+0·40$V, and $-0·45$ to $+0·20$V resp. (*versus* s.c.e.). After quant. oxidn. of Br^- in a 10-ml aliquot, the cathodic wave of BrO_3^- was recorded from $-1·0$ to $-1·90$V (*versus* s.c.e.). In absence of interfering ions, the accuracy (for *Fucus virsoides*) was within $\sim 5\%$. Results for 6 other species are reported.   W. J. Baker.

The 'Donnan Theory' in development of plant growth media from ion-exchange resins. E. O. SKOGLEY (*Agron. J.*, 1969, **61** (2), 317–322. 20 ref.).—The precision with which the composition of mixed cation-exchange–anion-exchange growth media could be calculated, using the Donnan Theory, to give preselected soln. phase compositions approximating to those in soils or Hoagland's nutrient soln. was studied. Treatment variables were used to test the functioning of the Donnan system in the resin media. Analysis of the exchanger and soln. phases showed that, although differences were found between theoretical and experimental values, the data are considered to support the functioning of the system. Wheat, tomato and sunflower plants grew at about the same rates in soil and resin media, whilst growth in nutrient soln. was slower. The contents of major and trace nutrient cations in resin-grown plants were essentially the same as those in soil-grown plants.
                                                                                 A. H. Cornfield.

Proposal for a method establishing the provenance of seeds of clover, alfalfa [lucerne] and grasses. J. KOVAČEVIĆ (*Bull. scient. Cons. Acads RSF Yougosl.*, A, 1959, **14** (9–10), 299–300. Engl., 9 ref.).—The types of weed seeds present in ten different specimens of red clover were detd. The incidence of *Setaria glauca*, a companion weed of red clover, was assessed numerically. The method used is similar to the phytocoenological methods of vegetation studies.   M. Allsebrook.

Rapid method of determining the hull content of safflower and sunflower seed. I. M. NUR (*Agron. J.*, 1969, **61** (2), 336–338. 3 ref.).—The method is based on separation of the hull from the seed after cutting the seed with a mechanical seed-cutter, which is

described. The method gave results similar to those obtained by separation of hulls after germination of seed in water or 5-vol. H_2O_2.   A. H. Cornfield.

Crops and Cropping

Field Crops

Foliar and soil application of nitrogen to wheat. B. S. MATHUR, P. S. BHATNAGAR and SARDAR SINGH (*Trop. Agric., Trin.*, 1969, **46** (3), 255–259. Engl., 16 ref.).—Field work with urea is reported. N increased the yield of both grain and straw, irrespective of whether it was applied to the soil alone, or as a foliar spray, or in a combination of both methods.   E. G. Brickell.

Autumn chiselling for annual cropping of spring wheat in the intermountain dryland region [U.S.A.]. T. W. MASSEE and F. H. SIDDOWAY (*Agron. J.*, 1969, **61** (2), 177–182. 12 ref.).—Available soil water in a loessial silt loam cropped annually to wheat over 6 yr was higher at seeding time where autumn chiselling (30 cm depth) was practised than in non-chiselled plots. In a wheat–fallow rotation, chiselling increased available soil water to only a small extent over chiselled, annually cropped plots. Average wheat-yields over 6 yr were highest from annually cropped, autumn-chiselled plots and lowest from the wheat–fallow chiselled rotation.
                                                                                 A. H. Cornfield.

Weed control and plant residue maintenance with various tillage treatments in a winter wheat–fallow rotation. C. R. FENSTER, C. E. DOMINGO and O. C. BURNSIDE (*Agron. J.*, 1969, **61** (2), 256–259. 12 ref.).—The effects of different tillage instruments operated at various times during the fallow year on weed control, plant residue maintenance and wheat yields are reported.
                                                                                 A. H. Cornfield.

Increase in yield of wheat treated with Mendok (sodium 2,3-dichloroisobutyrate). H. Y. MOHAN RAM and P. N. RUSTAGI (*Agron. J.*, 1969, **61** (2), 198–201. 15 ref.).—Application of Mendok (250 or 1000 ppm sprays applied 2–3 times starting at the flag leaf stage) decreased plant height and susceptibility to lodging and increased grain yield per plant. Increase in grain yield was due to greater numbers of earing tillers and grains per ear.
                                                                                 A. H. Cornfield.

Influence of water management and fertility on rice growth and yield. E. A. OELKE and K. E. MUELLER (*Agron. J.*, 1969, **61** (2), 227–230. 12 ref.).—A 4-cm depth of water during the growing season gave consistently higher yields of rice than did 4, 8 or fluctuating 4–18-cm depths. Responses to applied N level (67–134 kg/ha) were similar for two varieties under the different water-management treatments. Plant population, active leaf area, panicles per plant and total $N\%$ in shoots and grain were highest with the lowest depth of water, but lodging was greater and straw $N\%$ was lower. The greater no. of panicles per unit area was the main component which contributed to the higher yields under shallow water.   A. H. Cornfield.

Decline in vigour in second generation of double cross hybrid and synthetic maize. GHAZANFAR ALI SHAH (*W. Pakistan J. agric. Res.*, 1968, **6** (4), 24–32. Engl., 19 ref.).—Hybrid 59 deteriorated more in plant height, cob length, cob circumference and grain yield than did synthetic maize, but in no. of kernels per cob and 1000 kernel wt. the reverse was true. Synthetic maize was longer lasting compared to hybrids and could be used for sowing in its second generation.   E. G. Brickell.

Relationships between nitrogen response, plant population and row width on growth and yield of maize. R. NUNEZ and E. KAMPRATH (*Agron. J.*, 1969, **61** (2), 279–282. 18 ref).—Leaf area index (*LAI*) of maize increased, whilst leaf area per plant decreased, with increasing plant population (34,500–69,000 plants/ha). N rates (112–280 kg/ha) and row width (53–109 cm) had no effect on *LAI* or leaf area per plant. Efficiency of a given leaf area to produce grain increased with rate of applied N. Max. grain yields were obtained with 280 kg of applied N and 51,750 plants/ha and with *LAI* of 3·5–4·5.   A. H. Cornfield.

Effect of water table depth and flooding on yield of millet. R. E. WILLIAMSON, C. R. WILLEY and T. N. GRAY (*Agron. J.*, 1969, **61** (2), 310–313. 16 ref.).—Yields of millet in a loam and a fine sandy loam increased with depth of water table (15–76 cm). The $\%$ of N, P and K in the plant were lower with 15–30-cm water table depths than with greater depths. Significant root growth below the 8-cm depth occurred only where the water table was $\geqslant 46$ cm deep. 2 days of flooding 4 weeks before the first harvest reduced

the first harvest yield by 40%, but did not affect the second harvest yield. Flooding at earlier or later dates caused less yield variation.

A. H. Cornfield.

Effect of date of planting, varieties and spacings on yield of potatoes. M. A. SHUJA (*W. Pakistan J. agric. Res.*, 1968, **6** (4), 66–78. Engl., 24 ref.).—A field trial in 1967 is reported. Plantings of Arran Banner and Patroness on Mar. 13 and Apr. 12 gave > 99% germination but planting on May 15 gave significantly reduced population. A spacing of 15 cm, as opposed to 25 and 35 cm, also reduced germination significantly.   E. G. Brickell.

Effect of dates of planting, varieties and spacings on occurrence of internal brown spot in potatoes. M. A. SHUJA (*W. Pakistan J. agric. Res.*, 1969, **7** (1) [(5)?], 32–37. Engl., 9 ref.).—Incidence of disease was markedly decreased by delay in planting, the small tubers being the least affected. Spacing was without affect; Arran Banner was highly susceptible to the disorder but Patroness was almost unaffected.   E. G. Brickell.

Effect of nitrogen, phosphorus and zinc placement on yield and composition of potatoes. P. N. SOLTANPOUR (*Agron. J.*, 1969, **61** (2), 288–289. 16 ref.).—In furrow-irrigated calcareous soils in a semi-arid region, yields of potatoes and uptake of nutrients were much greater where the fertilisers (N with P and/or Zn) were banded beneath the seed than when broadcast and disked-in. Application of N increased the uptake of P and Zn. Application of P or Zn each decreased the uptake of the other, and this occurred even when the two nutrients were applied in separate bands.

A. H. Cornfield.

Sod-seeding and fertilisation for improving permanent pastures. A. M. DECKER, H. J. RETZER, M. L. SARNA and H. D. KERR (*Agron. J.*, 1969, **61** (2), 243–247. 17 ref.).—Sod-seeding permanent bluegrass pasture with crown vetch (I) and birdsfoot trefoil (II) increased average dry matter production during the following 3 yr. Sod-seeding with I was as effective as either complete renovation or application of 140 kg of N per ha annually. II was not as effective as I in improving dry matter yields.

A. H. Cornfield.

Factors affecting carbohydrate reserves of cool season turfgrasses. L. J. ZANONI, L. F. MICHELSON, W. G. COLBY and M. DRAKE (*Agron. J.*, 1969, **61** (2), 195–198. 11 ref.).—Soil temp. and N fertilisation were the most important factors that influenced seasonal fluctuations in total sol. carbohydrate levels of Kentucky bluegrass and three varieties of bentgrass. Bluegrass showed higher levels of sol. carbohydrate than did the bentgrass varieties at all sampling dates.   A. H. Cornfield.

Long-term fertility requirements of Coastal Bermuda grass, *Cynodon dactylon*. II. Nitrogen, phosphorus and lime. W. W. WOODHOUSE, JUN. (*Agron. J.*, 1969, **61** (2), 251–256. 12 ref.).— Yields of Coastal Bermuda grass over 11 yr on a sandy soil were increased by 45 kg (hay) per kg of N applied for rates up to 224 kg/ha/yr. Up to this rate, recovery of applied N in the forage ranged from 75 to 90%, but declined rapidly with higher N rates. There was little further yield response to N with application of up to 672 kg/ha, probably due to considerable reduction in soil pH in the lower profile. Liming (1120 kg of dolomitic limestone per ha) after 11 yr of cropping doubled forage yields. Forage yields were very low where no P was applied. An initial application of 49 kg of P/ha followed by 25 kg/yr was adequate for maintaining max. yields.   A. H. Cornfield.

Fertiliser studies on Pangola grass (*Digitaria decumbens* Stent.) in Trinidad. I. Description of experiments and effect of nitrogen. II. Effect of phosphorus, potassium and magnesium. N. AHMED, L. I. TULLOCH-REID and C. E. DAVIS (*Trop. Agric., Trin.*, 1969, **46** (3), 173–178. 14 ref. 179–186. 5 ref. Engl.).—In the absence of N, harvest was possible only at the fourth month of the expt. An N content of $2 \cdot 12 \pm 0 \cdot 17\%$, and a fertiliser [(NH₄)₂SO₄] rate of 80 lb of N/acre, for one month's growth at adequate soil moisture level, gave the highest yield with the risk, however, of increasing soil acidity to dangerous levels.

II. P up to 120 lb/acre had no significant effect on yield or P content of the grass. K at 120 lb/acre did not increase yield but did raise K content, and a content of $2 \cdot 23\%$ was associated with the largest yield response from 20 lb/acre of K. Mg applications up to 100 lb/acre increased neither yield nor Mg uptake. Neither P nor Mg fertilisation is therefore recommended on River Estate Loam.

E. G. Brickell.

Daily variation in carbohydrate content of forage crops. D. A. HOLT and A. R. HILST (*Agron. J.*, 1969, **61** (2), 239–242. 11 ref.).— The water-sol. carbohydrate content of lucerne increased from

6 a.m. to noon, but did not change to 6 p.m., whilst in bluegrass, bromegrass and tall fescue it increased from 6 a.m. to 6 p.m. Non-structural ($0 \cdot 2$N-H₂SO₄-sol. minus water-sol.) carbohydrate content in lucerne increased to a limited extent to noon and to a greater extent to 6 p.m., whilst in the grasses it increased linearly from 6 a.m. to 6 p.m. Water-sol. carbohydrate contents of lucerne and bromegrass were significantly lower under high than under low K nutrition.   A. H. Cornfield.

Factors affecting the alkaloid content of tall fescue, *Festuca arundinacea* Schreb. C. E. GENTRY, R. A. CHAPMAN, L. HENSON and R. C. BUCKNER (*Agron. J.*, 1969, **61** (2), 313–316. 9 ref.).— No alkaloids were found in the seed of three varieties of tall fescue, whilst three alkaloids were found in the seed of two other varieties. Alkaloid content differed between varieties and between different plant parts, and was higher at the pasture than at the hay stage. Application of P + K had little effect whilst application of N + P + K increased plant alkaloid content. In the mature plant, alkaloid content was higher in the leaf than in the stem.

A. H. Cornfield.

Effects of age, plant spacing, and other variables on growth, yield, and fibre quality of kenaf, *Hibiscus cannabinus* L. F. D. WILSON and J. F. JOYNER (*Tech. Bull. U.S. Dep. Agric. Res. Serv.*, 1969, (1404), 19 pp. 10 ref.).—Field trials at Lake Worth, Fla. in 1962 are reported. Differences in plant spacing had no significant effect on height (H), fibre yield (FY), or fibre quality; H, FY and stem dia. (SD) increased with age as expected. SD was lowest in the top section and highest in the basal section, as expected, but % fibre and FY were highest in the middle section. Tensile strength and tex were highest in the top section. Yields estimated from a combination of basal + top and middle + top regression equations agreed most closely with actual yields of all three sections.

E. G. Brickell.

Plant population and yield in Desi cotton. M. SIRAJUD-DIN KHAN and RAHMAT ALI SAROYA (*W. Pakistan J. agric. Res.*, 1968, **6** (4), 33–44. Engl., 8 ref.).—A short and coarse-linted variety of Desi cotton (*Gossypium arborium*), namely 231 Rosea, was studied. 9 in × 2 ft spacings (29,040 plants per acre) and two plants per hole gave the best average yield.   E. G. Brickell.

Water use by cotton from low and moderately saline static water tables. L. N. NAMKEN, C. L. WIEGAND and R. G. BROWN (*Agron. J.*, 1969, **61** (2), 305–310. 11 ref.).—Lysimeter studies showed that water tables at 91, 183 and 274-cm depths contributed 54, 26, and 17%, resp., of the total water use under cotton with high moisture treatment, and 61, 49, and 39%, resp., with low moisture treatment. Water use from the 274-cm water table decreased with increasing salinity level in the 183–274-cm zone.   A. H. Cornfield.

Horticultural Crops

Nutrition of nursery apple trees. C. M. RITTER (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 99–105. 3 ref.).—On a silty clay loam (pH $7 \cdot 2$) which had a wheat cover crop [fertilised with 300 lb/acre of 5–10–10 (N-P₂O₅-K₂O)] ploughed-in in May just before 1-yr-old apple trees were planted, further application of NPK or NH₄NO₃ did not improve nursery tree quality, irrespective of the rootstock used. The extra fertiliser treatments resulted in less fibrous root systems than in the control, whilst NH₄NO₃ prevented bud growth on EM 26 and MM 104 stocks. Scions on seedling stock were more vigorous than those on other stocks, regardless of fertiliser treatment.   A. H. Cornfield.

Influence of succinic acid mono(2,2-dimethylhydrazide) on growth, productivity, mineral nutrition, and quality of apples. F. W. SOUTHWICK, W. J. LORD and W. D. WEEKS (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 71–81. 20 ref.).—The rate of growth of fruit of McIntosh apples decreased with level of application of Alar (1000–5000 ppm sprays) and with increasing earliness of application (mid-June to mid-Aug.). Annual single spray treatments in mid-July to mid-Aug. over 2 yr had little effect on foliar levels of major and trace elements, fruit set, total yield or red colour of the fruit, but decreased pre-harvest drop and rate of flesh softening at harvest. The effect of Alar treatment on disease development during storage is also reported.   A. H. Cornfield.

Effectiveness of thinning sprays as related to fruit size at time of spray application. L. P. BATJER, C. G. FORSHEY and M. B. HOFFMAN (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 50–54. 13 ref.).—The extent of thinning in four varieties of apple at three locations by application of naphthaleneacetic acid or Sevin (carbaryl, 1-naphthyl N-methylcarbamate) was not related to fruit size (5–16 mm) at time of spraying.   A. H. Cornfield.

Relationship of date of application and size of fruit to the effectiveness of naphthaleneacetic acid for thinning apples. C. W. DONOHO, JUN. (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 55–62. 17 ref.).—Over 3 yr, NAA (10 ppm spray) was most effective in reducing the set of 'Jonathan' apple fruit when applied at petal fall or when the largest fruit reached 15–18 mm in length. No significant thinning occurred if the treatment was given when the length was $\geqslant$ 19 mm. Since rate of fruit elongation varied from year to year, application of NAA when fruit reached a particular length rather than after a particular time period from blooming would be the more useful index for timing the spray application. A. H. Cornfield.

Capsaicin content of capsicum fruits at different stages of maturity. S. I. BALBAA, M. S. KARAWYA and A. N. GIRGIS (*Lloydia*, 1968, **31** (3) 272–274).—Growth rate and development are generally faster in summer than in autumn plants; flowering is $\sim$ 6 weeks earlier and fruiting $\sim$ 1 week earlier. Wt. of mature fruits does not differ greatly, being $\sim$ 45% (av. of both crops) heavier in *C. minimum* than in *C. frutescens*. Colour begins to change 3 weeks after fertilisation from green to yellow green in both series. Pungency, detected by taste, commences in $\sim$ 4 weeks in summer and 5 weeks in autumn plants. Capsaicin is slightly higher in the fruits of the former than in the latter. C. V.

Phosphorus fertilisation of hops. L. C. BOAWN and P. E. FAS-MUSSEN (*Agron. J.*, 1969, **61** (2), 211–214. 8 ref.).—Application of P [112–1344 kg/ha spread over 3 yr to a fine sandy loam (pH 7·2)] increased the % of P in leaves and cones, but had no effect on fresh vine wt.; % of α-acid in the cones tended to decrease as the level of P fertilisation increased. The extent of the decrease in % of Zn in leaves and cones due to increasing P application was less when Zn-EDTA than when $ZnSO_4$ was applied. No visual Zn deficiency symptoms occurred even at the highest rate of P application. A. H. Cornfield.

Effect of gibberellin on almond flower bud growth, time of bloom and yield. J. R. HICKS and J. C. CRANE (*Proc. Am. Soc. hort. Sci.*, 1968, **92**, 1–6. 6 ref.).—Spray application of 100–200 ppm of gibberellin before or after flower bud differentiation (Aug. or Sept.) had no effect on differentiation, but drastically retarded development, which was followed by bud abscission, delay in blooming and reduction in yields the following year. A. H. Cornfield.

Manuring and spacing experiments on vegetables. A. B. WEBSTER (*N.Z. Jl agric. Res.*, 1969, **12** (2), 381–416. 21 ref.).—Responses of lettuce, leeks, beetroot, carrots, pole beans and cabbage to five levels of $(NH_4)_2SO_4$, serpentine superphosphate, and of K_2SO_4, were detd. annually for 3 yr, together with row spacings. Responses were detd. by statistical treatment of results. E. G. Brickell.

Spinach cation and oxalate contents and their interactions as influenced by fertilisation. V. N. LAMBETH, W. S. REGAN, J. R. BROWN and D. G. BLEVINS (*Fd Technol., Champaign*, 1969, **23** (7), 937–941. 18 ref.).—A field expt. which included 24 combinations of Ca, P, K and N, was conducted to study the effect of nutrient inter-relationships on spinach quality, as detd. by cation content, oxalates and cation–oxalate equivalency. Low spinach quality, indicated by large excesses of sol. oxalates, may result from low P, high K, high N level imbalances. I. Dickinson.

Plantation Crops

Effect of age of planting material on growth and production of Basrai banana (*Musa cavendishii* L.). SYED AHMED PASHA JAGIRDAR and AMIR HUSSAIN (*W. Pakistan J. agric. Res.*, 1968, 6 (4), 60–65. Engl., 5 ref.).—No significant differences were found in growth and production of Basrai banana raised from suckers between 1½ and 3½ months old; the latter gave better yield under conditions of severe frost. E. G. Brickell.

Determination of the state of ripeness of bananas by colorimetry. M. GOTTREICH, N. TEMKIN-GORODEISKI, A. PELED *et al.* (*Trop. Agric., Trin.*, 1969, 46 (3), 239–245. Engl., 12 ref.).—A modification of Sumner's method (*J. biol. Chem.*, 1924, **47**, 5–9) is described, extraction and reaction being combined into one operation. The fruit sample was placed directly in Sumner's reagent [$FeNH_4(SO_4)_2$ and dinitrosalicylic acid] and kept on a boiling water bath for 5 min. The reaction colour was compared with standards. Results correlated satisfactorily with respiration rates. E. G. Brickell.

Extent of economical ratooning of sugar-cane as compared to planted crop. MOHAMMAD IBRAHIM KHAN, GHULAM HASSAN and NAZAR HUSSAIN (*W. Pakistan J. agric. Res.*, 1969, **7** (1) [(5)?], 28–31. Engl., 5 ref.).—Data from a 2-yr study show that it is economical to have two ratoons from the yield. E. G. Brickell.

Forest Crops

No abstracts.

General Aspects

Effect of maize steep liquor for erosion control and vegetative establishment on highway backslopes. B. L. SCHMIDT, G. S. TAYLOR and R. W. MILLER (*Agron. J.*, 1969, **61** (2), 214–217. 7 ref.).—Although application of maize steep liquor slurry followed by a CaO slurry formed a thin, stabilised surface layer on backslope plots after seeding and helped to reduce initial erosion, the treatment was not as effective as a straw mulch in improving cover, plant growth and erosion control. The slurry treatment retarded germination of tall fescue up to 1 week and did not decrease evaporation of soil moisture. A. H. Cornfield.

Mathematical models in soil productivity studies, exemplified by the response to nitrogen. W. C. VISSER (*Pl. Soil*, 1969, **30** (2), 161–182. 4 ref.).—A mathematical model relating crop yields to favourable and adverse growth factors and their interactions is presented. The model is used to analyse yield data over 6 yr in which grass received varying levels of N, P and K. A. H. Cornfield.

Ecology of fire in grasslands. R. DAUBENMIRE (*Adv. ecol. Res.*, 1968, **5**, 209–266. 214 ref.).—The heat generated as herbaceous cover burns is discussed. Bush fires (those moving against the wind) are hotter and produce max. temp. near the ground. Soil surface temp. (measured in African Savannas) can be 720° but elsewhere in grasslands are $\gg$ 100°, a level much lower than in forest fires, From the surface down, the temp. gradient is so steep that direct alterations of soil structures or chemistry by fire is inconsequential in grasslands. The subsequent results are reviewed (N content, pH, etc.). Protein and mineral contents of post-burn grass shoots are higher, therefore such herbage is more nutritious for animals. Seedlings often appear in abnormal numbers after a grassland fire. C. V.

Increasing the sugar content of sugar-containing crops. E. I. DU PONT DE NEMOURS & CO. (Br. Pat. 1,161,189, 10.7.68. U.S., 31.7.67, 20.5.68).—0·25–25 kg/ha of an amino-oxyacid $OY(CHR)_n \cdot CHRX$ is applied to the crop 10–60 days prior to harvest, wherein n is 0 or 1; R is H, Me, or Et; X is CN, CO_2M, $CONR^IR^{II}$, $CO \cdot Z \cdot R^{III}$ or $CONR^{IV} \cdot O \cdot CHR \cdot CO_2R^{III}$ [M is H, alkali metal, or (substituted) NH_4; R^I-R^{II} are H, alkyl of 1–3 C, or R^{II} is pyrimidyl which may contain 1–2 Me, C_{2-3} cyanoalkyl, C_{3-5} alkoxyalkyl, or Ph (optionally substituted); Z is O or S; R^{III} is alkyl of 1–6C, alkenyl of 3–6 C, or (substituted) Ph; R^{IV} is H, alkyl of 1–4 C, Ph or halogenophenyl; Y is phthalimido, 1,2,3,6-tetra- or -hexa-hydrophthalimido, maleimido, succinimido, norborn-5-ene-2,3-dicarbonimido, or NR^VR^{VI}; R^V is as R^{IV}; and R^{VI} is H, alkyl of 1–4 C, or COR^{VII}; R^{VII} is H or various (substituted) alkyl, alkenyl, alkynyl, alkoxy, or Ph]. An example is $CO_2Me \cdot CH_2ONH_2$. Over 50 formulations are exemplified, and > 250 effective compounds are listed. F. R. Basford.

Organothiophosphates and their use as plant growth-promoters. NIPPON KAYAKU K.K. (Br. Pat. 1,165,846, 30.6.67. Jap., 1.7.66).—Esters of formula p-$SO_2R^{IV} \cdot R^{III} \cdot O \cdot PS(OR^I)R^{II}$ promote growth of plants, esp. cereals, potatoes and sweetpotatoes [R^{III} is benzene residue optionally further substituted by F, Cl, Br or Me; R and R^I are alkyl of 1–5 C; R^{II} is OR, NHR or NR_2; R^{IV} is OR, allyloxy, SR, allylthio, OR-substituted-Et,-OEt or -SEt, $X \cdot C_6H_{4-n} Y_n$ (X is O or S; n is 1–5; Y is H, F, Cl, Br, R, OR, SR, CNS or NCS), halogen, or $NR^VR^{VI}(R^V$–R^{VI} are H, R^I, or R^{VI} is alkyl or $C_6H_{5-n} Y_n$)]. An example is o-chloro-p-(p-anisyloxysulphonyl)phenyl Me_2 thionophosphate (prepn. described). F. R. Basford.

Protection of growing plants. N. V. HOLLANDSCHE DRAAD- EN KABELFABRIEK LTD. (Inventor: J. P. PIJST) (Br. Pat. 1,151,481, 28.3.68).—Growing seeds, bulbs or tubers are protected by covering the soil with a sheet of air- and water-permeable plastic material (e.g., a foamed polyurethane) which disintegrates under the influence of sunlight. S. S. Chissick.

Plant seed testing and sorting. G. LAUKIEN (Br. Pat. 1,151,988, 4.3.68. Switz., 3.3.67).—Seeds are non-destructively tested and sorted in an apparatus that automatically weighs one seed at a time, automatically determines the wt. of a given substance in this seed by a n.m.r. device and then calculates the specific content so that the seed may be sorted into the appropriate group. (4 drawings.)
S. D. Huggins.

Animal Husbandry

Feedstuffs

Nutritive value of corn [maize] silages treated with chemical additives for lactation. W. G. SCHMUTZ, L. D. BROWN and J. W. THOMAS (*J. Dairy Sci.*, 1969, **52** (9), 1408–1412. 16 ref.).—The nutritive value of maize silage containing added limestone (I), urea (II) or $(NH_4)_2HPO_4$ (III) or a combination of I with either II or III was investigated. II and III significantly increased the crude protein equiv. of the silage and the org. acid concn. of the silage was increased by all additives. Trials with lactating cows indicated that the nutritive value of the silage was not increased by the addition of I and III. The addition of $0\cdot5\%$ of II to silage resulted in some increase in nutritive value.
M. O'Leary.

Accumulation of fluoride by forage crops. D. C. MACLEAN, R. E. SCHNEIDER and L. H. WEINSTEIN (*Contr. Boyce Thompson Inst. Pl. Res.*, 1969, **24** (7), 165–166. 5 ref.).—Field trials with *Phleum pratense* L. var. 'Climax' and *Trifolium pratense* L. showed that F accumulation by forage is a poor indicator of fluoride pollution except when used for the protection of livestock or for the confirmation of suspected fluoride-induced foliar symptoms. Vegetation analyses cannot replace air monitoring since they do not indicate the duration, frequency, or concn. of air-borne fluoride.
E. G. Brickell.

Some methods in use in the study of the utilisation of protein by animals. R. A. EVANS (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 148–149).—The methods discussed are free and total amino acid analysis, and discrepancies in N retention, measured in two different ways.
S. S. Chissick.

Residual effect of feeding grain to grazing steers on the productivity of pasture. S. S. BENACCHIO, G. O. MOTT, D. A. HUBER and M. F. BAUMGARDNER (*Agron. J.*, 1969, **61** (2), 271–274. 14 ref.).—The residual effects on pasture productivity of feeding grain (4300–57,900 kg/ha) to grazing steers over the 4 previous yr, with no supplements being supplied during the test yr, were studied. For each 10,000 kg/ha of grain which had been supplied in the previous 4 yr, forage dry matter was increased by 224 kg, crude protein by 19 kg, carrying capacity by 53 steer days, and estimated total digestible nutrients consumed by 145 kg/ha.
A. H. Cornfield.

Effects of Diet and Environment on Livestock

Oral administration of Supracide to lactating cows: effect on consumption, production, ration utilisation, and residue levels in milk and certain tissues. C. E. POLAN, J. T. HUBER, C. N. MILLER and R. A. SANDY (*J. Dairy Sci.*, 1969, **52** (9), 1384–1387. 12 ref.).—The oral adminstration to dairy cattle of up to 30 ppm (of total dry feed consumed) of the organosphosphate insecticide GS-13005, Supracide, S-[(2-methoxy-5-oxo-$\triangle^2$-1,3,4-thiadiazolin-4-yl)methyl] O,O-dimethylphosphorodithioate, had no effect on voluntary hay or total feed consumption, milk production, blood cholinesterase, transaminase activity or digestibility of proximate components. Supracide and its analogue were not detected in the milk, urine or faeces at sensitivities of $0\cdot005$ and $0\cdot025$ ppm, resp. Pesticide residues were not detected in several samples of tissue analysed.
M. O'Leary.

Response of lactating cows to two levels of mill-run blackstrap molasses from cane grown on organic soil. J. M. WING and G. W. POWELL (*J. Dairy Sci.*, 1969, **52** (9), 1413–1414. 5 ref.).—Though the addition of $4\cdot2$ and $12\cdot6\%$ of mill-run molasses to high-concentrate rations resulted in depression of milk production and fat and *SNF* contents, the use of molasses is considered to be feasible when its low cost offsets the drop in production.
M. O'Leary.

Drylot feeding versus pasturing for milk production in dairy cattle. A. L. SENDAGI, R. H. KLIEWER, F. B. WOLBERG, *et al.* (*J. Dairy Sci.*, 1969, **52** (9), 1404–1407. 10 ref.).—The results of trials with 98 cows over a 4-yr period showed that animals adequately fed in drylot produced as much milk, *SNF* and protein as those on first

class grass–legume pastures. Reproductive performance and health of the cows were not adversely affected by drylot feeding.
M. O'Leary.

Effects of dietary palmitic and stearic acids on milk yield and composition in the cow. W. STEELE (*J. Dairy Res.*, 1969, **36** (3), 369–373. 10 ref.).—Expt. with 12 cows in mid-lactation showed that the isocaloric replacement of concentrate starch by 5% of stearic acid (I) resulted in increased yields of milk, milk fat, *SNF* and lactose, and in reduction of milk protein content. With 10% of I there was increase only in milk yield and reduction in the contents, but not in the yields, of *SNF* and protein. 10% of palmitic acid (II) reduced the contents of *SNF* and protein in the milk but increased yields of milk, milk fat and lactose, and increased milk fat content. Neither I nor II affected blood glucose concn.
M. O'Leary.

Effects of dietary palmitic and stearic acids on milk fat composition in the cow. R. C. NOBLE, W. STEELE and J. H. MOORE (*J. Dairy Res.*, 1969, **36** (3), 375–381. 16 ref.).—The inclusion of 5 or 10% of stearic acid in the concentrate mixture fed to cows, decreased the concn. and yields of 10 : 0, 12 : 0, 14 : 0, 14 : 1, 16 : 0 and 16 : 1 acids in the milk fat, and increased those of 18 : 0 and 18 : 1. 10% of palmitic acid decreased the concn. of 6 : 0, 8 : 0, 10 : 0, 12 : 0, 14 : 0, 14 : 1, 18 : 0 and 18 : 2 acids and increased those of 16 : 0 and 16 : 1. This treatment reduced yields of 6 : 0, 10 : 0, 12 : 0, 14 : 0 and 18 : 2 and increased yields of 4 : 0, 16 : 0, and 16 : 1 acids. 5% of stearic and 10% of palmitic acid both increased the yield of total milk fat.
M. O'Leary.

Influence of nitrogen source and fibre levels in the ration on the freezing point and chloride content of cow's milk. G. M. GIKONYO and D. H. KLEYN (*J. Dairy Sci.*, 1969, **52** (9), 1379–1383. 21 ref.).—The effects of rations containing five levels of urea (0, $2\cdot15$, $7\cdot55$, $12\cdot70$ and $15\cdot00$ kg/1000 kg) and five levels of fibre (11, 12, 15, 16 and 19%) on the f.p. and chloride content of cow's milk were determined. Urea content had no significant effect on f.p. depression and only a slightly significant effect on chloride content. Fibre content had a significant effect on both f.p. depression and chloride content.
M. O'Leary.

Effects on fatty acid compositions of lipids in cow's milk [arising from] grass and legume feeds. I–IV. T. SAITO, S. TAKADAMA, H. KASUGA and T. NAKANISHI (*Jap. J. Dairy Sci.*, 1969, **18** (1), A26–A36, 14 ref.; (2), A61–A66; (3), A78–A85. 18 ref.; (4), A126–A134. 10 ref. Jap.).—Engl. summ.
C. V.

Low-fat milk phenomenon in cows grazing pearl millet pastures. H. F. BUCHOLTZ, C. L. DAVIS, D. L. PALMQUIST and K. A. KENDALL (*J. Dairy Sci.*, 1969, **52** (9), 1388–1394. 14 ref.).—Sudan grass (I) and pearl millet (II) were compared with respect to chemical composition and animal performance. No significant differences were detected in cell wall constituents and lipid composition, but the oxalic acid content of II was significantly higher than that of I. Cows grazed on II produced milk significantly lower in fat, and the fat was more unsaturated, than that from animals on I. Grazing on II had no significant effect on the molar proportions of rumen acetate and propionate.
M. O'Leary.

Winter decline in the solids-not-fat content of herd bulk-milk supplies. J. A. WRIGHT, J. A. F. ROOK and J. J. PANES (*J. Dairy Res.*, 1969, **36** (3), 399–407. 15 ref.).—Three of four Yorkshire Friesian herds, surveyed from Oct. 1967 to May 1968, showed a decline in *SNF* content during the winter feeding period. Milk protein content (*MPC*) was at a min. in Jan.–Feb. but lactose content also declined and in two of the herds this was the major cause of the fall in *SNF*. The lactose decline was partly due to an advance in lactation and partly to an increase in the incidence of mastitis. In three of the herds, autumn-calving animals showed a marked fall in *MPC* in early lactation which was not observed in summer-calving animals. No increase in *SNF* or *MPC* was achieved by feeding of supplementary concentrates towards the end of the winter but the commencement of grazing in the spring resulted in an immediate increase in both these milk constituents.
M. O'Leary.

Studies on the origin of milk fat. A further study of bovine serum lipoproteins and an estimation of their contribution to milk fat. C. BISHOP, T. DAVIES, R. F. GLASCOCK and V. A. WELCH (*Biochem. J.*, 1969, **113** (4), 629–633. 23 ref.).—When [^{3}H]-palmitic acid combined in olive oil triglycerides was introduced into the rumen of a lactating cow, the specific radioactivity of the triglyceride fraction of the lipoproteins, pptd. by dextran sulphate, reached a max. both earlier and greater than that of the milk fat. This

fraction is the main circulating lipid precursor of milk fat; its contribution is calculated to be 36%. Since the milk fat contained 39% of C_{16} fatty acids and 32% of C_{18} fatty acids, appreciable amounts of these must have been derived from a source other than preformed circulating lipids, probably from acetate in the udder.

J. N. Ashley.

Effects of sodium sulphate and gluten supplements on the intake and digestibility of a mixture of spear grass and Townsville lucerne hay by sheep. M. J. PAYNE (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (39), 393–399. 23 ref.).—Gluten was used as protein and Na_2SO_4 as sulphur source in low quality forage; the supplements were used either singly or together. Increased intake and digestibility of feed in gluten-fed animals supports previous work. The intake of digestible dry matter increased by 62% with addn. of 14 g of Na_2SO_4 (in 45 ml of water) per day per animal. It is possible that the additional S available resulted in better utilisation of the N present. All sheep given supplements gained wt. whereas controls lost wt.   M. T. Rawnsley.

Comparison of the effects of crown vetch (*Coronilla varia*) and lucerne hays on the live-weight gain of sheep. P. J. REYNOLDS, C. J. JACKSON, JUN. and P. R. HENSON (*Agron. J.*, 1969, **61** (2), 187–190. 17 ref.).—Early cut crown vetch (I) and lucerne hays (II), which had similar chemical compositions, were compared in a 70-day feeding experiment using 6-month-old wethers, with the forages fed *ad lib.* in either pelleted or ground form. Live-wt. gain was greater on II than on I, and greater on pelleted than on ground forage. Consumption of pelleted forage was considerably greater than that of ground forage. Digestibility of I was less than that of II.   A. H. Cornfield.

Effects of 'marginal' deficiencies of copper and selenium on growth and productivity of sheep. M. K. HILL, S. D. WALKER and A. G. TAYLOR (*N.Z. Jl agric. Res.*, 1969, **12** (2), 261–270. 32 ref.).—Se (11·96 mg of anhyd. Na_2SeO_4; orally initially and at monthly intervals) induced highly significant improvements of 5% in hogget live-wt., 8% in hogget fleece wt., 2% in 2-tooth live-wt., and 5% in 2-tooth fleece wt. Cu (568 mg of $CuSO_4.5H_2O$) induced corresponding changes of −2, −5, −1 and 0%, resp. Cu + Se (at the above rates) induced highly significant improvements of 10% in hogget live-wt., 12% in hogget fleece wt., 5% in 2-tooth live-wt., 11% in 2-tooth fleece wt., and 10% in 2-tooth twinning rate. Significant ($P < 0.05$) Se–Cu interactions occurred in which live-wt., fleece wt. and fecundity responses were induced by Cu therapy, but only in Se-treated sheep. Animals were 5% heavier and clipped 4% more wool than hoggets, and were 3% heavier, clipped 6% more wool and produced more twins than 2-tooths.

E. G. Brickell.

Evaluation of formaldehyde-treated casein for wool growth and nitrogen retention. P. J. REIS and D. A. TUNKS (*Aust. J. agric. Res.*, 1969, **20** (4), 775–781. 8 ref.).—Untreated casein in the diet was inferior to untreated casein per abomasum and formaldehyde-treated casein in the diet for all parameters studied. Average increase in wool growth was 62% for both types of supplement; N retained was about 3 g/day, of which more than half was in wool. The treated casein was 90% digestible. E. G. Brickell.

Partial replacement of soyabean meal by amino acids in pig rations based on wheat and sorghum. R. M. BEAMES and P. M. PEPPER (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (39), 400–407. 21 ref.).—In five expt., L-lysine hydrochloride, with or without DL-methionine (98% strength), was used to replace half the 15% soyabean meal content of the pig rations (grain). In each case it resulted in poor performance of pigs, esp. young pigs. Sorghum grain + lysine was not as good as wheat + lysine.   M. T. Rawnsley.

Nutritional evaluation of diets containing meat meal for growing pigs. II. Effect of level of meat meal or meat and bone meal in wheat-based diets. E. S. BATTERHAM and J. M. HOLDER (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (39), 408–412. 10 ref.).—Results showed that the response to increasing dietary levels of meat meal and meat-and-bone meal vary according to the Ca and protein content of the meal. Leaner hams were produced in diets with 20% meat, or meat-and-bone meal, indicating that N tends to be retained at this level. The 20% level seems suitable when used with a wheat diet (restrictive rate) for the 40–160 lb growth range.

M. T. Rawnsley.

Factors affecting the chick's requirement for phosphorus. J. C. FRITZ, T. ROBERTS, J. W. BOEHNE and E. L. HOVE (*Poult. Sci.*, 1969, **48** (1), 307–320. 45 ref.).—Increasing levels of vitamin D (150–3000 ICU/kg) in the feed improved chick performance on a sub-optimum level (0·15% P in the diet) of NaH_2PO_4 and on all

levels (0·15–0·55% of P) of Ca phytate and soft rock phosphate. Growth and calcification were good with dietary Ca : P ratios of 1·0–2·5 where NaH_2PO_4 was used, but was best at a ratio of ∼ 1·0 where soft rock phosphate, Curaçao phosphate and $Ca_3(PO_4)_2$ were used. Similar relative biol. values were obtained with White Plymouth Rock (I) and White Leghorn chicks, except that on low-P diets, severe rickets developed in I. Similar relative biol. values were obtained with most sources of P by use of either calcification data or growth data, except that some of the less sol. forms gave unexplained growth depressions which were largely overcome as the chicks grew older. When NaH_2PO_4 was used with a practical diet, chicks required 0·45–0·50% of total P for max. growth during the first 3 weeks, and 0·65% of total P for max. calcification.

A. H. Cornfield.

Influence of dietary potassium on chick growth, food consumption and blood and tissue composition. K. E. RINEHART, W. R. FEATHERSTON and J. C. ROGLER (*Poult. Sci.*, 1969, **48** (1), 320–325. 14 ref.).—Decreases in feed consumption and wt. gains occurred within 24 h when 1-day-old chicks were put on a K-deficient diet (450 ppm K) compared with those receiving a normal diet (4000 ppm K). The differences increased with time. Plasma and skeletal muscle K decreased in K-deficient chicks; red blood cell, liver and heart muscle K were unaffected, and plasma total protein was increased.   A. H. Cornfield.

Interaction of rubidium and potassium in chick diets. L. B. SASSER, E. W. KIENHOLZ and G. M. WARD (*Poult. Sci.*, 1969, **48** (1), 114–118. 12 ref.).—Chicks made poor growth on a K-deficient diet (0·083% of K^+). Addition of up to 0·2% of Rb^+ to a K-deficient diet improved wt. gains to 4 weeks, but resulted in some mortality. 0·4% of Rb^+ caused complete mortality by the end of the second week. Addition of 0·2% of Rb^+ to a K-adequate diet (0·2% of K^+) had no effect on wt. gains, whilst 0·4% of Rb^+ caused no toxicity symptoms until the end of the second week. The Rb content in thigh, breast and liver tissue increased with the Rb level of the diet to a greater extent when the K-deficient than when the K-adequate diet was supplied.   A. H. Cornfield.

Haematological response of different stocks of chickens to iron-copper-deficient diets. K. W. WASHBURN (*Poult. Sci.*, 1969, **48** (1), 204–209. 10 ref.).—There were highly significant differences in packed erythrocyte vol. among four stocks of chickens when either adequate or marginally deficient levels of Fe and Cu were supplied. Texas-50 Leghorns were more responsive to the effects of Fe–Cu deficiency than were the other stocks.   A. H. Cornfield.

Soyabean oil *versus* rapeseed oil in turkey starter diets. R. E. SALMON (*Poult. Sci.*, 1969, **48** (1), 87–93. 16 ref.).—The addition of up to 6% of rapeseed oil (I), replacing an equal % of soyabean oil (II), in a 9% II–32% protein diet had no effect on wt. gains of poults to 41 days of age, but complete replacement of II by I decreased wt. gains. 7–8% of I gave satisfactory results providing 1–2% of beef tallow was also added. Feed efficiency was decreased by the higher levels of I to 14 days of age, but not in the later growth periods. The presence of I in the diet resulted in deposition of high levels of erucic and eicosenoic acids in the body fat and also caused a decrease in linolenic and increases in stearic and oleic acids.   A. H. Cornfield.

Effect of diet and breed of chicken on the metabolic efficiency of nitrogen and energy utilisation. J. J. BEGIN (*Poult. Sci.*, 1969, **48** (1), 48–54. 13 ref.).—Efficiency of N utilisation when a maize-soyabean meal diet was fed from 1 to 3 weeks of age was not significantly different among three breeds of chicken, but efficiency of metabolisable energy utilisation was lower for New Hampshires than for White Plymouth Rocks or White Leghorns. When the diet had 66% of the calories supplied by maize oil or cerelose, efficiency of both N and energy utilisation was higher for White Plymouth Rocks than for the two other breeds. There were no significant differences in any breed between the use of cerelose or maize oil.   A. H. Cornfield.

Protein and sulphur-amino acid requirement of the laying hen as influenced by dietary formulation. R. H. HARMS and B. L. DAMRON (*Poult. Sci.*, 1969, **48** (1), 144–149. 8 ref.).—The hen required 0·25–0·28 g of methionine daily, provided it was supplied with a total of 0·53 g of S-amino acids. This requirement was met by a level of 0·268% of methionine and 0·533% of total S-amino acids in a diet containing 2887 kcal of metabolisable energy per kg.

A. H. Cornfield.

Cottonseed meal as a source of protein in diets for laying hens. D. G. SAVILLE, L. SMITH and P. NICHOLLS (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (39), 314–416. 14 ref.).—The influence of

cottonseed meal (I) on egg production, egg quality and hatchability, was studied together with the effects of FeSO₄ (II) and lysine. The cottonseed meal contained 0.041% of free gossypol, which is known to cause depression in egg production. Results (unlike those of previous work) showed that even low levels of I depress egg production and hatchability. This could be partly offset by addn. of II.   M. T. Rawnsley.

Effect of protein level on carcass composition of turkeys. E. G. BIXLER, G. F. COMBS and C. S. SHAFFNER (*Poult. Sci.*, 1969, **48** (1), 261–266. 13 ref.).—Poults fed low-protein diets (20.2% during 2–4 weeks, decreasing to 17.5% during 6–8 weeks of age) showed lower wt. gains and feed efficiency than did those fed adequate protein (27.4% during 2–4 weeks, decreasing to 23.7% during 6–8 weeks of age). The treatment resulted in increased body fat and decreased body protein and water at 4 weeks of age. Differences due to protein level in the performance of fast- and slow-growing families suggest that genetic selection, based on rate of gain of poults fed a low-protein diet, may be useful in improving the ability of turkeys to fatten.   A. H. Cornfield.

Broiler pigmentation of neoxanthin and violaxanthin relative to lutein. D. D. KUZMICKY, G. O. KOHLER, A. L. LIVINGSTON *et al.* (*Poult. Sci.*, 1969, **48** (1), 326–330. 10 ref.).—When supplied orally each day for 5 days to pigment-depleted male chicks, neoxanthin (I) was only 8% as potent as lutein in skin pigmentation efficiency (as measured by toe-web analysis and visual scoring). Violaxanthin (II) was virtually ineffective. Traces of other xanthophylls were found in the skin of chicks that had been supplied with I and II.   A. H. Cornfield.

Absorption and distribution of ethylenediaminetetra-acetic acid (EDTA) ingested by the chick. R. D. KEALY, D. E. GREENE, P. W. WALDROUP and E. L. STEPHENSON (*Poult. Sci.*, 1969, **48** (1), 94–99. 7 ref.).—Studies with orally administered 2-¹⁴C-labelled Na₄EDTA showed that about 50% of the amount fed was excreted within 24 h. 55–65% of the EDTA supplied was retained by the chick. Most of the activity found in the blood was present in the plasma. Activity in the blood and liver declined rapidly after 2 h, although some activity was retained in the liver longer than in the blood. Activity found in the urine was in the non-urate part.   A. H. Cornfield.

Requirement of turkey poults for biotin and effect of deficiency on incidence of leg weakness in developing turkeys. L. S. JENSEN and R. MARTINSON (*Poult. Sci.*, 1969, **48** (1), 222–230. 9 ref.).—The biotin (I) requirement of male turkeys to 3 weeks of age was 231–284 μg/kg of diet. When birds were fed a I-deficient diet to 4 weeks of age, growth rate was decreased and incidence of leg weakness increased during the 4–24 week period even when adequate I was supplied during the latter period.   A. H. Cornfield.

Effects of dimetridazole and antibiotics on growth and reproduction in turkeys. S. L. BALLOUN, D. L. MILLER, L. G. ARENDS and G. M. SPEERS (*Poult. Sci.*, 1969, **48** (1), 171–176. 6 ref.).—Addition of 0.015–0.020% of dimetridazole (I) to the diet increased wt. gains and feed conversion of Large White turkeys to 24 weeks of age in four tests. 110 ppm of streptomycin–penicillin was also effective in one test, whilst 50 ppm of Zn-bacitracin improved feed conversion but not wt. gains. The effects of I and the antibiotics were partly additive. I and Zn-bacitracin–penicillin, alone or in combination, had no effect on reproductive performance.   A. H. Cornfield.

Influence of an anti-ovulatory compound on the expression of vitamin D deficiency symptoms in laying hens. S. I. CHANG, J. McGINNIS and M. H. PUBOLS (*Poult. Sci.*, 1969, **48** (1), 154–159. 10 ref.).—Laying hens on a vitamin D-deficient diet exhibited decreased egg production and bone ash and higher mortality than did hens receiving 1200 ICU of vitamin D₃ per kg of feed. When egg production was prevented by addition to the feed of the anti-ovulatory compound chlormadinone acetate, vitamin D deficiency symptoms did not appear even where the vitamin D-deficient diet was supplied. The vitamin D requirement of adult non-laying hens was very low or non-existent. Vitamin D-deficient laying hens maintained normal oviduct size, ovum numbers and levels of plasma Ca and lipids.   A. H. Cornfield.

Turkey lipid characteristics: influence of sex, age and oestradiol 17β-monopalmitate. W. E. OSBORN, R. E. MORENG and T. E. HARTUNG (*Poult. Sci.*, 1969, **48** (1), 274–283. 18 ref.).—The lipids in skin tissue of turkey carcasses were more susceptible to oxidation in males than in females. Turkey fat was more stable at 28 than at 20 or 24 weeks of age. Neck implantation with oestradiol 17β-monopalmitate (0.03 g) at 15 weeks of age retarded oxidation of lipids in skin fat, increased the proportion of palmitoleic and oleic acids, and decreased the proportion of stearic and linoleic acids in the lipid fatty acids. Total body fat content was not related to stability of lipids.   A. H. Cornfield.

Effect of oestradiol 17β-monopalmitate on yields and quality of chicken roasters. M. A. MEGALLY, R. B. HARRINGTON and W. J. STADELMAN (*Poult. Sci.*, 1969, **48** (1), 130–136. 11 ref.).—The hormone treatment (0.01 g per bird injected in a polyethylene glycol paste carrier) had no effect on wt. gains but improved eviscerated yields, tenderness and flavour.   A. H. Cornfield.

Influence of oral administration of orally active oestrogen and progestin on oviduct development of the immature pullet. T. J. WOODY, G. C. HARRIS, JUN., P. W. WALDROUP and J. N. BEASLEY (*Poult. Sci.*, 1969, **48** (1), 124–130. 11 ref.).—The wt. and length of the oviduct were increased when pullets received dienoestrol diacetate (I, 0.070–0.140 g/kg of diet) and were decreased by chlormadinone acetate (II, 0.0022–0.0088 g/kg) supplied from 14 to 18 weeks of age. I increased feed consumption and wt. gains, whilst II decreased wt. gains, but had no effect on feed consumption.   A. H. Cornfield.

Fluoride toxicity in the chick. C. W. WEBER, A. R. DOBERENZ and B. L. REID (*Poult. Sci.*, 1969, **48** (1), 230–235. 18 ref.).—Chicks fed a diet containing 500 ppm of F⁻ (NaF) to 4 weeks of age showed 8% and those receiving 1000 ppm of F⁻ showed 21% reduction in body wt. gains. The treatments did not affect feed conversion, total plasma protein or lipoproteins, dietary metabolisable energy, $\%$ fat digestion or $\%$ of fat in several body organs. Dietary F⁻ did not alter enzyme activities in liver and kidney, but heart cytochrome oxidase levels were increased by 500 ppm of F⁻ and plasma alkaline phosphatase by 1000 ppm of F⁻.   A. H. Cornfield.

Symposium: physiological response and stress [in birds]. I. Introduction. D. POLIN. II. Nervous system of birds. A. VAN TIENHOVEN. III. Immunobiological control of the immune response of the fowl. B. GLICK. IV. Environmental stress and physiological compensating mechanisms in fowl—temperature and respiratory regulation. H. S. SIEGEL (*Poult. Sci.*, 1969, **48** (1), 9–10; 10–16, 54 ref.; 17–22, 55 ref.; 22–30, 79 ref. Critical review papers.   A. H. Cornfield.

Effects of debeaking, floor space and dietary energy levels on broiler growth. L. D. ANDREWS and T. L. GOODWIN (*Poult. Sci.*, 1969, **48** (1), 191–196. 15 ref.).—The effects of debeaking at 1 and 10 days of age, floor space (3.7–7.4 dm² per bird) and dietary energy level (2149–2314 kcal/kg) on growth of 13 strains of broilers to 8 weeks are reported.   A. H. Cornfield.

Liver, heart and adrenal weights of broilers reared under constant temperatures. J. W. DEATON, F. N. REECE, E. H. McNALLY and W. J. TARVER (*Poult. Sci.*, 1969, **48** (1), 283–288. 8 ref.).—Male and female broilers reared from 1 day to 8 weeks of age at temp. ranging from 15.6 to $32.2°$ had smaller livers, hearts and adrenals than did those reared at $7.2°$. Differences due to temp. did not become apparent until after 4 weeks of age. Body wt. increased at a greater rate than did the wt. of organs during the first 4 weeks, but at approx. the same rate during the last 4 weeks.   A. H. Cornfield.

Dietary calcium and phosphorus and variations in plasma alkaline phosphatase activity in relation to physical characteristics of egg shells. H. S. PAUL and D. C. SNETSINGER (*Poult. Sci.*, 1969, **48** (1), 241–251. 22 ref.).—Plasma alkaline phosphatase activity (*PAPA*) increased with dietary Ca level (1.75–4.25%) but was not affected by varying dietary P level (0.35–0.80%). High *PAPA* were associated with thicker shells, but *PAPA* was not associated with egg-shell sp. gr. or shell breaking strength. 24 h cyclic changes in *PAPA* were observed with a peak approx. 10 h post-oviposition, whilst peak plasma Ca levels occurred 1 h post-oviposition. In a particular 24 h cycle, *PAPA* and plasma Ca levels were not related to shell characteristics.   A. H. Cornfield.

Variation in initial quality of chicken eggs. IV. Certain characteristics of hens chosen for quality of eggs produced. J. H. SKALA (*Poult. Sci.*, 1969, **48** (1), 164–171. 23 ref.).—Within each breed or strain, those hens that produced high quality eggs (high Haugh units) tended to reach sexual maturity later and to produce larger eggs than did those hens that produced low quality eggs. In spite of the tendency of the former hens to be larger in adult body wt., their feed efficiency equalled that of the latter hens when calculated on the basis of total wt. of eggs produced.   A. H. Cornfield.

Effect of wavelength of light on growth and reproduction of Japanese quail, *Coturnix coturnix japonica*. A. E. WOODARD, J. A. MOORE and W. O. WILSON (*Poult. Sci.*, 1969, **48** (1), 118–123. 12 ref.).—Female quail brooded under red or white light had higher body wt. at 5 weeks than did those under green or blue light. Birds under red light reached 50% rate of lay 2 weeks earlier than those under blue or green light, and maintained a higher rate of production to 16 weeks. At 5 weeks of age, testes of males brooded under red light were 2–3 times the size of those of birds under green or blue light. Fertility of eggs laid by birds kept under blue light was significantly lower than that of eggs from birds kept under green, red or white light. Colour of light had no effect on hatchability, egg wt. or feed efficiency. A. H. Cornfield.

Raising ducks. W. J. ASH (*Fmrs' Bull.*, 1969, *U.S. Dep. Agric.*, 1969, (2215), 14 pp.).—Breeds, breeding stock, incubation, brooding and rearing, nutrition, marketing and diseases, are discussed.
E. G. Brickell.

Analysis and Other Aspects

Colorimetric determination of protein in feed and forage crops. A. J. MACKENZIE and E. R. PERRIER (*Agron. J.*, 1969, **61** (2), 332–333. 5 ref.).—The method involves shaking 0·5 g of dried (70°) and ground (40 mesh) plant material with 25 ml of Orange G soln. (1 g of dye, corrected for assay, is dissolved in a soln. that contains 21 g of citric acid and 2·5 ml of 10% thymol in EtOH, with dilution to 1000 ml) for 1 h. After filtration, the extent of reduction in transmittance (i.e., the extent of dye-binding by the plant material) is measured spectrophotometrically at 475 nm. There was high correlation between extent of dye binding and total N% with each of six crop species tested. A. H. Cornfield.

Detection of prophylactic drugs in animal feeding stuffs by thin-layer chromatography. P. W. HAMMOND and R. E. WESTON (*Analyst, Lond.*, 1969, **94** (1123), 921–924. 6 ref.).—Using only two solvent systems (for group separation) and five detection agents, the presence of acinitrazole, 2-chloro-4-nitrobenzamide, amino-nitrothiazole, amprolium (I), N^4-acetyl-4′-nitrosulphanilanilide, buquinolate, decoquinate, dimetridazole, 3,5-dinitrobenzamide, ethopabate, furazolidone, metichlorpindol, nitrofurazone, pyrimethamine, Me benzoquate, sulphaquinoxaline and zoalene were detected. With the exception of **I**, the drugs were extracted with MeCN–CHCl₃; the extract was chromatographed on an Al₂O₃ column to yield three fractions which were then submitted to t.l.c. on SiO₂ gel with CHCl₃–MeOH (9: 1) as solvent system. **I** was extracted with MeOH, using EtOH–N–HCl (1 : 1) as solvent system. Detection agents used were 1,2-diaminoethane, Ehrlich's reagent, Dragendorff's reagent, diazotisation soln., and u.v. light plus citric acid–H₃BO₃. Identification was based on R_F values and colours; results are discussed. Detection limits correspond approx. with estimated min. commercial usage. W. J. Baker.

Improvements in the official first action method for extraction and assay of nystatin in animal feeds. T. B. PLATT, J. D. LEVIN and M. A. MASSEY (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 675–678. 5 ref.).—Quant. recoveries of nystatin from animal feeds were obtained by simplifying the official method ('Official Methods of Analysis', 10th Ed., Washington: A.O.A.C. Secs. 33.144, .145, .147); the hexane wash was eliminated and the MeOH was removed by vac. distillation. Feed blank extracts were prepared by autoclaving at 121° for 20 min. D. I. Rees.

Modifications of the assay of bacitracin in feeds. G. H. CRAIG (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 681–685. 4 ref.).—Two modifications to the method previously described (*Idem, ibid.*, 1965, **48**, 256) are described. An improved clean-up was obtained by Soxhlet extraction with petroleum ether (b.p. 38–54°) for 2 h. Larger zones of growth inhibition were produced on plates seeded with *Micrococcus flavus* by adding to the agar a sub-inhibiting amount of neomycin sulphate. Amounts as low as 0·01 unit per ml of bacitracin could be detected. D. I. Rees.

Microbiological assay of erythromycin thiocyanate in feeds. H. S. PERDUE, J. A. GORDON and J. MANNEBACH (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 672–675).—The feed (≡5–20 g/ton of erythromycin thiocyanate) is extracted with Me₂CO which is then evaporated, and the residue is washed with n-hexane followed by MeOH. The combined washings are partitioned with phosphate buffer (pH 8·0) prior to assay with *Sarcina lutea* ATCC 9341. Recoveries averaged 106%. D. I. Rees.

Microbiological assay of streptomycin in feeds. J. J. MAYERNIK (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 679–681. 4 ref.).—The method previously described (*Idem, ibid.*, 1961, **44**, 33) was found

to give recoveries of 84–89% of low levels of streptomycin in feeds. Unsuccessful attempts to improve these recoveries by various compensation methods and by ion-exchange treatment are described. D. I. Rees.

Extraction and g.l.c. determination of mixed sulphonamides in feeds. A. FRAVOLINI and A. BEGLIOMINI (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 767–769. 10 ref.).—The feed (20 g ≡ 50–100 mg of each sulphonamide) is stirred with dimethylformamide and the residue obtained on evaporating the filtrate is hydrolysed with H₂SO₄. After neutralising with Ba(OH)₂ and filtering, the resulting amines are analysed by injecting an aliquot of the aq. filtrate on to a glass column (80 cm × 3 mm) of 3% of Carbowax 20M on 80–100 mesh Chromosorb W at 132° with He as carrier gas (100 ml/min) and flame ionisation detection. Quant. analyses of seven sulphonamides were obtained with 94–104% recoveries.
D. I. Rees.

Gas chromatographic determination of chlormadinone acetate in cattle tissues. A. L. DONOHO, W. S. JOHNSON, J. R. KOONS and R. E. SCROGGS (*J. Ass. off analyt. Chem.*, 1969, **52** (4), 770–773. 8 ref.).—Muscle, liver or kidney sample (20 g) is extracted with MeOH and the hormone is partitioned into CCl₄. A fat sample (15 g) is dissolved in hexane and the hormone is partitioned into MeCN. Muscle, liver and fat extracts are purified by silica gel column chromatography and kidney extracts on Al₂O₃ columns. The g.l.c. analysis is done on a glass column (16 in × 4 mm) of 1·5% of XE-60 on 80–100 mesh Gas Chrom Q at 230° with Ar–CH₄ as carrier gas (9 : 1; 190 ml/min) and pulsed tritium electron capture detection. Recoveries of added 0·05 ppm of the hormone were 80–110% in muscle, liver and kidney, and 70–80% in fat.
D. I. Rees.

Daily urinary excretion of oestradiol-17β and oestrone by the hen. R. S. MATHUR and R. H. COMMON (*Poult. Sci.*, 1969, **48** (1), 100–104. 13 ref.).—Urinary excretion over 24 h was 2·42–3·61 μg of oestrone (**I**) and 3·10–3·56 μg of oestradiol-17β (**II**) for laying hens and 0·27–0·93 μg of **I** and 1·41–2·26 μg of **II** for non-laying hens. A. H. Cornfield.

Influence of hen oviduct homogenates on the motility, oxygen uptake and fertilising capacity of fowl spermatozoa. A. R. LEHRER and H. SCHINDLER (*Poult. Sci.*, 1969, **48** (1), 30–36. 16 ref.).—The influence of fresh homogenates from different regions of the hen oviduct on rate of O₂ uptake and preservation of motility and fertilising capacity of fowl spermatozoa was studied.
A. H. Cornfield.

Simple method for determining concentrations of ammonia in animal quarters. S. G. MOUM, W. SELTZER and T. M. GOLDHAFT (*Poult. Sci.*, 1969, **48** (1), 347–348. 9 ref.).—The concn. of NH₃ in the atm. of animal houses can be determined by hanging moistened pH indicator paper in the room for 15 sec. Under these conditions, pH of the paper ranges from 6 to 11 as atm. NH₃ concn. increases from 0 to 100 ppm. The accuracy of the method is ± 5 ppm. A. H. Cornfield.

Losses of energy and nitrogen on drying poultry excreta. D. W. F. SHANNON and W. O. BROWN (*Poult. Sci.*, 1969, **48** (1), 41–43. 3 ref.).—Vac. oven drying at 40° gave the highest loss of N (16–40%) and of energy (7–17%). Freeze-drying and forced air oven-drying at 60° gave the lowest losses of N (av. 4·8% and 4·6%, resp.). Lowest loss of energy (av. 1·3%) occurred with freeze-drying. Loss of both N and energy increased with increasing temp. (60–120°) of forced air oven-drying. A. H. Cornfield.

Odour transport by particulate matter in high density poultry houses. W. E. BURNETT (*Poult. Sci.*, 1969, **48** (1), 182–185. 9 ref.).—G.c. analysis of the volatiles carried by particulate matter filtered from the air of a poultry house showed the presence of a number of individually odoriferous compounds.
A. H. Cornfield.

Bacterial population of an indoor poultry lagoon. L. J. CABES, JUN., A. R. COLMER, H. T. BARR and B. A. TOWER (*Poult. Sci.*, 1969, **48** (1), 54–63. 17 ref.).—The type and no. of bacteria, total suspended solids, pH and B.O.D. of the waste water from an indoor poultry lagoon (aerated channel for treating poultry manure in a laying house) are presented. The % reduction in B.O.D. varied over 5 months, but averaged 74%. Possible methods of improving the quality of the waste water are discussed.
A. H. Cornfield.

Animal feed compositions. MONSANTO CO. (Br. Pat. 1,162,232, 24.10.66. U.S., 24.10.65).—Cellulosic roughage for ruminants

contains $0 \cdot 0001 - 0 \cdot 1\%$ by wt. of $H_2N \cdot (R \cdot NH)_xH$ ($x = 2$–20, R = alkylene of 2–40C) or $H_2N \cdot R^I(NH_2)[(O)_nH]$ (R^I is a 2–40C aliphatic hydrocarbon, $n = 0$ or 1). Particularly effective are compounds where R and R^I are derived from dimeric linoleic acid and x is an integer, to give a polymer of mol. wt. ~ 8000. A feed composition contains $41 \cdot 66\%$ of ground maize, $5 \cdot 0\%$ of soyabean meal, $0 \cdot 88\%$ of defluorinated phosphate rock, $0 \cdot 45\%$ of trace minerals, $8 \cdot 00\%$ of molasses, $44 \cdot 00\%$ of chopped lucerne, $0 \cdot 01\%$ of vitamins A and D and $0 \cdot 01\%$ of polymeric amine of mol. wt. 3000.
S. D. Huggins.

Animal feed supplements. BOOTS PURE DRUG CO. LTD. (Inventors: R. G. TOWLERTON and J. G. LORD) (Br. Pat. 1,165,776, 13.6.67. Divided out of Br. Pat. 1,165,775).—A lick for very young piglets (2–8-day) which contains assimilable Fe [Fe^{II} fumarate (I)] and is useful for the prevention of Fe-deficiency anaemia, is prep. from a porous hard block of palatable material containing the I and sugar by coating it with a soft, orally acceptable humectant [glycerol (II)]. In an example, I, sucrose, gums acacia and tragacanth and water are mixed and molasses (III) is added. The damp mixture is compressed in a mould, dipped into water containing II and III and dried (1 h at 60°).
S. S. Chissick.

Malic acid and process for its preparation. ALLIED CHEMICAL CORP. (Br. Pat. 1,160,777, 20.7.67. U.S., 21.7.66).—A malic acid (I) composition, useful as a food acidulant and fit for animal consumption, consists of I plus $0 \cdot 5$–5 wt.-% of succinic acid and $< 0 \cdot 03$ wt.-% of an unsaturated carboxylic acid. Aq. I which contains fumaric (II) and maleic acids is contacted with H_2 in the presence of a catalyst at 200 (40–150) psi and at 25–100 (25–60)° and the composition is separated therefrom. In an example, aq. crude I is heated at 200° and at 153–157 psi for 3 h and then cooled, evaporated and filtered to remove solid II. The filtrate is mixed with a slurry of 5% Pd on C and treated with H_2 at 100 psi and 50°. On working up, the composition is obtained.
S. S. Chissick.

Steroid compositions. UPJOHN CO. (Br. Pat. 1,160,024, 10.2.67. U.S., 9.3.66).—Inhibition of egg-laying in poultry and other birds is effected by adding to the feed a compound $NR^IR^{II} \cdot [CH_2]_2 \cdot A \cdot CHR^{VII}R^{VIII}$ or $NR^VR^{VI} \cdot [CH_2]_2 \cdot A \cdot CHMeR^{IX}$ wherein R^I-R^{II} are H, C_{1-8}-alkyl which may contain OH, or C_{2-8}-alkenyl or NR^IR^{II} is heterocyclyl with 4–6 ring atoms; R^{VII} is Me and R^{VIII} is 3-OR-androst-5-en-17-yl (R is H, acyl of 1–18 C, or $[CH_2]_n$-NR^IR^{II}; n is 2–6), but when R^I-R^{II} are H or alkyl then R is only $[CH_2]_nNR^IR^{II}$; or R^{III} is H and R^{VIII} is 3-OR^{III}-androstan-17-yl (R^{III} is H, acyl of 1–18 C, or $[CH_2]_nNR^IR^{II}$); R^V-R^{VI} are C_{1-8}-alkyl, C_{2-8}-alkenyl, or NR^VR^{VI} is 4–6-membered heterocyclyl; R^{IX} is 3-SR^{IV}-androst-5-en-17-yl or 3-Z-androst-3-en-17-yl; R^{IV} is H or $[CH_2]_2NR^IR^{II}$; Z is oxo, OH or :NOH; and A is O or CH_2. The compounds may be used in the form of acid addition salts or N-oxides. A typical agent is 25-azacholesterol hydrochloride.
F. R. Basford.

2.—FOODS

Cereals, Flours, Starches, Baking

Nutritive value of barley varieties assessed with the confused flour beetle. S. R. LOSCHIAVO, A. J. McGINNIS and D. R. METCALFE (*Nature, Lond.*, 1969, **224** (5216), 288. 6 ref.).—Newly hatched larvae of *Tribolium confusum* were provided with 1 g of finely ground barley and kept at 30° and 70% r.h.; the intervals to pupation and adult emergence were detd. after 17 days. Different varieties of barley were characterised by different rates of larval development. Apparent digestibility was also detd. for adult beetles; the values ranked in same order as for duration of larval period. Factors responsible for the differential rates are as yet unknown.
W. J. Baker.

Cooking–extrusion–expansion of rice. H. H. MOTTERN, J. J. SPADARO and A. S. GALLO (*Fd Technol.*, *Champaign*, 1969, **23** (4), 567–569).—The method and equipment for the extrusion process are described. The product showed a slightly rancid flavour after 6 months storage at 22°, but this was not apparent when it was mixed with milk and sugar. The product was mixed with glandless cottonseed concentrate 10, sugar 5, salt 4% and malt syrup, which made a pleasant beverage. The rate of dispersion can be controlled by particle size. The mixed gelatinisation–dextrinisation of the product permits use of high concn. in a beverage without producing excessive viscosity yet holding oilseed flours in suspension.
I. Dickinson.

Japanese miso-type products prepared by using defatted soyabean flakes and various carbohydrate-containing foods. J. ILANY (FEIGENBAUM), J. DIAMANT, S. LAXER and A. PINSKY (*Fd Technol.*, *Champaign*, 1969, **23** (4), 554–556. 14 ref.).—The prep. of miso and miso-type products was studied. Defatted, commercially available soyabean flakes were substituted for whole soyabeans, and wheat, maize, barley, sorghum, oats, potatoes, beets or bananas were substituted for rice in koji. Differences were observed in the development and growth of the mould, time of fermentation, appearance, colour, odour, flavour, taste, ratio of total to sol. N and reducing sugars, and in the end-product, according to the cereal or carbohydrate-contg. food used as substrate in the prepn.
I. Dickinson.

Water absorption in wheat flour. P. MEREDITH (*Baker's Dig.*, 1969, **43** (4), 42–45, 63. 14 ref.).—Flours vary in the amount of water that they can absorb and efficiently carry through to the loaf of bread. Many factors that affect this absorption are fixed by considerations of price and trade. The damaged starch content, however, can be varied, within limits, by adjustments within the flour mill.
I. Dickinson.

Process for protein–starch separation in wheat flour. D. A. FELLERS, P. H. JOHNSTON, S. SMITH, *et al.* (*Fd Technol.*, *Champaign*, 1969, **23** (4), 560–564. 5 ref.).—A wet process is described for the centrifugal separation of starch from a thick water/flour slurry. The protein concentrate remained liquid throughout the process and could be handled easily. It had better nutritional quality, because vitamins, minerals and sol. proteins (higher in lysine content than gluten) were retained. A high quality food-grade starch (protein content $< 0 \cdot 3\%$) was readily prep. by a simple washing operation.
I. Dickinson.

Distribution of hydroxyethyl groups in commercial hydroxyethyl starch. II. H. C. SRIVASTAVA, K. V. RAMALINGAM and N. M. DOSHI (*Stärke*, 1969, **21** (7), 181–183. Engl., 10 ref.).—An alternative method was used to confirm previous findings (viz. in a commercial sample of hydroxyethyl starch with degree of substitution of $0 \cdot 1$, the C-2 OH was substituted to the extent of 84%, the remaining ether groups being located in the C-6 position). The sample was acid hydrolysed and the glucose removed by fermentation. The non-fermentable fraction contained four components which were separated by paper chromatog. R_G (rate of movement relative to glucose) values were used to identify the 6-O-, the 2-O- and 3-O-deriv.; the fourth component was identified by the periodate–benzidine reagent. Quant. fractionation on charcoal columns confirmed the distribution of hydroxyethyl groups found previously.
J. B. Woof.

Tailoring starches for the baking industry. R. R. HAHN (*Baker's Dig.*, 1969, **43** (4), 48–53, 64. 11 ref.).—The physicochem. nature of starch, its composition and structure, swelling and gelatinisation, and consistency, are discussed, together with various techniques for the modification of properties that yield speciality starches for baking.
I. Dickinson.

Effects of dioctyl sodium sulphosuccinate on the softness of bread and cake. T. A. LEHMANN and W. G. BECHTEL (*Baker's Dig.*, 1969, **43** (4) 57–60. 9 ref.).—The use of this compd. (I) at 50 or 70 ppm permitted reduction of emulsifier from 8 to 6 or 7% of the shortening, without sacrifice of cake quality. The effects of I were more pronounced in leaner cakes. I did not affect dough fermentation or bread quality; the effect on bread softness was inconclusive. It appeared to be effective in sponge and dough bread, but ineffective in bread made by continuous mixing process.
I. Dickinson.

Review of some recent studies on prevention of mould spoilage in bakery products. Y. POMERANZ (*Baker's Dig.*, 1969, **43** (4), 61–62, 70. 23 ref.).—Phys. methods, e.g., use of CO_2 or vac., radiation, heat and microwaves are discussed together with biol. and chem. methods.
I. Dickinson.

Preparing starch products. NATIONAL STARCH & CHEMICAL CORP. (Br. Pat. 1,160,356, 8.2.68. U.S., 17.2.67).—The process comprises (*i*) reacting a starch (I) base with an inhibiting reagent, e.g., adipic, succinic or acetic anhydride, (*ii*) swelling I, (*iii*) reacting the swollen I with an enzyme at pH 3–10 such that the 1,4- (but not the 1,6-) linkages of the amylopectin (II) are split and the II-substituent linkages remain intact, e.g., β-amylose (III) or α-1,4-glucosidase and (*iv*) isolating the product, whose aq. dispersions are stable and resistant to syneresis and gelling when stored at low temp. In an example, waxy maize starch is reacted with epichlorohydrin, slurried with water and gelatinised and dried. The product in aq. solution is treated with III at pH $4 \cdot 8$ and at 55°, and after 1 h, $HgCl_2$ is added and the suspension is spray-dried. When used in

the prep. of a cranberry sauce, the sauce could be frozen and thawed repeatedly without change.   S. S. Chissick.

Process for making bread. MAPLE LEAF MILLS LTD. (Inventors: J. H. HULSE and R. E. HANNAH) (Br. Pat. 1,151,985, 28.2.68. Can., 14.6.67).—A bread dough is claimed which requires no fermentation and which can be divided, moulded, proofed and baked immediately after mixing and resting. Into the standard bread mix are incorporated: (*i*) > 1 acid, e.g., lactic, acetic, citric, tartaric, acid phosphate, (*ii*) > 1 bromate, of Na, K and Ca, (*iii*) > 1 compd. chosen from an iodate (of Na, K or Ca), Ca peroxide, acetone peroxide, azodicarbonamide, ascorbic acid (I), dehydro-I, and the whole is mixed for slightly longer than the normal mixing time. E.g., flour, yeast, sugar, salt, regular shortening, skim-milk powder, $KBrO_3$, $Ca(IO_3)_2$, lactic acid and water are mixed for 12 min; the final temp. is adjusted to 90°F. After resting (8–10 min) the mix is moulded and then proofed (60 min, 105°F) and baked (30 min, 400°F).   S. S. Chissick.

Preparation of succinyl half esters of mono-acylated polyhydric alcohols. NATIONAL DAIRY PRODUCTS CORP. (Inventor: E. H. FREUND) (Br. Pat. 1,165,373, 4.10.66).—The esters, of use in baked goods (to improve grain, texture, softness and moisture, and to increase vol.) are prepared by reacting succinic anhydride with a mono-C_{14-24}-acyl C_{2-6}-polyhydric alcohol at < 115° but above the m.p. of the latter.   F. R. Basford.

Fumaric acid derivatives and their use as bread softeners. CHAS. PFIZER & CO. INC. (Br. Pat. 1,160,255, 26.10.66. U.S., 18.11.65 and 27.9.66).—A method of improving the shelf-life of yeast-leavened bakery products comprises incorporating in the raw dough an effective amount (> 2 wt.-% of flour) of finely divided mono-stearyl fumaric acid (I) and/or its salts with Na, K, Ca or Mg, prep. by slowly cooling a hot soln. of the acid/salt. In an example, Na I is slurried with water, heated to 90° and then (*i*) rapidly chilled to 70° and (*ii*) cooled to 45° over 30 min. After filtering, drying, etc., the salt is incorporated into a bread mix and the final product has improved shelf life and grain texture and remains soft for longer than usual.   S. S. Chissick.

Sodium stearyl fumarate in chemically leavened bakery products. CHAS. PFIZER & CO. INC. (Br. Pat. 1,164,919, 18.12.67. U.S. 28.2.67).—The addition of > 0·1 (2) wt.-% of Na stearyl fumarate to high sugar, low shortening, chemically leavened bakery products gives a product similar to that obtained by using a high shortening content plus eggs.   S. S. Chissick.

Bread-improving composition and method. J. R. SHORT MILLING Co. (Br. Pat. 1,167,675, 21.6.68. U.S., 27.9.67).—The composition is a mixture of active lipoxidase-containing material (I), 5–92, and enzyme-peroxidisable fat II, 3–30, supported on a powdery solid material (III), 5–50 (5–15), comprising mainly sugars and protein, e.g., dairy whey solids, and optionally a compatible carrier 0–87 wt.-%. II is used in the form of a coating on particles of III, and I and II are dispersed in water, with *in situ* peroxidisation of II by mixing.   S. S. Chissick.

Cookies and biscuit-like foods. KYOWA HAKKO KOGYO CO. LTD. (Br. Pat. 1,151,986, 29.2.68. Jap., 14.3.67).—Products having improved and lasting flavour are prep. by kneading (at ≤ 40°) suitable raw materials including sugar, lysine hydrochloride (I) (0·01–1% by wt. of wheat flour used) and $NaHCO_3$ (at least stoicheiometrically equiv. to amount of I used), to make a dough which is then oven baked at 170–220° for 10–20 min.   S. S. Chissick.

Sugars, Syrups, Confectionery

Degree of contamination of commercial refined sugar and isolation of the 'flat-sour' bacteria. B. RECKNITZ and L. JANOTA-BASSALIK (*Roczn. Technol. Żywn.*, 1969, **15**, 57–63. Pol., 7 ref.).—Microbiological analysis of refined sugar was carried out using the standard method of thermophilic bacterial spores determination. The no. of thermophilic spores in refined sugar was generally 50% lower, and the no. of 'flat-sour' spores was in most cases over 50% lower, than that permissible by common international standards. *Bacillus stearothermophilus* was isolated.   A. Lewin.

Moisture in honey: review of chemical and physical methods. J. W. WHITE, JUN. (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 729–737. 38 ref.).—The review presented includes determination by the Karl Fischer titration and by several physical methods. A corrected table of Wedmore's revision (*Bee Wld*, 1955, **36**, 197) of the data of Chataway (*Can. J. Res.*, 1933, **8**, 435) is given.   D. I. Rees.

G.l.c. flavour profile of maple syrup. J. C. UNDERWOOD, V. J. FILIPIC and R. A. BELL (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 717–719. 7 ref.).—The sample (2 quarts) was shaken with $CHCl_3$ (1 l) for 30 min, the $CHCl_3$ layer was separated off and conc. to a final vol. of 5 ml. A 500 μl aliquot was analysed by g.l.c. with use of a stainless steel column (4 ft × 0·25 in) of 20% of Carbowax 20 M on 60–80 mesh Chromosorb W temp. programmed from 50 to 240° at 3·5°/min with He as carrier gas (50 ml/min) and with thermal conductivity detection. The major components identified were pyruvic alcohol, isomaltol, Cyclotene, α-furanone, hydroxymethylfurfural, vanillin, syringaldehyde and dihydroconiferyl alcohol. The flavour profiles of 5 syrups analysed were found to be similar, indicating that the technique can be used to detect adulteration.   D. I. Rees.

Processing and preserving ginger by syruping under atmospheric conditions. II. Effects of temperature, flowrate and sucrose: reducing sugar ratios on the processing of ginger in invert syrup. III. Processing techniques and syrup concn. for maximum drained weight recovery of syruped ginger. B. I. BROWN (*Fd Technol., Champaign*, 1969, **23** (7), 953–956; 969–972. 24 ref.).—II. The relative rates of absorption of sugars by the ginger from vat syrup were largely independent of vat temp. and inversion of sucrose in the syrup. The actual rate of syrup flow had little effect on the processing characteristics of the ginger. Best overall results were obtained when the vat syrup of 75% total sol. solids, contained 27–30% of reducing sugars.

III. Immersion of ginger in 20% total sol. solids for 21 h gave the most satisfactory increase in drained wt. with min. shrinkage.   I. Dickinson.

Preparing a granular, free-flowing sugar product. AMERICAN SUGAR Co. (Inventors: M. D. M. M. COHEN and C. P. GRAHAM) (Br. Pat. 1,163,694, 15.1.68).—A maize syrup [sucrose (I) content > 85%] is concentrated at 120–130° to a solid content of 91–97% and then subjected to simultaneous impact heating and forced gas flow. In an example, a feed syrup (93% of I) is introduced into a Turbulizer at 125° and a forced flow of air introduced. The product contains aggregates of fondant-sized I crystals and has improved shelf life, flavourability and cooking properties.   S. S. Chissick.

Additives for use in sugar manufacture. FABCON INC. (Inventor: J. A. CASEY) (Br. Pat. 1,157,294, 6.3.67).—An additive, consisting of ≮ 1 water-sol. salt of a sulphosuccinate ester dissolved in 1 : 1 aq. EtOH and/or propylene glycol mixture, improves the efficiency of the crystallisation of sugar from cane or beet. Suitable sulphosuccinates (I) include the diacetyl K or NH_4, and di-isobutyl, dihexyl or ditridecyl Na, K and NH_4 salts. The I is added to the pans during boiling of the 'massecuites', in the form of a 50% soln. in sufficient amount to provide, e.g., 1·5–7·5 ppm of I in a 15% soln. of glucose.   S. S. Chissick.

Malting, Brewing and Alcoholic Beverages

Germinating capacity and water sensitivity of brewing barley. G. KRAUSS and M. A. DJALALI (*Mschr. Brau.*, 1969, **22** (9), 248–253. Ger., 9 ref.).—A water steeping method, in conjunction with the germinator test of Pollock (using 4 ml water per dish), was found to be the most satisfactory for evaluation of germination capacity and water sensitivity. Good agreement with pilot maltings was obtained. Germinating ability increased with increasing ripening time in the field; sowing times and threshing procedures had little effect. Varietal differences and environmental conditions were important factors. Water sensitivity increased with decreasing temp. and increasing rainfall and humidity. Water-sensitive barleys had high O_2 requirement; non-water-sensitive barleys had low malting loss, low extract differences and high wort viscosities.   J. B. Woof.

Simple methods for the gas chromatographic examination of flavour compounds in malts and similar materials. S. ENGAN (*Z. analyt. Chem.*, 1969, **246** (5), 324. Ger.).—Finely ground malt is packed dry into a glass column, the flavour compounds are eluted with MeCN and the eluate is evaporated in vac. The residue is dissolved in Et_2O and an aliquot of this soln. is injected into a gas chromatograph equipped with a flame ionisation detector, with Aeropak 30 as solid support, 7% FFAP as stationary phase and N_2 as carrier gas. The lower boiling compounds are partly or completely lost, but they may be examined using a head-space technique. Both methods give fast and reproducible results and distinguish

clearly between different types of malt, and even between malts made by the same malting process but from different barleys.

J. Korkisch.

Rapid conductometric analysis of hops and hop extracts. P. DE CEUSTER and J. STRUYVELT (*Brauwissenschaft*, 1969, **22** (9), 362–364. Ger., 6 ref.).—20-g samples of hops were blended with MeOH and an aliquot of the extract was evaporated under vac. The residue was then extracted with a mixture of MeOH (4), water (8) and hexane (20 ml), and acidified with H_2SO_4. After dilution with MeOH, this extract was titrated conductometrically with 4% $Pb(OAc)_2$. Hop extracts were analysed similarly but, prior to extraction with hexane and subsequent titration, water-sol. components were extracted with $HCONMe_2$, filtered and acidified with 2% H_2SO_4.

J. B. Woof.

Yields from commercial hop extraction. K. KÄRNBACH (*Mschr. Brau.*, 1969, **22** (9), 241–243. Ger., 15 ref.).—The high analytical tolerances and sampling difficulties give doubtful significance to yields evaluated on the basis of a single batch. Av. yield figures indicate a probable actual loss of < 3% during extraction. Spent hops should contain no detectable α-acid when carefully extracted. Qual. comparison of hops and extract is possible in terms of % of α-acid in the total resin, and the oil constituents as indicated by g.c.

J. B. Woof.

Thiamine content, carboxylase activity and fermentation rate. F. WEINFURTNER, F. ESCHENBECHER and H. HINTERBERGER (*Brauwissenschaft*, 1969, **22** (8), 326–331; (9), 368–377. Ger., 103 ref.).—A literature review covering thiamine (I) content of yeast, its form within the cell, the mode of action of I pyrophosphate and the N content of yeast and its effect on fermentation. I detn. based on the microbiological method of Schopfer and by the thiochrome method is outlined. I pyrophosphate detn. by a manometric method is described. Precautions necessary for carboxylase activity detn. are discussed. The second part of the paper discusses the conditions under which I is formed. Top fermentation yeasts and non-flocculent bottom fermenters accumulated I better than flocculent bottom fermenters. Addn. of I to the culture medium enhanced accumulation of the vitamin both in resting and budding cells. The availability of N in the medium directly affected I content when the medium was free of I, but indirectly affected it when the medium contained I. Increased N-nutrition increased carboxylase activity as well as I synthesis. Lack of meso-inositol had a slight effect in reducing carboxylase but sub-optimal levels of biotin and pyridoxine had no effect. Fermentation velocity was not affected by I or pyridoxine but addn. of the former to the medium sometimes enhanced N assimilation.

J. B. Woof.

Miniature brewery investigations on the effect of different mashing procedures on the properties of beer. I. High temperature, two and three mash procedures. L. NARZISS and H. HEISSINGER (*Brauwissenschaft*, 1969, **22** (9), 353–361. Ger., 40 ref.).—A detailed investigation of the compn. of worts and beers, prepd. by various decoction mash procedures, was carried out. The 'jar' method previously described (*ibid.*, 1969, **22** (8), 331) was carried out using the following mashing temp. sequences: 62/70/77°, 50/65/77° and 35/50/65/77°. The programme and duration at each temp. affected enzyme activity and hence final wort compn. The effects of proteolytic activity, amylases, glucanases and phosphatases are considered.

J. B. Woof.

Production of pito, a Nigerian fermented beverage. J. A. EKUNDAYO (*J. Fd Technol.*, 1969, **4** (3), 217–225. 26 ref.).—Local production of pito was investigated under controlled lab. conditions. Maize, sorghum or a mixture was soaked for two days and malted for five days in baskets lined with damp banana leaves. It was shown that both cereal amylases and fungal activity are important in bringing about saccharification. The grains were then mashed with water and rough filtered; the filtrate, left overnight, was soured by atm. lactobacilli and then concentrated (by boiling). Addn. of a starter culture from the previous brew, contg. mainly *Candida* spp. and *Geotrichum candidum*, brought about the main fermentation. The ability of the various organisms, isolated from local brews, to hydrolyse starch and produce acids was detd.

J. B. Woof.

Rapid determination of proline in grapes and wines. C. S. OUGH (*J. Fd Sci.*, 1969, **34** (3), 228–230. 16 ref.).—0·5 ml of the samples (contg. 0·05–0·50 μmole/ml of proline) + 0·25 ml HCOOH and 1 ml of 3% ninhydrin soln. were heated (in sealed containers) over a boiling water bath for 14–15 min. During cooling, 5 ml of 1 : 1 Pr^iOH–water were added. After cooling for > 5 min

absorbance readings were made at 517 nm. Lysine caused greatest interference. The use of Me Cellosolve improves the method—possibly eliminating the usual extraction of reaction product by benzene.

M. T. Rawnsley.

'Brennwein' investigation according to Micko and Wüstenfeld and possibilities of improvement. II. Reproducibility of the Micko distillation and investigation of improvements. K.-G. BERGNER and H.-A. MEEMKEN (*Dt. Lebensmitt Rdsch.*, 1969, **65** (9), 282–288, Ger., 4 ref.).—The compn. of fractions obtained in the normal Micko distillation and those from fast and slow distillations, were detd. Factors such as heating rate and room temp. caused variations in compn. The use of a double fractionation column gave a more reproducible fractionation.

J. B. Woof.

Preparing a hop extract. SWAN BREWERY CO. LTD. (Br. Pat. 1,162,488, 14.8.68. Australia, 21.8.67).—Whole hops are washed with hot (200°F) water and then heated with water to 220–230°F (pressure 4 psig) after the pH has been adjusted to 9·0 by the addition of Na_2CO_3. The extract is separated and stored under CO_2; optionally the spent hops are washed with water and the washings added to the extract.

S. S. Chissick.

Manufacture of hop extracts. BUSH BOAKE ALLEN LTD. (Inventor: W. MITCHELL) (Br. Pat. 1,161,787, 17.11.65).—An isohumulone-containing hop extract is prep. by extracting hops with a water-immiscible solvent in which the solubility of alkali metal humulate salts is less than in water, e.g., C_6H_6 or petroleum ether, (I), and contacting the soln. with a dil. [> 5 (3)%] aq. alkali, e.g., a carbonate, at a pH such that the α-acids but not the lupulones are transferred to the aq. phase. The latter is separated and the humulates are isolated, optionally as the isohumulates. In an example, ground dried hops are extracted with I (b.p. 75–95°) at 30° and 80 vol.-% of the solvent is distilled off. The resulting concentrate is vigorously stirred with 2·5% (w/v) K_2CO_3 soln. (final pH 9·0–9·2), and the humulates are pptd. from the aq. phase by addition of $CaCl_2$.

S. S. Chissick.

Preparing hop extract and hop extract products. BLUE STAR CHEMICALS N.V. (Inventor: P. DE CEUSTER) (Br. Pat. 1,151,465, 13.10.67. Belg., 14.11.66).—A process for adjusting the α-resin content of hop extract (I) to a predetermined value and increasing the preservation time of the extract consists of mixing the I with at least one inert, solid material (SiO_2, $CaCO_3$, $BaSO_4$, $CaSO_4$, an insol. silicate or phosphate). E.g., a pure I sample containing 36% of α-resins is mixed with one part of $Ca_3(PO_4)_2$ to give a I with a standard content of 18% of α-resins.

S. S. Chissick.

Malting. A.B.M. (MALTING) Ltd. (Inventors: K. C. STOWELL and P. M. HOWLETT) (Br. Pat. 1,163,067, 21.7.67 and 27.2 and 30.5.68).—Grain, e.g., barley, is dehusked under conditions such that most rootlet growth is prevented and the aleurone layer is mainly undamaged (apparatus described), and then malted, optionally in the presence of gibberellic acid or a bromate (K, Na, Ca).

S. S. Chissick.

Starch-hydrolysing enzyme system for use in grain alcohol fermentation. HIRAM WALKER & SONS INC. (Br. Pat. 1,161,419, 1.12.66. U.S., 13.12.65).—For the saccharification and fermentation to an alcohol of a starch product, a glutamylase ferment, prep. by cultivating *Aspergillus niger* strain NRRL 3112 or 3122 at 85–98°F and pH 4–7 in a dealcoholised liquid grain residue under aerobic conditions for 3–7 days, is added.

S. S. Chissick.

Method and apparatus for the accelerated and continuous fermentation and maturation of beer wort. INSTITUT FÜR DIE GAERUNGS-U. GETRAENKEINDUSTRIE (Inventors: G. BOSEWITZ, H. EHLIES and R. DICKSCHEIT) (Br. Pat. 1,160,297, 10.8.66).—Batteries of fermentation and maturing tanks are arranged in series into which yeast and wort are supplied, and after 10 days an acceptable beer is obtained.

S. S. Chissick.

Continuous fermentation and maturing of beer. FORSCHUNGS-INSTITUT FÜR DIE GAERUNGSINDUSTRIE ENZYMOLOGIE UND TECHNISCHE MIKROBIOLOGIE (formerly INSTITUT FÜR DIE GAERUNGS-U. GETRAENKEINDUSTRIE) (Inventors: H. C. WOLTER, P. LIETZ, P. STEFFEN, *et al.*) (Br. Pat. 1,163,825, 13.4.67).—The process comprises conducting a stream of filtered and cooled wort through a yeast propagation vessel, mixing the fermented wort with a stream of unfermented wort, passing the mixture vertically down a fermentation column, first at a decreasing fermentation temp. and then at a higher temp., and finally deep-cooling the beer prior to colloidal stabilisation. Optionally air and CO_2 are introduced at various stages of the process.

S. S. Chissick.

Maturation of beer. SWAN BREWERY CO. LTD. (Br. Pat. 1,162,489, 16.8.68. Australia, 21.8.67).—Beer from fermenter tanks (yeast count $\not> 15 \times 10^6$ cells/ml) is treated with pure (99·9%) CO_2 introduced via orifices of $\not> 10$ (5) μm dia. at 40–45°F (flow rate 1500–2500 lb of CO_2 per 5000 gal of beer per 24 h), the dissolved O_2 content being const.　　　　　　　　S. S. Chissick.

Beer of increased stability. DEUTSCHE GOLD- UND SILBER-SCHEIDEANSTALT (Br. Pat. 1,151,476, 3.4.68. Ger., 4.4.67).—Beer, of increased stability and clarity, contains finely divided SiO_2 modified with an org. polymer and obtained by pptg. silicate soln. with acid in presence of a water-sol. vinylpyrrolidone (or an alkyl analogue) homo- or co-polymer. E.g., unfiltered, output ripe beer is sampled for treatment with the stabiliser (100 g/hl of beer). After mixing, the beers are left standing for 24 h at 1° and filtered. Beer containing polyvinylpyrrolidone K30 on SiO_2 has a stability of 295 days, and one containing polyvinylpyrrolidone K90 on SiO_2 is stable for 341 days, compared with a control value of 87 days.
　　　　　　　　S. D. Huggins.

Pasteurising canned beer. ETABLISSEMENTS J. B. GABRIELS S.P.R.L. (Br. Pat. 1,167,930, 22.11.67. Belg., 5.6.67).—An apparatus is described for heat-pasteurising canned beer. Cylindrical cans are filled to 95–98% of capacity at 0–3°, sealed, supported at top and bottom and then heated to a suitable temp., e.g., 72°, followed by cooling to ambient temp.　　S. S. Chissick.

Chemical composition and method for the removal of beer stone. NIKEX NEHEZIPARI KULKERESKEDELMI VALLALAT (Br. Pat., 1,162,230, 28.10.66. Hung., 30.10.65).—The composition, useful for the removal of beer stone from the surface of brewing equipment made from Al, enamelled Fe or coated Fe(II) concrete, comprises an alkaryl or aryl sulphonic acid, e.g., Me·C_6H_4·SO_3H, and a soln. saturated with and containing a suspension of NH_4SH, plus optionally either $(NH_4)_2SO_4$ or H_2SO_4 and optionally an alkali metal (Na) chromate or dichromate. The surface is coated with the composition at or above ambient temp. and contact is maintained until the incrustation of beer stone is sufficiently decomposed to permit removal.　　　　　　S. S. Chissick.

Accelerating the ageing of distilled liquors. J. T. LIMPE (Br. Pat. 1,164,074, 24.5.68).—The process claimed comprises subjecting the liquor, e.g., whisky, after storage for 30–240 days in white oak barrels, to vigorous mechanical agitation for 1 h periods with a rest of 10 min after each agitation, for 8 h per day. The time of storage and the no. of days agitation determine the accelerated age, e.g., 150 days storage plus 10 days agitation produce a quality distilled liquor equiv. to 5 years of age.　　S. S. Chissick.

Alcoholic beverages. NISSHIN SANGYO K.K. and M. YAMADA, H. KOMODA and F. MANO (Br. Pat. 1,165,206, 20.10.66. Jap., 22.6.66).—The gas evolved during the fermentation of an alcoholic beverage is introduced into an alcoholic soln. at 30 to −60°, optionally in the presence of glycerine (I) or propylene glycol as evaporation retardant, and a flavouring agent (Et palmitate or stearate or 2-phenylethanol). The beverage and soln. are mixed at a later stage. In an example, the gas evolved during the fermentation of whisky mash is bubbled into 79% EtOH containing I at 3° over 3 days and the two liquids are finally combined.
　　　　　　　　S. S. Chissick.

Production of a distilled malt beverage. HIRAM WALKER & SONS (SCOTLAND) LTD. (Inventors: J. W. LAWRIE and A. A. CUNNINGHAM) (Br. Pat. 1,151,783, 26.4.66).—A process for the economic concn. of pot ale (I), obtained as a waste product in the production of malt whisky, is claimed. The I is concentrated by evaporation and the resulting syrup dried to recover the grain solids. The hot vapour produced in the process is passed to a heat exchanger. A suitable apparatus is described (1 diagram).　　S. S. Chissick.

Fruits, Vegetables and Their Products

Determination of moisture in dried prunes. H. R. BOLIN and F. S. NURY (J. Ass. off. analyt. Chem., 1969, 52 (4), 858–864.).—The rapid method, involving a commercial dried fruit moisture tester based on the change in conductance that occurs with change in moisture, and the AOAC method 20·009 involving measurement of wt. loss on drying in a vac. oven, were studied collaboratively. Similar results were obtained by the two methods.　　D. I. Rees.

Free amino acids and other nitrogenous substances of table grape varieties. W. M. KLIEWER (J. Fd Sci., 1969, 34 (3), 274–278. 24 ref.).—Amino acids detd. were α-alanine, γ-aminobutyric acid, arginine, aspartic acid, glutamic acid, proline, serine and threonine. At early maturity total free amino acids were 1·04–5·53, and at

late maturity 1·24–6·45 nM/100 ml juice (according to variety). These eight compd. accounted for up to 96% of the free amino acids.　　　　　　　　M. T. Rawnsley.

Influence of citric acid, ascorbic acid and EDTA on the quality of canned guava (Psidium guajava). L. SETTY, K. V. NAGARAJA and S. RANGANNA (Indian Fd Packer, 1968, 22 (2), 27–45).—During storage, a light pink to brown colour may develop, the syrup becoming viscous. Citric acid, 0·25%, both enhances the discoloration and impairs the flavour. In plain syrup, these changes are not found at room temp. Addition of 0·05% of ascorbic acid ensures colour and flavour retention but the pH falls slightly; EDTA is equally effective but a cloudiness of syrup results. No treatment is effective in preventing these changes under prolonged storage at 37°.　　　　　　　　C. V.

Chemical composition of mango fruit. TAJ MUHAMMAD CHAUDHRY and M. A. R. FAROOQUI (W. Pakistan J. agric. Res., 1969, 7 (1) [(5) ?], 103–107. Engl., 13 ref.).—Sindhri mango contained the highest % of dry matter, ash, sugars, and total sol. solids, while Bombay Alphonso contained most vitamin C. Sugar/acidity ratio decreased in the following order: Baganpali > Sindhri > Bombay Alphonso > Seedling; only the last was unsuitable for dessert purposes.　　　　　　E. G. Brickell.

Rapid routine method for determination of total pectin in citrus products. J. J. MONSELISE (Israel J. Technol., 1969, 7 (3), 271. Engl., 6 ref.).—Modification of the method of Fellers & Rice (Ind. Engng Chem. analyt. Edn, 1932, 4, 628) permits detn. of sol. and insol. pectic compd. in the undil. sample (citrus juice) or dil. sample (for conc. or comminuted products). The total pectic compd. are solubilised with 10% NaOH, the mixture is filtered, and the filtrate (plus 2 ml of 10% HCl) is then heated at ~98°, cooled and centrifuged. The vol. of the pectic acid ppt. is converted into % pectin by reference to a calibration graph for known pectin soln. Results are generally ~20% higher than those for the time-consuming Ca pectate method.　　　　　　　W. J. Baker.

Citrus juice characterisation. Analysis of the phosphorus fractions. C. E. VANDERCOOK and H. C. GUERRERO (J. agric. Fd Chem., 1969, 17 (3), 626–628. 15 ref.).—The P compounds were fractionated into groups, including total P, inorg. P, lipid P and an EtOH-insol. P fraction containing nucleic acids and phosphoproteins. The av. of total P for orange, lemon and grapefruit juices were 19·3, 11·1 and 13·6 mg of P per 100 ml, resp. The av. % of total P distributed among the groups for these three juices were, resp., 65·3, 55·0 and 69·5 for inorg. P; 7·3, 11·6 and 7·9 for lipid P; 15·6, 17·5 and 11·0 for EtOH-insol. P. An inverse correlation was observed between the % of inorg. P and EtOH-insol. P of all juices, but particularly orange. Possible applications to juice characterisation are discussed.　　I. Dickinson.

Influence of some chemical agents on resistance to radiation of anthocyanin pigments in fruit juices. J. WILSKA-JESZKA (Roczn. Technol. Chem. Żywn., 1969, 15, 65–78. Pol., 16 ref.).—Juices from frozen black- and red-currants, bilberries, black lilac berries and strawberries were subjected to γ-radiation from ^{60}Co. The stability of the irradiated juice was proportional to the log. of the anthocyanin concn. $CuSO_4$, $FeCl_3$, hydroquinone, thiourea and tannin all inhibited the decomp. of anthocyanins induced by radiation. Best effects were obtained with Cu^{2+} ions which acted at 10^{-4} mole/l, whilst similar effects with urea required a concn. of 10^{-2} mole/l.　　　　　　　A. Lewin.

Phenolic compounds of blackcurrant juice and their protective effect on ascorbic acid. III. Mechanism of ascorbic acid oxidation and its inhibition by flavonoids. K. A. HARPER, A. D. MORTON and E. J. ROLFE (J. Fd Technol., 1969, 4 (3), 255–267. 35 ref.).—The loss of ascorbic acid by oxidn. was followed by a polarographic method in citrate buffer at pH 2·9, in the presence and absence of Cu^{2+}. Flavonoids present in blackcurrant juice were used as antioxidants in the absence of Cu^{2+}; quercetin (I) and dihydro-I were most effective, rutin the least. Anthocyanins increased the oxidn. rate. In the presence of Cu^{2+}, I showed increasing antioxidant activity up to a I : Cu^{2+} ratio of 7·8 : 1, followed by a decreasing activity at higher levels.　　　　　　J. B. Woof.

Composition of Montmorency cherry essence. I. Low-boiling components. E. E. STINSON, C. J. DOOLEY, V. J. FILIPIC and C. H. HILLS (J. Fd Sci., 1969, 34 (3), 246–248. 12 ref.).—Two g.l.c. units with flame ionisation detectors were used, together with i.r. analysis. The most abundant components were EtOH and MeOH (90,000 and 5000 ppm, resp.); others identified were AcOH 485, EtOAc 295, Et_2O 140, MeOAc 32, Me_2CO ~16, isobutyraldehyde ~16 and

propionaldehyde < 5 ppm. Methods of identification, etc., are given.   M. T. Rawnsley.

Autoxidation of fatty acid lipids and carotene of freeze-dried avocado salad. B. J. LIME (*Fd Technol., Champaign*, 1969, **23** (4), 569–572. 20 ref.).—Peroxides of unsatd. Me esters, formed in the presence of carotene, react with the carotenes and thus have a limited effect on the overall oxidn. of the unsatd. fats (*UF*). This may explain the stability of the *UF* in the salad stored in an air atm.   I. Dickinson.

Objective colour method for the determination of tomato maturity. J. B. HUTCHINGS, F. W. WOOD and R. YOUNG (*J. Fd Technol.*, 1969, **4** (1), 45–49. 6 ref.).—Assessment of whole tomatoes was carried out by tristimulus colour measurements using a Colorcord colorimeter, and correlation established between a subjective scale of maturity (1–10), the dominant wavelength and the luminance. The correlation indicated that the maturity could be assessed from the luminance value only, in the subjective maturity range of 4–10, to an error of $\pm\frac{1}{2}$ maturity units. The influence of commonly occurring colour vision defects on subjective assessments makes the objective method particularly useful.   J. G.

Carotenoid degradation in bleached paprika. R. R. DE LA MAR and F. J. FRANCIS (*J. Fd Sci.*, 1969, **34** (3), 287–290. 23 ref.).—Bleached and normal paprika samples were extracted and separated; column chromatog. revealed 54 and 37 pigments, resp. Not all were identified. Bleached paprika gave more isomeric and oxidative products.   M. T. Rawnsley.

Pectic substances of the pigmented onion skins. I. Characteristics and acid decomposition products. A. F. ABDEL-FATTAH and M. EDREES (*J. Chem. Un. Arab Repub.*, 1968, **11** (3), 383–388. Engl., 18 ref.).—Four pectic samples characterised by low ash contents, a high degree of esterification and slight reducing effects, were isolated from the skin by extraction with NH₄ oxalate (various concn. at 90 or 100°). A fifth sample, highly degraded, was isolated by extraction with aq. HCl at 100°. The acid hydrolysate was also found to contain arabinose and rhamnose.   S. S. Chissick.

Relationship between maturity and quality of canned broad beans (*Vicia faba* L.). V. D. ARTHEY and C. WEBB (*J. Fd Technol.*, 1969, **4** (1), 61–74. 13 ref.).—Studies showed that (*i*) there were no differences in flavour in canned beans harvested at tenderometer readings (*T*) of 90–200, (*ii*) texture deteriorated linearly as beans matured, (*iii*) alcohol-insol. solids content of raw and canned beans increased linearly as beans matured, (*iv*) *T* were proportional to sample size and (*v*) consumers preferred canned beans harvested at *T* = 120 or 160 equally well. Optimum maturity for canning was determined to be at *T* = 136 using a 5-oz sample of raw beans.   P. C. W.

Origin of off-odours in frozen green beans. L. CHOW and B. M. WATTS (*Fd Technol., Champaign*, 1969, **23** (7), 973–974. 7 ref.).—Malonaldehyde, an index of unsaturated fatty acid oxidn., and acetaldehyde, a product of anaerobic fermentation, increase to a max. and then decrease during frozen storage of raw vegetables. Both the lipid oxidn. and anaerobic fermentation are contributing reactions to flavour deterioration. Secondary reactions of these aldehydes with other food constituents may also be involved.   I. Dickinson.

***In situ* acrylamide polymerisation effect on appearance and rehydration of dehydrated vegetables.** S. SCHWIMMER (*Fd Technol., Champaign*, 1969, **23** (7), 975–976. 13 ref.).—After polymerisation and dehydration, treated vegetables increased in dry wt. and, when rehydrated, absorbed more water than control samples. The treated vegetables retained original shape in both the dehydrated and rehydrated forms.   I. Dickinson.

Application of dinitrophenol and anthrone in the colorimetric determination of sugars in potato tuber juice. B. SAMOTUS and M. KUJAWSKI (*Roczn. Technol. Chem. Żywn.*, 1969, **15**, 5–16. Pol., 8 ref.).—A quick method is described for determining the sum of glucose and fructose using dinitrophenol, the sum of glucose, fructose and sucrose using anthrone at 100° and fructose compounds using anthrone at 60°. After a short inversion, the method can also be used to determine maltose using a dinitrophenol reagent. The procedure can be carried out without prior pptn. of colloidal substances. The method has high sensitivity and can be used for long series, thus being useful for routine analysis of small samples as well as determination of sugar contents in many samples simultaneously.   A. Lewin.

Baked food products. GENERAL FOODS CORP. (Br. Pat. 1,167,909, 12.12.66. U.S., 21.12.65).—The products are packaged food-filled comestibles adapted to be warmed and eaten after removal from a package, and comprise a semi-moist filling of high moisture content having a plastic condition when warmed to above ambient temp., a baked crust of lower moisture content enveloping the filling, and a moisture-proof package containing the filling and crust envelope. The relative moisture contents of the filling and baked crust are such that moisture is transferred from the crust to the filling when the comestible is removed from the package and rapidly warmed, the filling thus increasing in moisture content. The filling is, e.g., a fruit product, esp. apple powder, which may contain a humectant (e.g., glycerol) and a bacteriostatic level of solutes such as sugars.   S. S. Chissick.

Air-stable dehydrated potato products. AMERICAN POTATO CO. (Br. Pat. 1,163,906, 5.10.66. U.S., 7.10.65).—A process is claimed for prep. the title products which are resistant to oxidative deterioration of the fat fraction when stored in air. Butylated hydroxytoluene (I) dissolved in EtOH is added to the cooked potato prior to or during the mashing operation and the dehydration is effected at ≯ 150°F after a time lapse of ≮ 15 min. The final product contains 13–35 ppm of I.   S. S. Chissick.

Non-alcoholic Beverages

Rapid screening procedure for determination of benzoic acid and sorbic acid in fruit beverages. W. M. GANTENBEIN and A. B. KARASZ (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 738–741. 3 ref.).—The beverage is acidified with HCl and treated with Et₂O, petroleum ether and Na₂SO₄ and the mixture is filtered. The filtrate is diluted with Et₂O and the contents of benzoic and sorbic acids are determined by measuring the absorbances at 225 and 250 nm, resp. and comparing with calibration curves. Recoveries of 82–142% were obtained for 0·06–0·95 mg/ml of each acid in orange and apple juice beverages.   D. I. Rees.

Food standards committee report on soups. MINISTRY OF AGRICULTURE, FISHERIES & FOOD. 1968, 29 pp., 2s. 9d. (London: H.M.S.O.).—Recommendations for the compositions of a wide variety of canned and powdered soups are presented. Codes of practice for canned soups and soup mixes are appended.   P. C. W.

Instant coffee. PROCTER & GAMBLE CO. (Br. Pat. 1,165,656, 16.5.68. U.S., 16.5.67).—Instant coffee flakes of thickness 2×10^{-3} to 10^{-2} in and *d* 1 to 1·5 g/cm³, containing 0·1–20 (0·2–1)% of an aromatising coffee oil, are prep. by a roll-milling process (apparatus described). The flakes can be used as such or combined with ≯ 95% of conventional instant coffee particles.   S. S. Chissick.

Manufacture of beverages containing living lactic acid bacilli. K. K. YAKULT HONSHA (Inventors: M. SHIROTA, H. ENDO and M. SHIIZUKA) (Br. Pat. 1,157,135, 23.11.66).—The process comprises autolysing fresh, unicellular green algae [chlorellae (I) or scenedesmus], extracting the product with water, mixing the resulting aq. soln. with dextrose or sugar and animal milk, inoculating and cultivating, e.g., *Lactobacillus acidophilus* (II) on this soln. and finally adding sweetening materials and essences. In an example, I are concentrated by centrifugal pptn. and autolysed at pH 7 and 37° for 24 h. After adding water and grinding, the aq. extract is mixed with skim-milk powder and dextrose and then heat sterilised and inoculated with II and cultivated at 37° for 72 h. Finally, the culture broth is mixed with a cane sugar syrup and homogenised to give a composition of good flavour in which the II content is const. over 5 days at room temp. and which is mixed with twice its own vol. of water to yield the beverage.   S. S. Chissick.

Milk, Butter, Other Dairy Products, Eggs

Heat stability of milk as affected by variations in pH and milk salts. P. A. MORRISSEY (*J. Dairy Res.*, 1969, **36** (3), 343–351. 15 ref.).—The min. stability exhibited by milk at pH ~ 6·9 was shown to arise from heat-induced deposition of Ca phosphate on a caseinate/β-lactoglobulin complex (I). The reaction makes I sensitive to pptn. by Ca²⁺. The phenomenon was found to be independent of the initial colloidal phosphate content of the milk, the characteristic micellar structure of milk caseinate and the pH at which I is formed.   M. O'Leary.

Milk-clotting and proteolytic activities of rennet, and of bovine pepsin and porcine pepsin. P. F. Fox (*J. Dairy Res.*, 1969, **36** (3), 427–433. 14 ref.).—Milk-clotting and proteolytic activities of rennet (I), bovine pepsin (II) and porcine pepsin (III) were compared; II resembled I more closely that III in many important characteristics. It is concluded that II merits further investigation as a possible I substitute.   M. O'Leary.

Effects of ultra-high temperature (*UHT*) processing and of subsequent storage on the vitamin content of milk. J. E. Ford, J. W. G. Porter, S. V. Thompson, *et al.* (*J. Dairy Res.*, 1969, **36** (3), 447–454. 24 ref.).—*UHT* processing, and subsequent storage of milk for 90 days at 15–19°, caused no loss in the contents of vitamin A, carotene, vitamin E, thiamine, riboflavin, pantothenic acid, biotin or nicotinic acid. Processing caused little or no loss of vitamins B_6 and B_{12}, but up to 50% of these vitamins was lost during the 90 days storage. All the dehydroascorbic acid and $\sim 20\%$ of the ascorbic (I) and folic acid (II) were lost on processing. In the absence of O_2 there was no further loss of I and II on storage, but the presence of O_2 in stored milks caused rapid losses in these vitamins.   M. O'Leary.

Occurrence and biochemical origin of aliphatic lactones in milk fat. Review. P. S. Dimick, N. J. Walker and S. Patton (*J. agric. Fd Chem.*, 1969, **17** (3), 649–655. 55 ref.).—The objectionable flavour in stored forms of milk and the attractive flavour properties of butter as a cooking and baking additive, can be traced to the same homologous series of aliphatic lactones. Radiochemical studies show that these trace flavour compounds are products from the endogenous biosynthesis of satd. fatty acids via delta-oxidation in the mammary gland. This formation of aliphatic lactones and their relationship to lipid metabolism have challenging practical implications.   I. Dickinson.

Detection of vegetable fats in milk fat by gas chromatography of the sterols. G. Cerutti, G. Volonterio and P. Resmini (*Riv. ital. Sostanze grasse*, 1969, **46** (7), 356–362. It., 28 ref.).—The sample of fat or emulsion was saponified with 2N alc. KOH; unsaponifiable matter was separated by continuous extraction with hexane. After evaporation of hexane the sample was dissolved in EtOH and heated with an alc. soln. (1·5%) of digitonine. Pptd. digitonides were heated with hexamethyldisilazane and trimethylchlorosilane, in presence of tetrahydrofuran, for 3 h at 55° in a sealed tube. The total product was examined directly by g.l.c. Vegetable oil ($\sim 1\%$) in butter was revealed by the presence of β-sitosterol. The method can be used to determine the origin of fats used in margarines, and the raw materials used for monoglyceride emulsifiers for ice cream manufacture.   L. A. O'Neill.

Sialic acid in milk. T. Okuyama (*Jap. J. Dairy Sci.*, 1968, **17** (4), A55–A76. Jap., 105 ref.).   C. V.

Variation of ^{90}Sr and ^{137}Cs in milk, some factors affecting the radionuclide concentration, and the effect of remedial actions. B. Underdal (*Thesis, Dep. Fd Hyg., vet. Coll. Norway, Oslo*, 1969, 122 pp. Engl., 139 ref.).—Levels of ^{90}Sr and ^{137}Cs were determined in milk from 110 farms in 28 Norwegian dairy districts during 1965–67. ^{90}Sr content varied from 11 to 271 pCi/l, av. ~ 30 pCi/l. Significant correlations were found between ^{90}Sr content and (*i*) area of farm land per 1000 l of milk and (*ii*) annual pptn. ^{90}Sr levels decreased considerably from 1965 to 1967. ^{137}Cs levels varied between 27 and 3282 pCi/l, and were higher in farm than in dairy milk. Significant correlations were found between ^{137}Cs level and (*i*) annual pptn. and (*ii*) milk yield per cow. The ^{137}Cs : ^{90}Sr ratio varied from district to district; it was higher in summer than winter. No correlation was found between stable Sr and ^{90}Sr levels. Changing farming and feeding practices had a significant effect on ^{137}Cs but not ^{90}Sr levels. Remedial action is discussed.   P. C. W.

Frozen storage of milk. R. Tamate and F. Ohtaka (*Jap. J. Dairy Sci.*, 1969, **18** (2), A37–A47. Jap., 10 ref.).—Effects of addition of various salts, removal of ionic Ca, ultrasonic treatment, removal of milk fat and substitution with vegetable fat on the quality of frozen milk were studied. Protein flocculation was markedly retarded by Na or K citrate and by removal of ionic Ca ($\ll 9\cdot8\%$), but addition of Na or K phosphate, ultrasonic treatment, fat removal or substitution by vegetable fat did not improve milk life. Other aspects are discussed. (From Engl. summ.)   C. V.

Transportation of raw milk through plastic pipelines. S. A. Dachi (*Jap. J. Dairy Sci.*, 1968, **17** (3), A37–A47. Jap., 40 ref.).   C. V.

Inter-relationship of the viscosity, fat content and temperature of cream between 40° and 80°. L. W. Phipps (*J. Dairy Res.*, 1969, **36** (3), 417–426. 17 ref.).—The dependence of the viscosity (η) and density (*d*) of cream on its fat content in the range 0–50% (fraction of dispersed oil phase $\varphi = 0$–$0\cdot5$) and temp. (40–80°) was investigated. The results are presented in the form of empirical equations from which η and *d* may be interpolated; nomograms are constructed to allow ready detn. of η and *d*.   M. O'Leary.

Influence of free fatty acids on sweet cream butter flavour. M. R. McDaniel, L. A. Sather and R. C. Lindsay (*J. Fd Sci.*, 1969, **34** (3), 251–254. 11 ref.).—A statistical evaluation of test panel results is given. Butyric acid had the lowest total av. flavour threshold. A correlation between threshold and chain length exists, and interaction of components at sub-threshold levels seems to occur.   M. R. Rawnsley.

Recent advances in lactic acid bacteria research in Japan. T. Nakae (*Jap. J. Dairy Sci.*, 1968, **17** (1), A5–A15. Jap., 104 ref.).   C. V.

Proteolytic action of dairy lactic acid bacteria in production of fermented dairy products. K. Ohmiya and Y. Sato (*Jap. J. Dairy Sci.*, 1968, **17** (6), A118–A128. 67 ref.; 1969, **18** (1), A1–A11, 44 ref. Jap.).—Engl. summary.   C. V.

Effect of delays in pour plating on bacterial counts [in dairy products]. J. M. Berry, D. A. McNeill and L. D. Witter (*J. Dairy Sci.*, 1969, **52** (9), 1456–1459. 3 ref.).—The results of an experiment with *Pseudomonas fluorescens* as test organism and tripticase soy agar as plating medium showed that delays in excess of 10 min between measuring of the sample into petri dishes and pouring of the medium gave significant reductions in the resulting plate count. The reduction is considered to be due to adsorption of cells on to the petri dish surface, with the formation of bottom spreaders.   M. O'Leary.

Yoghurt production. J. Knox (*Fd Process. Ind.*, 1969, **38** (456), 54–56).—The review covers: prepn. of the acidification cultures of *Streptococcus lactis* or *Lactobacillus bulgaricus*; prepn. of the base from skimmed milk and milk powder, with the addn. of fruit and flavouring, and subsequent processing and packaging; control tests and product faults such as flavour defects and syneresis; coagulation and coliform infection tests.   J. B. Woof.

Application of some biotechnical methods to increase the decomposition of lactose or prevent reacidification of the mould-ripened fat quarg cheese. E. Pijanowski, M. Długewski, E. Jakubczykowa, E. Korolczukowa, A. Pilarska and A. Skrżyńska (*Roczn. Technol. Chem. Żywn.*, 1969, **15**, 101–114. Pol., 8 ref.).—The use of some selected yeast strains together with the mould *Oospora lactis* results in complete decomp. of sugars in the ripened quarg cheese. This method is most promising for preventing the reacidification of the ripened cheese. Trials with nisin showed that this antibiotic does not prevent reacidification of cheeses ripened with *O. lactis*.   A. Lewin.

Comparative study on different methods for preserving the quality of eggs. Khawaja Nasim Ahmed and M. Rafique Chaudhry (*W. Pakistan J. agric. Res.*, 1969, **7** (1) [(5)?], 119–122. Engl., 9 ref.).—Liquid paraffin dipping maintained pH and albumen thickness but was less effective than Na_2SiO_3 treatment in preventing wt. loss and air cell increase. Thermostabilisation (15 min in water at 135°F) was the least effective.   E. G. Brickell.

Fresh and frozen egg yolk protein fractions: emulsion stabilising power, viscosity and electrophoretic patterns. E. M. Davey, M. E. Zabik and L. E. Dawson (*Poult. Sci.*, 1969, **48** (1), 251–260. 35 ref.).—The emulsifying properties and the effects of freezing and thawing on the emulsifying properties of egg yolk and three crude egg yolk protein fractions (lipovitellin, lipovitellenin and livetin), separately and in combination, were studied.   A. H. Cornfield.

Method of treating milk. Alfa-Laval A.-B. (Inventors: C. O. Claesson and E. M. Claesson) (Br. Pat. 1,151,963, 20.1.67. Swed., 28.1.66).—The process consists of treating milk with gaseous or solid CO_2 to adjust the pH to $6\cdot0$–$6\cdot5$ ($6\cdot4$–$6\cdot5$). The process results in standardisation of the properties of different batches of milk., and the treated milk is very suitable for processing into cheese, etc. (2 drawings.)   S. S. Chissick.

Treating [and storing] milk or milk products. AB Tetra Pak (Br. Pat. 1,166,338, 30.10.67. Swed., 16.11.66).—A sterile and high quality product is obtained by heat-treating the milk or milk product to which has been added a gas (CO_2) or a volatile liquid which reduces the pH to 5–$6\cdot8$ and packing in a container which is

permeable to the gas or volatile liquid vapour and impermeable to bacteria (polyethylene or polypropylene). S. S. Chissick.

Manufacture of co-precipitates of milk proteins. COMMONWEALTH SCIENTIFIC & INDUSTRIAL RESEARCH ORGANIZATION (Br. Pat. 1,151,879, 16.9.66. Australia, 17.9.65).—The continuous flow process consists of (*i*) heating skim-milk, optionally previously treated with $CaCl_2$, to cause interaction of the proteins, (*ii*) allowing the interaction to occur, (*iii*) passing through a pptg. stage at which $CaCl_2$ and/or an acid (HCl, H_2SO_4) is added and thoroughly mixed, (*iv*) allowing the co-ppt. to form, and (*v*) separating the co-ppt. The products are useful as nutritional supplements, as emulsifiers, etc. (2 drawings.) S. S. Chissick.

Method and apparatus for manufacturing Cheddar or like cheese. COMMONWEALTH SCIENTIFIC & INDUSTRIAL RESEARCH ORGANIZATION (Br. Pat. 1,150,403, 28.6.67. Aust., 6.7.66).—The method comprises (*i*) forming drained cheese curd into slabs, (*ii*) moving the slabs along a first path to allow partial consolidation of the curd by gravitation, (*iii*) inverting the slabs and moving along a second path to further consolidate the slabs and allow initial fibre development and (*iv*) compressing the slabs. Apparatus for carrying out the process is described. S. S. Chissick.

Edible Oils and Fats

Quality problems of edible oils. G. JACINI (*Riv. ital. Sostanze grasse*, 1969, **46** (7), 342–346. It.).—Two classes of edible oils are discussed: (*i*) naturally edible oils and (*ii*) oils rendered edible by refining. L. A. O'Neill.

Characterisation of Israel lemon oil and detection of its adulteration. A. LIFSHITZ, Y. STEPAK and H. B. BASKER (*J. Fd Sci.*, 1969, **34** (3), 254–257. 6 ref.).—Standard methods and g.l.c. were used and results were statistically evaluated. Sophisticated adulterations of 10–20% were clearly detectable. M. T. Rawnsley.

Fatty acid composition of cocoa butter oil by urea fractionation and programmed temperature gas chromatography. J. L. IVERSON, P. G. HARRILL and R. W. WEIK (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 685–688. ref.).—The urea fractionation procedure previously described (Iverson & Weik, *ibid.*, 1967, **50**, 1335) was used to detect trace amounts (0·001–0·1%) of Me esters obtained from cocoa butter oil; g.l.c. on a packed DEGS column was used. The contents of saturated acids (C_{10}–C_{28}), branched acids (C_{16}–C_{24}), monounsaturated acids (C_{16}–C_{24}) and linoleic and linolenic acids found in six cocoa butter oils are listed. D. I. Rees.

Thin-layer and gas–liquid chromatography of cholesterol in fats and oils. I. Development of method. II. Collaborative study. C. W. THORPE, L. POHLAND (I) and D. FIRESTONE (I) (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 774–778, 9 ref.; 788–781, 6 ref.).—I. A CHCl₃ extract of the unsaponifiable matter is streaked on a silica gel layer and developed with Et₂O–petroleum ether (1 : 1). The steroid band (R_F 0·2–0·3) is located under u.v. light, extracted with CHCl₃ and the residue obtained on evaporation is dissolved in EtOAc containing cholestane as internal standard and an aliquot is analysed by g.l.c. on a glass column of JXR, OV-1 or OV-17 on Gas Chrom Q at 210–240° with N₂ as carrier gas and flame ionisation detection. The determination of cholesterol by this means allowed the detection of 2–3% of animal fat in vegetable oil. II. The method was subjected to collaborative study and the result obtained confirmed the previous findings. D. I. Rees.

Recovery and measurement of volatiles from lipids. Hydrocarbons in irradiated fats. W. W. NAWAR, J. R. CHAMPAGNE, M. F. DUBRAVCIC and P. R. LeTELLIER (*J. agric. Fd Chem.*, 1969, **17** (3), 645–648. 20 ref.).—The higher boiling compd. were collected by cold-finger vac. distillation and measured quant. by relating their g.c. peak areas to that of an appropriate internal standard. The lower boiling compd. were recovered on a short precolumn packed with stainless steel helices, fitted inside the g.c. oven before the inlet of an Al₂O₃ column. Recovery data are given for C_1–C_{22} hydrocarbons. I. Dickinson.

Differential thermal analysis as a control instrument in edible fat hydrogenation. D. E. B. STEELE and J. M. V. BLANCHARD (*J. Fd Technol.*, 1969, **4** (3), 291–302. 3 ref.).—D.t.a. was used as a technique for following the progressive hydrogenation of groundnut oil, to ensure the consistent quality of the product. Apparatus and thermograms are described for detn. of the solid fat index at different temp. The method compares favourably in speed and reproducibility with dilatometry. J. B. Woof.

Meat, Poultry, Fish

Effect of sample dimensions on the cleaving of meat in the objective assessment of tenderness. C. L. DAVEY and K. V. GILBERT (*J. Fd Technol.*, 1969, **4** (1), 7–15. 14 ref.).—A tenderometer was used to examine the effect of sample dimensions on shear-force values. When shear-force values were corrected to a 1-cm² sample cross-section at the line of proposed cleavage, characteristic values were obtained which did not require assessment of statistical significance. Thus inter-animal and intra-muscular variations in tenderness can be studied, and also the effect of processing conditions. The theoretical conclusion drawn from the results is that the tensile strengths of meat fibres determine to a large degree the cleavage load, and the shear-force values will therefore increase with the fibre no. and hence with the cross-sectional area of the samples. J. G.

Temperature changes in meat heated by radiowaves (2450 MHz) and in hot water. C. KIEREBIŃSKI (*Roczn. Technol. Chem. Żywn.*, 1969, **15**, 171–187. Pol., 16 ref.).—Theoretical considerations, confirmed by experimental results, indicate a logarithmic relationship for both conventional and microwave heating of meat. Only at lower temp., i.e., at the start of heating, does the logarithmic relationship not hold. Using microwaves, the increase of temp. per unit time is 5–20 times greater than by conduction (in water), the quality of meat being satisfactory. The temp. increase is greater in the outer layers of meat, the meat tissue showing strong absorption of microwave radiation. A. Lewin.

Meat pigment changes in intact beef samples. G. L. ZIMMERMAN and H. E. SNYDER (*J. Fd Sci.*, 1969, **34** (3), 258–261. 16 ref.).—Reflectance spectrophotometry was used to study pigment changes in beef which had (*i*) been oxygenated (80 psig O₂ for 12 h or 12 days) and wrapped in an O₂-impermeable film, (*ii*) been treated with K₃Fe(CN)₆, and (*iii*) either treatment (*i*) or (*ii*) plus malonic acid (I). Metmyoglobin (*M*) was increased in (*i*) but I inhibited O₂ utilisation and *M*-reducing activity. M. T. Rawnsley.

Dielectric loss factor of reconstituted ground beef. Effect of chemical composition. D. VAN DYKE, D. I. C. WANG and S. A. GOLDBLITH (*Fd Technol.*, Champaign, 1969, **23** (7), 944–946. 6 ref.).—Below a critical moisture content (20%), water had little effect on the dielectric loss factor (ϵ''_r); between 20 and 45%, the water concn. had a dramatic effect; above 45% its influence was negligible. Addn. of NaCl to the samples caused an increase in the ϵ''_r values. At const. protein : ash ratios and water content > 45%, an increase in the fat content resulted in a decrease in ϵ''_r. I. Dickinson.

Quality of pre-chill canned porcine muscles. S. GOPAL REDDY and R. L. HENRICKSON (*Fd Technol.*, Champaign, 1969, **23** (7), 941–943. 14 ref.).—More free fluid and/or gel was exuded from the post-chilled cured canned muscles (post-*CCCM*), exhibiting a highly significant treatment effect, Data revealed non-significant quant. differences between pre- and post-*CCCM* with regard to the press fluid, total moisture and nitroso-pigments. A highly significant increase in shear force was attributed to pre-chill canning; this was evident in all pre-*CCCM*. I. Dickinson.

Sensory evaluation of lamb and yearling mutton flavours. O. M. BATCHER, A. W. BRANT and M. S. KUNZE (*J. Fd Sci.*, 1969, **34** (3), 272–274. 16 ref.).—Subtle flavour differences due to age or sex were detected by panel members using triangle tests. This may be due to trace flavour components in the fat. Differences in flavour were less evident in broths or patties. M. T. Rawnsley.

Calorimetric properties of lamb and other meats. A. K. FLEMING (*J. Fd Technol.*, 1969, **4** (3), 199–215. 20 ref.).—Phys. factors governing heat transfer rates, during cooling and freezing of meats, were studied using an automatic adiabatic calorimeter. Samples of the more common export grades of New Zealand lamb, brains, kidneys and veal were frozen to −40° and known pulses of heat injected. A computer program was used to convert voltage readings from thermocouples directly into temp.-enthalpy curves for adiabatic conditions. Water content of the samples correlated well with fat content and latent heats of freezing. In low fat meat, 11% of the total water was not frozen at −20° and this proportion increased with increasing fat content. J. B. Woof.

Kind and concentration of soluble protein extract and their effect on the emulsifying capacity of poultry meat. A. J. MAURER, R. C. BAKER and D. V. VADEHRA (*Fd Technol.*, Champaign, 1969, **23** (4), 575–577. 11 ref.).—The concn. of sol. protein (*P*) soln. significantly influenced the vol. of oil emulsified; sol. *P* at low concn. were more efficient as emulsifiers than at higher concn. As the total amt. of

available P in soln. was increased, the vol. of oil emulsified per 100 mg of P, decreased. P extracted in the presence of salt had a greater emulsifying efficiency than those extracted with water at the P concn. investigated. Addn. of NaCl to the P extracts had an enhancing effect on emulsion formation.   I. Dickinson.

Organoleptic evaluation of low-dose irradiated chicken stored under refrigeration conditions. C. M. MACLEOD, F. A. FARMER and H. R. NEILSON (*Fd Technol.*, *Champaign*, 1969, **23** (7), 964–968. 13 ref.).—From triangle tests in which odour, taste, colour and texture of irradiated (0·46 Mrad) stored cooked chicken were compared with non-irradiated, non-stored chicken, the unmatched sample (*US*) was correctly selected. The panel also identified the *US* when a dose of 0·69 Mrad was used for cooked chicken. No dislike was expressed for irradiated samples of raw chicken.
     I. Dickinson.

Tenderness of freeze-dried chickens treated with proteolytic enzymes. L. E. DAWSON and G. H. WELLS (*Poult. Sci.*, 1969, **48** (1), 64–70. 20 ref.).—The optimum concn. of enzymes (papain, ficin, bromelin and Rhozyme P-11), temp. and pH of rehydration soln. were determined for production of satisfactory tenderness in freeze-dried white meat from old hens.   A. H. Cornfield.

Oxidation of chicken tissue lipids as influenced by age and sex. J. E. MARION (*Poult. Sci.*, 1969, **48** (1), 301–304. 9 ref.).—There were no differences due to sex in the extent of lipid oxidation of tissues during refrigerated storage of broiler carcasses. Lipid oxidation declined with increasing age of birds (4–20 weeks) at slaughter.   A. H. Cornfield.

Sulphydryl content of excised chicken breast muscle during *post mortem* **ageing.** K. A. CALDWELL and H. LINEWEAVER (*J. Fd Sci.*, 1969, **34** (3), 290–291. 10 ref.).—No significant changes in total or non-protein sulphydryl concn. were detected during the first 6 h *post mortem*. The detection agent used was 5,5′-dithiobis(2-nitrobenzoic acid).   M. T. Rawnsley.

Hydrogen sulphide in cooked chicken meat. L. J. PARR and G. LEVETT (*J. Fd Technol.*, 1969, **4** (3), 283–289. 14 ref.).—Chicken meat from breast and leg was minced and powdered by grinding in liquid N_2. Portions were heated under N_2 in sealed cans which, after cooling, were punctured, ice-cold water being drawn in through a needle to absorb H_2S and mercaptans. The charge was then transferred to a gas entrainment apparatus. The H_2S content was detd. by entrapping in bismuth nitrate, reaction with acid N,N-diphenyl-p-phenylenediamine followed by colorimetric detn. with $FeCl_3$–HNO_3 reagent. Levels of 0·2–1·0 ppm were found (20–100-fold higher than the odour threshold).   J. B. Woof.

Collaborative study of a rapid electrophoretic method for fish species identification. R. J. LEARSON (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 703–707. 2 ref.).—The method described involves applying an aq. extract of the flesh on to a cellulose acetate strip soaked in Veronal buffer (pH 8·6) and observing the protein patterns after electrophoresis and staining with Ponceau S reagent. Unsuccessful identification of the fish species by the collaborators with use of photographic standards indicated the necessity for analysing authentic samples at the same time as unknown samples.
     D. I. Rees.

Key to the identification of canned salmon species by scale characteristics. R. T. NEWTON and J. L. BURNETT (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 696–702. 3 ref.).—The method described involves microscopic examination (20–30 × magnification) of the scales. A key based on characteristic scale patterns is given to distinguish between five species of canned salmon.   D. I. Rees.

Effects of high-energy radiation on the lipids of fish. M. F. DUBRAVCIC and W. W. NAWAR (*J. agric. Fd Chem.*, 1969, **17** (3), 639–644. 24 ref.).—The volatile compd. formed in the lipid fraction of mackerel by γ-irradn. under vac. at 0·3, 2 and 6 Mrad and at 0 and 25°, were investigated. Using g.c. and mass spectrometry, 56 compd. were identified. The major products of irradn. were the longer chain compd., probably originating from radiolytic cleavage of the fatty acids near the carbonyl groups.
     I. Dickinson.

Lipid analysis of the greater silver smelt, *Argentina silus* **(Ascinius), and an evaluation of its potential for food and fish meal production.** P. R. MACKIE and R. HARDY (*J. Fd Technol.*, 1969, **4** (3), 241–253. 13 ref.).—Lipid compn. of the greater silver smelt was investigated in relation to the cold storage life of the fish and the nutritional properties of meal made from it. Lipids were extracted with $CHCl_3$–MeOH–H_2O (10 : 10 : 9), and then analysed by t.l.c. and g.l.c., after initial separation into classes by chromatog. on silicic acid columns. The flesh was acceptable by taste panels

but the high content of unsatd. fatty acids indicated that rancidity would occur on storage if precautions were not taken. The amino acid content indicated that meal can be produced which is at least as nutritious as herring meal.   J. B. Woof.

Rate of phospholipid hydrolysis in frozen fish. J. OLLEY, J. FARMER and E. STEPHEN (*J. Fd Technol.*, 1969, **4** (1), 27–37. 27 ref.).—The rates of free fatty acid (*FFA*) formation in frozen haddock (**I**) and lemon sole (**II**) were measured between −7 and −29°. *FFA* formation (from phospholipid breakdown) was much faster in I than in II. A rapid first order reaction was observed with I and the hydrolysis proceeded to an asymptote whose value decreased with temp. The amount of free water available in the frozen state had an important bearing on the hydrolysis. Preferential hydrolysis of $C_{16:0}$, $C_{18:1}$ and $C_{20:5}$ phospholipids was observed. Determination of the activation energy was not possible owing to the different behaviour of the various mol. species. The relevance of these findings to protein denaturation is indicated, as the rate of *FFA* formation could be more important than abs. amounts in modification of cell texture.   J. G.

Connective tissues of fish. II. Gaping in commercial species of frozen fish in relation to *rigor mortis.* R. M. LOVE, J. LAVÉTY and P. J. STEEL (*J. Fd Technol.*, 1969, **4** (1), 39–44. 4 ref.).—Seven commercially important species of fish were studied to investigate the factors which affect gaping, i.e., the failure of sheets of connective tissue to hold blocks of muscle together. Gaping was assessed on a subjective scale of 1–10 and related to the time interval between freezing and *rigor mortis*. Fish that were frozen immediately after death gaped least and a marked increase accompanied the onset of *rigor mortis*. Thereafter, a steady, unaccountable increase was noted. Gaping was more marked in well nourished than in poorly nourished fish. A marked difference in gaping was also noted between species, haddock being most prone and catfish and skate not at all.   J. G.

Determination of degree of heating of fish muscle. J. J. DOESBURG and D. PAPENDORF (*J. Fd Technol.*, 1969, **4** (1), 17–26. 19 ref.).— To enable development of testing to control max. temp. in cookers, the extractability and coagulation properties of proteinaceous materials were studied. The results corresponded with the relationship between the max. temp. (T_m) of the heat treatment and the coagulation temp. (T_c), given by the equations $T_m = [1·02T_c − 0·2]$ (±2·0) for lean fish (hake) at 60–100°, and $T_m = [T_c + 0·1]$ (±2·6) for oily fish (mackerel and pilchard) at 60–80°. Details of the coagulation tests used and factors which affected the results (extraction pH, effect of salts) are discussed, and a modified method is described.   J. G.

Drained weight determination of frozen Alaska king crabmeat. G. A. MILLER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 692–695. 5 ref.).—The method described involves thawing a sample in eight times its wt. of water at 80°F. The drained wt. is calculated from the declared wt. and the wt. of the thawed sample. Recoveries were 96·2%, compared with 94·6% by the method of Werren and Weik (*ibid.*, 1967, **50**, 275).   D. I. Rees.

Food Additives

Preservatives, Colouring Matter

Nicotinamide and nicotinic acid in colour preservation of fresh meat. J. L. KENDRICK and B. M. WATTS (*J. Fd Sci.*, 1969, **34** (3), 292–294. 15 ref.).—Results show that addn. of nicotinic acid to meats may have adverse physiological effects. Metmyoglobin is quickly formed on exposure to air. Nicotinamide would need to be used in concn. of ⩾ 60 mg-%.   M. T. Rawnsley.

Processing refrigerated fresh peach slices. E. K. HEATON, T. S. BOGGESS, JUN. and K. C. LI (*Fd Technol.*, *Champaign*, 1969, **23** (7), 956–960. 9 ref.).—Ascorbic acid protected the peach colour more effectively than did a peroxidase inhibitor, oxygen acceptor enzyme or water emulsion of butylated hydroxyanisole. Na benzoate was equal or superior to K sorbate, $NaHSO_3$, diethyl pyrocarbonate, or mixtures of these, for preventing microbial spoilage. Stability of flavour and texture varied among the different varieties between 10 and 24 weeks.   I. Dickinson.

Natural colour enhancers—orange peel carotenoids for orange juice products. S. V. TING and R. HENDRICKSON (*Fd Technol.*, *Champaign*, 1969, **23** (7), 947–950. 15 ref.).—A method of extraction, concn. and purification of carotenoids is described. The carotenoids recovered from 90 lb of 'Pineapple' orange were sufficient to substantially increase the colour of 15–30 gal of orange juice. The use of orange oil for formulating the purified pigments

makes it possible to fortify the colour and flavour of the product in one operation.   I. Dickinson.

T.l.c. separation and spectrophotometric determination of 2-amino-anthraquinone in D & C Blue No. 9. S. J. BELL (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 831–833).—The dye was boiled with Me₂SO for 10 min, the soln. was filtered and the conc. filtrate was quant. transferred to a t.l.c. plate with a silica gel layer which was developed with Et₂O. The yellow band with R_F corresponding to that of 2-aminoanthraquinone was scraped off, slurried with HOAc and the absorbence read at 428 nm.   D. I. Rees.

Spices, Flavours, Other Additives

Microscopy of east asiatic spices. IV. (a) Indian lemon leaves (*Citrus hystrix* DC) 'Daoen Djeroek Poeroet' and (b) Indian laurel leaves (*Eugenia polyantha* Wight) 'Daoen Salam'. D. STRAUSS (*Dt. LebensmittRdsch.*, 1969, **65** (9), 288–290. Ger., 10 ref.).—The cellular structure of these plants is described.   J. B. Woof.

Differentiation of certain types of cassias and cinnamons by measurement of mucilaginous character. W. H. STAHL, J. N. SKARZYNSKI and W. A. VOELKER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 741–744. 5 ref.).—The method described involves preparing a slurry of a certain particle-size fraction of the spice with a Pr^{i}OH–H₂O mixture at 80° and measuring the height of the sediment after 2 h standing in a graduated cylinder. Korintji and Batavia cassias gave a column height of ∼ 85 ml whilst Seychelle cinnamon and Saigon cassia gave a column height of ∼ 25 ml.   D. I. Rees.

Extraction and concentration of volatiles from dilute aqueous and aqueous alcoholic solution using trichlorofluoromethane. P. J. HARDY (*J. agric. Fd Chem.*, 1969, **17** (3), 656–658. 10 ref.).—CCl₃F (Freon 11) was tested as a solvent for use in the analysis of food flavours, esp. those containing high concn. of alcohol. The results of trial extractions of 1-alkanols, 2-alkanones and Et alkanoates from H₂O and 10% EtOH in model systems are presented, together with a method of removing higher alcohols from the Freon 11 extracts using propylene glycol, which allows the remaining esters and carbonyl compd. to be concentrated further.   I. Dickinson.

Influence of intermolecular action between vanillin and ethyl-vanillin on the technology and quality of aromatic substances for foodstuffs. R. SZCZEPANIK and W. SARZYŃSKI (*Roczn. Technol. Chem. Żywn.*, 1969, **15**, 87–99. Pol., 9 ref.).—The relationship between mol. of vanillin and ethylvanillin was studied and the mutual influence of their solubilities in alcohol/water mixtures containing 0–100% of alcohol was investigated. The vanillin-ethylvanillin liquid–solid phase system and the influence of the strength and aroma of the ingredients were considered. The physiochemical influence of mol. of the aromatic substances and their solutions allows the choice of optimum conditions.   A. Lewin.

Estimation of monosodium glutamate in food products. E. FERNANDEZ-FLORES, A. R. JOHNSON and V. H. BLOMQUIST (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 744–746. 9 ref.).—The method described involves treating the sample, e.g., powdered or conc. soup, with C (to remove fat) and Me₂CO (to ppt. starch) and, after standing for 30 min, filtering. The filtrate is transferred to a column of Dowex 50W–X8 (H$^+$ form), the glutamate being retained and eluted with 1N-HCl. The glutamate content is determined by titration with HCHO. Recoveries ranged from 93 to 102% for dried soups and 92 to 105% for liquid soups.   D. I. Rees.

Detection and determination of synthetic emulsifiers in foods. J. M. MURPHY and H. R. HIBBERT (*J. Fd Technol.*, 1969, **4** (3), 227–234. 47 ref.).—The review covers various analytical procedures suitable for many of the emulsifiers used in foods.   J. B. Woof.

Carrageen: a seaweed colloid controlling the consistency of foods. J. K. PEDERSON (*Indian Fd Packer*, 1968, **22** (2), 39–45).—A brief survey of sources of raw material, processing, behaviour and applications.   C. V.

Atomic absorption analysis of dimethylpolysiloxanes in the presence of silicates. P. NEAL (*J. Ass. off analyt. Chem.*, 1969, **52** (4), 875–876. 4 ref.).—It is proposed that the polymer can be extracted from the foodstuff with petroleum ether and analysed by the atomic absorption method described. Test samples of the polymers, which are used as antifoaming agents during food processing,

showed 96% recoveries at the 5 $\mu g/ml$ level in the presence of inorg. silicates and 2% of cottonseed oil.

Preservation of fruits and vegetables. C. ILLOUZE (Br. Pat. 1,158,571, 7.8.66).—Vegetable products (e.g., grapefruit, grapes) are preserved by enclosing the product with a bactericidal gas generator comprising an alkali or alkaline earth metal metabisul-phite (0·001–5% of the vegetable product) and alum in an imperm-eable (plastic) envelope which has an opening of sufficient size to allow the product to respire.   E. Enos Jones.

Flavouring compositions. TAKEDA CHEMICAL INDUSTRIES LTD. (Br. Pat. 1,167,183, 14.10.66. Jap., 14.10.65, 8.2 and 19.5.66).—The title compounds contain as the main flavouring ingredients one or more compounds of formula $CH_2 \cdot S \cdot X \cdot S \cdot S$, where X is $CH_2 \cdot S$, $CH_2 \cdot S \cdot CH_2$ or $S \cdot CH_2 \cdot S$, prep. by the interaction of, e.g., hydrogen polysulphide and an active methylene compd. In an example, S is dissolved in hot aq. Na₂S, the soln. is mixed with HCHO and CHCl₃, and AcOH is added. The CHCl₃ layer is worked up to yield lenthionine ($X = S \cdot S \cdot CH_2$), which can be used, e.g., to enhance the flavour of Chinese noodle soup.   S. S. Chissick.

Synthesis of dihydropyrone and pyrone derivatives. MONSANTO Co. (Br. Pat. 1,164,697—9, 15.5.67. U.S., 16.5.66).—(A) 2,3-Dihydro-4-pyrone deriv. (I) are prep. by reacting a 3,4-epoxy-2-alkanone with an oxalic diester in a non-aq. medium and in the presence of a base at < 50°. In an example, Et₂ oxalate and then 3,4-epoxy-2-pentanone are added to NaOEt in C₆H₆ at 0–5° and the mixture is worked up to yield 2-methyl-3-hydroxy-6-carbethoxy-2,3-dihydro-4-pyrone (II). (B) 2-Alkyl-3-hydroxy-6-(carboxylic ester)-4-pyrone (III) is prep. from the corresponding I compound by reaction with a free-O₂-containing gas in a protic medium at 20–120°. In an example, the reaction mixture from the prep. of II is treated with air for 3 h in the presence of EtOH and at 68° to yield 2-methyl-3-hydroxy-6-carbethoxy-4-pyrone (IV). (c) 2-Alkyl-3-hydroxy-4-pyrone is prep. from III by heating in an inert atm. at 300–600 (450–550)°. In an example, IV is distilled at reduced pressure and the vapour carried in a stream of N₂ through a quartz tube heated at 550°. The effluent stream deposits 2-methyl-3-hydroxy-4-pyrone (maltol), useful as a flavour enhancer in breads, cakes, coffee, etc.   S. S. Chissick.

Stabilisation of flavouring and odorant compositions. H. B. TAYLOR Co. (Inventors: A. A. LEVINSON, L. C. RADTKE and K. B. BASA) (Br. Pat. 1,165,750, 8.11.66).—Flavouring or odorant (e.g., perfume) compositions (containing aliphatic polyhydric alcohols and carbonyl compounds are stabilised with a proton acceptor (to inhibit dioxolane formation without promoting a Cannizzaro condensation), e.g., Na₂CO₃. Thus, addition of 1% of 0·1 M-NaHCO₃ to a mixture of propylene glycol (95) and PhCHO (5 ml) appreciably minimises dioxolane formation.   F. R. Basford.

Treatment of [cubic common] salt [of low bulk density]. CEREBOS FOODS LTD. (Inventor: S. E. CROW) (Br. Pat. 1,163, 888,20.1.66).—The salt (obtained by the foamed salt technique described in Br. Pat. 1,016,743 and 1,130,854) is treated with 2–100 ppm of an aq. soln. of an alkali metal ferro- or ferri-cyanide, e.g., K₄Fe(CN)₆. The tendency for the salt to lose its low bulk d during storage at high temp. and r.h. is thereby reduced.   J. M. Jacobs.

Food Processing, Refrigeration, Packaging and Storage

Determination of acid phosphatase activity in canned hams as an indicator of temperature attained during cooking. E. H. COHEN (*Fd Technol.*, Champaign, 1969, **23** (7), 961–964. 8 ref.).—Residual acid phosphatase activity, in 65 hams, was related to max. internal temp. attained during cooking. Temp. in the range 59·4–70° were detd. by the relationship $T = 74 \cdot 75 - 5 \cdot 10$ log (acid phosphatase activity), standard deviation $\pm 1 \cdot 46°$. The method lacked accuracy when applied to hams processed to higher internal temp.   I. Dickinson.

Chemistry and technology of deep fat frying. H. U. RAO and B. S. BHATIA (*Indian Fd Packer*, 1968, **22** (1), 6–12. 87 ref.).—A review.   C. V.

Rôle of tissue amino components and environmental factors in colouring the surface of foods during smoke curing. Z. ZIEMBA (*Roczn. Technol. Chem. Żywn.*, 1969, **15**, 139–151. Pol., 21 ref.).—Intensive and permanent colouring of smoked products is the result of reaction between components of the smoke and proteins of the product. The state of the surface proteins, pH, O₂, light, time

and temp. influenced the coloration in the same way both in amino and non-amino systems. The formation of 'smoked' colour involves three types of non-enzymic browning: amino, non-amino and that due to oxidation, this last being produced by smoke which is starved of air. A. Lewin.

Rôle of the chemical constituents of wood smoke in colouring the surface of foods during smoking. Z. ZIEMBA (*Roczn. Technol. Chem. Żywn.*, 1969, **15**, 153–169. Pol., 41 ref.).—The 'smoked' colour intensity both in amino and non-amino systems is accompanied by a decrease in total carbonyl and changes in carbonyl compounds contained in the wood smoke. Changes are stronger in amino systems. Phenols with *o*- and *p*-OH groups, in weak alkali, give stable colour to the protein surface. Typical 'smoked' colour formation is due mainly to carbonyl–amino reactions. The acid constituents of smoke may intensify the coloration by hydrolysis of proteins and increasing the concn. of reactive amino groups. A. Lewin.

Possibility of treating dried soups with ionising radiation. N. PAUL, T. GRÜNEWALD and J. KUPRIANOFF (*Dt. LebensmittRdsch.*, **65** (9), 279–281. Ger., 6 ref.).—Reduction in cooking time of soups based on pease meal was achieved by low radiation doses (> 500 krad). The radiation caused a reduction in η of the aq. suspension; this became objectionable at > 1 Mrad. At 300 krad, the cooking time was reduced from 19 to 9 min and there were no substantial flavour changes; these however were evident at 500 krad. J. B. Woof.

Rapid freezing of tuna by immersion in dichlorodifluoromethane. L. CRAWFORD, R. FINCH and J. J. DALY, JUN. (*Fd Technol., Champaign*, 1969, **23** (4), 549–553. 7 ref.).—The speed of freezing, the amt. of refrigerant carried over into the final product and the effect on quality after canning were detd. Results were compared with those for fish frozen by the present brine immersion method. The use of CCl_2F_2 gave a const. improved uniformity of quality, as good as or better than the brine-frozen tuna. The prevalence of blood streaks and increased scorch, however, may outweigh the advantages, as they detract from the appearance of the finished product. I. Dickinson.

Secondary bleaching of brined cherries with sodium chlorite. D. V. BEAVERS and C. H. PAYNE (*Fd Technol., Champaign*, 1969, **23** (4), 573–575. 10 ref.).—The effects of pH and temp. were investigated in relation to quality and bleaching efficiency. Complete colour removal was accomplished in all bleached samples without loss in texture or development of off-flavour. Reduction of bleaching time and chlorite consumption was most effective where the bleaching soln. was at < 110°F and in the pH range 4·0–6·0. I. Dickinson.

New method of shelling green peas for processing. R. S. MITCHELL, L. J. LYNCH and D. J. CASIMIR (*J. Fd Technol.*, 1969, **4** (1), 51–60. 6 ref.).—A commercial machine is described. The pods are preheated in steam and then fed to a shelling section by means of a vibratory conveyor which aligns them for end-on presentation to a pair of rubber-covered rollers. Shelled peas are separated from unshelled pods. Comparisons are made with other methods; the new method is superior. Damage, quality assessment and size measurements are discussed. P. C. W.

Peeling of tomatoes for canning. B. JUVEN, Z. SAMISH and A. LUDIN (*Israel J. Technol.*, 1969, **7** (3), 247–250. Engl., 12 ref.).—Tomatoes peeled in 18% aq. NaOH at ~ 95° for 20–25 sec gave the most satisfactory results in respect of ease of peeling, highest quality of peeled fruit and min. peeling losses. Size and maturity of tomatoes (Roma, No. 54 and VF 145-21-4) affected the peeling efficiency. Addn. of 0·2% of surfactant improved the peeling process and decreased peeling losses by > 40% without affecting the natural colour and firmness of the peeled fruit. This improvement was unrelated to any surfactant effect on the surface tension of the NaOH soln. W. J. Baker.

Testing requirements for plastics. Migration behaviour of u.v. absorbers from food packaging. W.-J. UHDE, H. WOGGAN, G. ZYDEK and U. KÖHLER (*Dt. LebensmittRdsch.*, 1969, **65** (9), 271–278. Ger., 20 ref.).—Sheets of various plastics contg. u.v. absorber > 0·5%, were placed in contact with a range of solvents for 10 days at 45°. T.l.c. was then used to estimate the concn. of hydroxybenzophenone (I) and hydroxyphenylbenzotriazole (II) in the soln. (I) was also detd. spectrophotometrically at 290 nm. (II) was estimated polarographically. Little absorber was extracted by any solvent from PVC and low pressure polyethylene. High migration rates from high pressure polyethylene were observed with EtOH and sunflower oil. n-Heptane and EtOH extracted

large amt. of absorber from polystyrene and polymethacrylate. J. B. Woof.

Evaluation of the dry-pack and ice-pack for storing fresh broilers. R. C. SHANTZ, G. W. FULLER, H. L. ORR and G. W. ANDERSON (*Poult. Sci.*, 1969, **48** (1), 266–273. 6 ref.).—Moisture loss from broiler carcasses stored for up to 6 days at 4·4 or 7·3° was not significantly different for storage under dry- and ice-pack conditions. The dry-pack method was suitable for storing fresh, eviscerated carcasses for 4–5 days at 4·4° and 3 days at 7·2°, and the ice-pack method for 6 days at 4·4° and 4–5 days at 7·2°. Carcass colour was acceptable after 7 days of storage under both conditions. A. H. Cornfield.

Storage properties of dehydrated apple sauce made from explosion-puffed pieces. N. H. EISENHARDT, M. J. CALHOUN, F. B. TALLEY and E. S. DELLAMONICA (*Fd Technol., Champaign*, 1969, **23** (4), 557–560. 18 ref.).—N_2 storage of samples prolonged shelf-life and allowed room-temp. storage for nine months. Air-packed samples developed off-flavours in five months. At 100°F storage, both air- and N_2-packed samples were unacceptable in flavour at six months. Severe caking occurred at 100 but not at ≤ 73°F. I. Dickinson.

Heat of respiration of fresh produce as affected by controlled atmosphere. R. TOLEDO, M. P. STEINBERG and A. I. NELSON (*J. Fd Sci.*, 1969, **34** (3), 261–264. 14 ref.).—A calorimetric method was used to determine the heat of respiration (*HR*) of peas, lima beans, sweet-corn, and apples, in a controlled atm. (CO_2 10 or 5%, O_2 2%). Results showed that *HR* is ~ 30% of that in air. These direct measurements are probably more reliable than calc. values. M. T. Rawnsley.

Sorption isotherms of maize meal. P. VAN TWISK (*J. Fd Technol.*, 1969, **4** (1), 75–82. 17 ref.).—The equil. moisture contents (*EMC*) of three grades of maize meal were determined at 30° at r.h. 1·4–90·7%. *EMC* decreased as the fat content of the meal increased, at any specific r.h. Both desorption and adsorption isotherms were sigmoid, and hysteresis occurred in all cases. Mould growth occurred in meals during prolonged storage at r.h. ≥ 84%; the max. r.h. at which meals could be stored without mould contamination can be predicted from the sorption isotherms. P. C. W.

Manufacture of foodstuff bars. N. SLIKKERVEER (*Br. Pat.* 1,161,548, 7.5.68. Neth., 8.5.67).—The process, which results in space-saving, shape-retaining bars of dehydrated food that can be stored for prolonged periods without decay and without losing their ability to reconstitute in water, comprises (*i*) mixing dehydrated, non-pulverised foodstuff pieces and optionally other solid ingredients, e.g., seasoning, spices, with 25 wt.-% of an aq. binder soln., e.g., gelatin (I), agar, a saccharide, and (*ii*) moulding into bars and drying to a predetermined moisture content (8–11%). In an example, dehydrated roasted onions are mixed with seasoning and beef broth and then 2% aq. I is mixed in. After moulding, the mixture is dried to ~ 8% moisture content and wrapped in moisture-proof film material. These bars are suitable for use as additives to fried rice in order to prep. a flavoured rice dish. S. S. Chissick.

Production of breaded, deep-fried foodstuffs. NATIONAL STARCH & CHEMICAL CORP. (*Br. Pat.* 1,161,358, 9.6.67. U.S., 10.6.66).—The process comprises spraying a cereal starch with a solution of an oxidising agent, e.g., NaOCl, applying the product as a slurry to the foodstuff, applying a breading, and cooking in oil, In an example, pre-fried frozen fish which requires further cooking (3 min at 365°F) prior to consumption is prep. by dipping raw, frozen, skinless codfish fillets into an aq. batter mixture containing maize starch previously sprayed with NaOCl, at 70°F, draining off the excess batter, coating in breadcrumbs and pre-frying at 385°F for 45 sec. When prep. for eating, the appearance, texture and adhesion are excellent. S. S. Chissick.

Gelled food products and process for their preparation. CORN PRODUCTS CO. (*Br. Pat.* 1,163,598, 3.11.66. U.S., 15.11.65).—The products have an amylose (I) or starch (II) congealing agent base and are prep. from a thin-boiling I or II having exceptionally fast congealing properties, a fluidity of 30–80 and a fat content of > 0·3 wt.-%. In an example, a mixture of maize syrup, sucrose, defatted maize starch and water is heated to 106–108° and then pumped through a steam injection cooker at 129–135° (cooking time 26–28 sec). Citric acid, lemon flavour and yellow colour are added and the hot mixture is transferred to a mould and allowed to set at room temp. to produce a gum confection. S. S. Chissick.

(A) **Manufacture of frozen foodstuffs.** (B) **Seasoning foodstuffs.** W. R. GRACE & CO. (Br. Pat. 1,163,948—9, 11.11.66. U.S., 8.12.65).—(B) A method of seasoning fish and meat products comprises adding a flavouring agent in the form of a water-in-oil emulsion containing vegetable oil or animal fat, water and edible non-ionic hydrophilic and lipophilic emulsifier. In an example, vegetable oil, beeswax and monoglycerides are blended at 43° and protein autolysate, dehydrated onion, spice mixture, sugar, sorbic acid, Na benzoate, parsley and water are blended together and added. The composition may be used, e.g., to flavour chicken by basting during roasting. (A) A method for prep. a frozen coated foodstuff comprises heating the flavour agent claimed in B to 27–66°, contacting it with the frozen foodstuff for a time sufficient to form a coating 0·25–2·5 mm thick and then freezing. In an example, frozen chicken pieces are dipped into the emulsion described in B at 38° until the coating is equiv. to 18% by wt. of the chicken. The frozen pieces are stored at −17°.   S. S. Chissick.

Sterilisation of food products. EXPRESS DAIRY CO. (LONDON) LTD. (Inventors: T. R. ASHTON, V. C. H. COTTLE and D. JACKSON) (Br. Pat. 1,164,275, 19.10 and 2.11.65 and 23.2.66).—Prior to packaging in a gas-impermeable container, the dissolved O_2 (I) content of the product is reduced to a level sufficient just to support the oxidation of sulphydryl and other oxidisable compounds present. The desired level is obtained by, e.g., a flash evaporation cooling process. In an example, food cooled by indirect heat exchange (normal I content) is added to food cooled by a flash evaporation stage (no I) in suitable proportions.   S. S. Chissick.

Installation for sterilisation or pasteurisation of commodities packed in containers. GEBR. STORK & CO'S APPARATENFABRIEK N.V. (Br. Pat. 1,151,966, 16.2.67. Neth., 9.3.66).—A device is described whereby carriers conveying tins or glass jars of foodstuffs, which have accidentally remained too long in the sterilising chamber, are indicated by mark members.   J. M. Jacobs.

Packaging meat and fish. BADISCHE ANILIN- & SODA-FABRIK A.-G. (Inventor: W. KRAFT) (Br. Pat. 1,158,413, 20.10.66. Ger., 21.10.65).—Meat or fish which is subject to spoilage or deterioration is packed in a close-fit, multi-part container, i.e., in which the component parts fit together so closely that any flow of air from the atm. through the joints is prevented, made by expanding and fusing together particulate expandable styrene polymer. The components have complementary inter-engaging projections and recesses to prevent them slipping, and the bottom of the container has drainage holes.   J. M. Jacobs.

Wax–polymer coating compositions. SHELL INTERNATIONAL RESEARCH MIJ N.V. (Inventor: K. G. ARABIAN) (Br. Pat. 1,156,597, 27.5.68. U.S., 29.5.67).—The compositions, homogeneous at coating temp., and with excellent scuff- and freezer burn-resistance, contain (a) paraffin wax of m.p. 130–150°F (50–70) (b) a scuff-resistant wax of m.p. 170–190°F (15–35) (c) polyethylene of mol. wt. 1500–21,000 (5–15) and (d) an ethylene (74–76)–vinyl acetate (24–26) copolymer (3–15%).   E. Enos Jones.

Nutrition, Proteins, Amino Acids, Vitamins

Determination of protein in biological materials and foodstuffs. T. T. GORSUCH and R. L. NORTON (*J. Fd Technol.*, 1969, **4** (1), 1–6. 4 ref.).—Three convenient methods of protein analysis, (Folin, nitration and dye-binding methods) were studied to determine their usefulness for the estimation of proteins in mycelial systems, flour and dried egg. Calibration curves varied widely with the protein standard used, and the methods gave results which differed considerably for the same material even when the same protein standard was used. The ratio of the Folin results to either of the others remained approx. const. for a single type of sample even if the protein standard was altered. The ratios varied widely with different types of samples. The Folin method is considered to be the most suitable for routine use for the materials examined, but the results are thought to have no absolute validity.   J. G.

Methionine loss during protein hydrolysis of plant material. D. M. JENNINGS and O. A. M. LEWIS (*J. agric. Fd Chem.*, 1969, **17** (3), 668–669. 3 ref.).—The loss was demonstrated by comparing methionine (I) recovery figures from leaf material hydrolysed with and without prior oxidative protection treatment. The range of I loss was 30–59%, indicating that prior I protective treatment is necessary for the accurate detn. of this amino acid in hydrolysates of plant material.   I. Dickinson.

Studies on the use of Opaque-2 corn [maize] in vegetable protein-rich foods. R. BRESSANI and L. G. ELÍAS (*J. agric. Fd Chem.*, 1969, **17** (3), 659–662. 13 ref.).—Opaque-2 maize (I) was tested as a substitute for common maize in vegetable protein mixtures developed to prevent protein malnutrition. Studies on the complementation between the proteins of I and those of soyabean (II), cottonseed and black beans are reported. Mixtures with a lysine deficiency were improved in protein quality when prep. with I, but mixtures whose main protein source was II were not improved. The proteins of I and cottonseed flour did not complement each other. I and II protein complement each other in the range of 80–40% of the dietary protein from II, and 20–60% of the same from I.   I. Dickinson.

Trial of a vegetable protein-rich food (peanut [groundnut]–chickpea–sesame mixture) in protein-calorie malnutrition. M. M. HANAFY, Y. SEDDIK and M. K. AREF (*J. trop. Med. Hyg.*, 1968, **71** (7), 167–175. 42 ref.).—36 cases were studied. Such a vegetable protein-rich mixture with a balanced amino acid pattern was able to initiate and complete the cure of hospitalised cases of protein-calorie malnutrition. The mixture was also able to improve the quality of 'poor' diets devoid of animal protein.   C. V.

International Symposium: Development of high protein foods to meet world needs. (*J. Dairy Sci.*, 1969, **52** (3), 409–424).—Includes the following papers: **Potential of animal, fish and certain plant protein sources.** H. L. WILCKE (409–416. 13 ref.). **Protein from hydrocarbons.** C. F. FELDMAN (416–418). **What are the prospects for milk products as sources of protein?** P. A. PUTNAM (419–422).   P. P. R.

Progress in chemical studies on artificial and synthetic foods V. M. BELIKOV, S. V. ROGOZHIN, G. L. SLONIMSKIĬ, *et al.* (*Usp. Khim.*, 1969, **38** (9), 1569–1596. Russ., 135 ref.).—Five sections cover (*i*) synthetic food production in the 1960s; (*ii*) food proteins, their natural sources, vitamins, and the basic composition of food proteins and microbiological proteins; (*iii*) basic amino acids in proteins; (*iv*) chemistry of food flavour and odour; and (*v*) physicostructural problems of synthetic food, acceptable food structure, incorporation of fat, minerals and enzyme action on food.   L. A. Haddock.

Relationship of amino acid composition and wheat protein properties. L. H. KRULL and J. S. WALL (*Baker's Dig.*, 1969, **43** (4), 30–39. 36 ref.).—The unique cohesive properties of the gluten protein arise from intermol. interactions between amino acid functional groups present in the protein chains. Single H bonds may be weak, but when multiplied by the vast no. possible in wheat gluten, these forces are effective in producing cohesion. Salt and pH produce electrostatic effects between protein mol. The high mol. wt. of the glutenin proteins, resulting from interchain disulphide bonding, further increase the no. of sites for association, and contribute to the elastic quality of the gluten. Agents effective in modifying gluten protein behaviour in soln. also alter flour doughs.   I. Dickinson.

Spectrophotometric determination of riboflavine in urine. NAGI WAHBA (*Analyst, Lond.*, 1969, **94** (1123), 904–908. 16 ref.).—The urine (adjusted to pH between 3 and 6) was treated with 2 wt.-% $Zn(OAc)_2$ and 40% HCHO soln. After filtration, it was passed through a 20-cm column of large particle size (20–50 μm) talc on which riboflavine was selectively and quant. adsorbed. After removal of urinary pigments, etc., (0·01 N-HCl followed by 5% dioxan), the riboflavine was eluted with 20% dioxan and measured spectrophotometrically at 444 nm. Recovery of riboflavine was quant. because of its strong retention on the large particle size talc. Lower limit of detn. was 0·2 mg/100 ml of urine.   W. J. Baker.

Spectrophotometric determination of pyridoxal and pyridoxal 5'-phosphate with 3-methyl-2-benzothiazolone hydrazone hydrochloride, and their selective assay. K. SODA, T. YORIFUJI, H. MISONO and M. MORIGUCHI (*Biochem. J.*, 1969, **114** (3), 629–633. 11 ref.).   P. C. W.

Marine protein concentrate. GULF COAST INSTITUTE OF RESEARCH & TECHNOLOGY INC. (Br. Pat. 1,156,500, 4.10.67. U.S., 10.10.66).—The process comprises contacting raw fish with a mild org. acid, e.g., lactic or citric, optionally produced *in situ*, and extracting the fats and oils with an org. solvent, e.g., EtOH or Pr^{i}OH, at 30–100 (30–75)°. In an example, *Lactobacillus acidophilus* is cultivated on coarsely ground anchovy at 40–50° for 3–5 h and the mixture is pressed and dried and extracted with Pr^{i}OH to yield a residue free from odour and flavour, which is dried and can be used as a flour, food supplement, etc.   S. S. Chissick.

Unclassified, Tobacco

The essence of flavour. J. H. MORIARTY (*Baker's Dig.*, 1969, **43** (4), 54–56, 65. 7 ref.).—The chem. and physiol. aspects of flavour are discussed. Methods of flavour measurement, including physiochem. means and those based on human analysis, are considered.   I. Dickinson.

Colorimetric determination of volatile sulphur compounds in foods. II. Reaction with bis(*p*-nitrophenyl) disulphide. H. G. MAIER (*Z. analyt. Chem.*, 1969, **247** (1–2), 46–48. Ger., 3 ref.).—Volatile mercaptans and H_2S are distilled from a buffer soln. of pH 7 into acetone at 0°, and S is determined spectrophotometrically. Down to 0·1 mmole/kg can be determined with a standard deviation of 8·4%.   R. Waspe.

Determination of ammonia in foodstuffs. II. Re-investigation of vacuum distillation. H. THALER and W. STURM (*Z. analyt. Chem.*, 1969, **246** (5), 315–319. Ger.).—In a vac. apparatus with modified receiver, the optimum conditions for a procedure for the quant. liberation of small amounts of NH_3 were tested. For the release of NH_3, an alkalinity of at least pH 9 was necessary. Using this method with foods containing protein, NH_3 was released also, though only in small amounts. This depends not only on the type of substance, but also on the alkalinity of the soln. and the duration of distillation. Therefore, variations in the relationship between the amount of sample and the soln. used for alkalisation cause a change of alkalinity and hence different yields of NH_3. For detn. of NH_3 in mixtures of substances with unstable N compounds, the macro-vac. apparatus is superior to steam distillation at normal pressure, but cannot be recommended as a reliable method.   J. Korkisch.

Lithium contents of some consumable items. R. P. HULLIN, M. KAPEL and J. A. DRINKALL (*J. Fd Technol.*, 1969, **4** (3), 235–240. 20 ref.).—It is possible that Li alleviates manic depressive psychosis symptoms by displacing retained Na. The extent of Li ingested with water and various common dietary constituents, was studied using atomic absorption spectroscopy. Water from different locations was found to contain 0·003–0·018 (Harrogate spa water 1·8), salt 1–3·42, lettuce 0·26–2·04 (dry wt.) and potato 0·07–0·28 ppm (dry wt.) of Li. Some may be ingested through smoking, as tobacco ash contains 23–148 ppm of Li.   J. B. Woof.

Extraction and atomic absorption analysis of lead in plant and animal products. W. L. HOOVER, J. C. REAGOR and J. C. GARNER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 708–714. 11 ref.).—The method described involves digesting the dried sample (< 10 g ≡ > 3 μg of Pb) with HNO_3, H_2SO_4 and $HClO_4$. The Pb released is entrained with added $SrSO_4$. The SO_4^{2-} ppt. is centrifuged off and converted to the carbonate by addition of $(NH_4)_2CO_3$ soln. The carbonate ppt is dissolved in 1 N-HNO_3 and the Pb is determined by atomic absorption spectrophotometry with either 2833·06 or 2169·99 Å resonant wavelength. Quant. recoveries were obtained except with samples of high Ca content. Good recoveries were obtained after removal of the Ca by a procedure described. Moderate quantities of Al, Fe, P, Sn, Ca, B and Tl did not interfere.   D. I. Rees.

Rapid method for routine determination of sodium benzoate and/or potassium sorbate in foodstuffs. J. J. MONSELISE (*Israel J. Technol.*, 1969, **7** (3), 263. Engl., 2 ref.).—The benzoic and sorbic acids were removed by steam distillation of the sample (1 g) with conc. H_3PO_4 and Na_2SO_4, and were then detd. spectrophotometrically at 228 and 254 nm, resp. In the presence of both preserving agents, the concn. can be calc. by equations given. This improved method, (*Idem, ibid.*, 1967, **5**, 223), is specific for distillates of citrus juices, fruit syrups, comminuted bases, jams and ketchup.   W. J. Baker.

Determination of non-biogenic acetic acid in vinegars by measuring the natural radioactivity of radiocarbon. F. MECCA and G. VICARIO (*Chimica Ind., Milano*, 1969, **51** (9), 985–986. It., 3 ref.).—The method of Simon *et al.* (*Z. Lebensmittelunters. u.-Forsch.*, 1968, **136**, 279) for detg. ^{14}C in the HOAc of vinegars was modified to enable fermentation vinegars to be differentiated from those obtained from synthetic HOAc. Two stages were involved: (1) prepn. from the sample of a concentrate contg. ≮ 96% HOAc and (2) detg. the specific radioactivity by liquid-phase scintillation.   J. I. M. Jones.

Continuous steam distillation of [food] materials completely miscible with water. A. A. WILLIAMS (*Chemy Ind.*, 1969, (42), 1510–1511. 6 ref.).—Limitations of steam distillation at atm.

pressure to obtain the aroma extracts of foods, were eliminated by use of apparatus described and illustrated. The steam distillate was continuously extracted by the solvent before it was returned to the distillation flask. In this way distillation was continued with a relatively small vol. of H_2O, all the aromatic oil eventually passing into the solvent for further concn. if necessary. 32 h distillation was necessary for removal of > 90% of coumarin (0·3 g) in 500 ml of a 5% ethanolic soln.; this time would necessitate an unmanageable vol. of H_2O by conventional methods. Steam-volatile fractions from pentane–ether extracts of strawberry and pentane extracts of cider were obtained by this method.   W. J. Baker.

Trace analysis of *N*-nitroso compounds. I. Liquid–liquid distribution in acetonitrile–heptane as clean-up method. G. EISENBRAND, P. MARQUARDT and R. PREUSSMANN (*Z. analyt. Chem.*, 1969, **247** (1–2), 54–55. Engl., 11 ref.).—Extraction from n-heptane into MeCN is shown to be generally satisfactory for removal of lipids from, e.g., food or plant extracts prior to determination of *N*-nitroso compounds.   R. Waspe.

Semimicro method for determining total lipids in fish meal. M. E. AMBROSE, B. J. ROCHE and G. M. KNOBL, JUN. (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 688–692. 10 ref.).—The method described involves extracting the fish meal (2 g) with a $CHCl_3$–MeOH mixture, drying the extract under N_2 at 50° and finally over P_2O_5 in a desiccator and weighing. The method is a modification of the method of Smith *et al.* (*Comml Fish. Rev.*, 1964, **26**, 1), sample size and solvent amounts being reduced by 80%. Results averaged 99·0% of those obtained by the AOAC method 22·037.   D. I. Rees.

Production of lipids and sterols by *Aspergillus fumigatus*. II. Utilisation of some industrial products and byproducts for the microbiological production of fats and sterols. H. G. OSMAN, M. ABDEL-AZIZ and A. H. EL-REFAI (*J. Chem. Un. Arab Repub.*, 1969, **12** (1), 99–105. Engl., 22 ref.).—Samples of unhopped sweet wort and molasses–maize steep liquor (cheap, natural media) were sterilised, inoculated with a spore suspension of *A. fumigatus*, and incubated at 28° for 10 days under stagnant conditions. Various amt. of lipids and sterols were isolated, according to the initial concn. of nutrient.   S. S. Chissick.

Recovery of oils and fats from protein-containing waste water. A/S APOTHEKERNES LABORATORIUM FOR SPECIALPRAEPARATOR (Br. Pat. 1,159,948, 21.10.66. Denmark, 21.10.65).—Waste water from slaughter houses, dairies and wool-washing, fish product and bone-meal factories, is adjusted to pH 2–4·5 by the addition of lignosulphonic acid or salt prior to flotation separation (e.g., by centrifuging) at, e.g., 80–100°.   J. A. Sugden.

Factors governing recovery and quality of pectin from fresh mandarin orange waste (peel and pomace). P. C. AGARWAL and J. S. PRUTHI (*Indian Fd Packer*, 1968, **22** (4), 5–15).—Effects of HCl and citric acid and their concn. and vol., no. of extractions, time of heating and effect of additions of polyphosphate were studied, 2·5% of the latter on the basis of peel wt. increasing the yield of pectin without harming quality.   C. V.

Trimethylsilyl derivatives of commercial pectins. R. C. WILEY and M. TAVAKOLI (*Fd Technol., Champaign*, 1969, **23** (4), 565–566. 8 ref.).—The enzyme hydrolytic products of commercial pectins were basically D-galacturonic acids. They were separated together with standardisation sugars, and were quantified as trimethylsilyl deriv. Deriv. recovery was 90–110% of the original wt. of the pectins.   I. Dickinson.

Enzyme regulation. Ed. G. WEBER (*Adv. Enzyme Regulation*, 1968, **6**, 517 pp.).—Regulation of carbohydrate and lipid metabolism. (5 papers); Steroids: mechanism of action. (2 papers); Isozymes: action of vitamin K. (2 papers); Activation and feedback in enzyme regulation. (3 papers); Regulation of dihydrofolate reductase. (3 papers); Cyclic AMP and enzyme regulations. (5 papers); Regulation of cancer. (2 papers); and Effects of ethanol on the metabolic activities of the liver. (1 paper).   C. V.

Collaborative study of the g.l.c. determination of glycerine and propylene glycol in tobacco. J. A. GILES and R. H. CUNDIFF (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 753–756. 2 ref.).—The method of Cundiff *et al.* (*Tob. Sci.*, 1964, **8**, 163) was modified by using Diatoport S as column support and amethole as internal standard. The collaborative results showed unacceptable high systematic error standard deviations.   D. I. Rees.

Collaborative study of a colorimetric determination of nitrate in tobacco. C. J. ROSENE (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 756–759. 2 ref.).—The ground sample (1·5 g ≡ 0·5–2·4% of

NO_3^-) is extracted with water and an aliquot of the filtrate is treated with 80% H_2SO_4 and 2,4-xylenol soln. The 6-nitro-2,4-xylenol formed is distilled off and measured spectrophotometrically at 450 nm. Although the precision standard deviation of the results was acceptable, the systematic error standard deviation was unacceptable.   D. I. Rees.

Effect of nitrates in tobacco on the catechol yield in cigarette smoke. A. G. KALLIANOS, R. E. MEANS and J. D. MOLD (*Tob. Sci.*, 1968, **12** (1), 125–129. 14 ref.).—Only 10% of the catechol (I) is nitrated and 4-nitro-I is absent in the condensed smoke. Other possible modes of action must therefore be considered.   C. V.

Reaction products of nicotine with ethylene oxide and their pyrolysis. Y. OBI, Y. SHIMADA, K. TAKAHASHI, K. NISHIDA and T. KISAKI (*Tob. Sci.*, 1968, **12** (1), 70–74).—Nicotine (I) in tobacco leaves was apparently reduced by ethylene oxide (II) (which is used as a steriliser and insecticide), but a II-treated cigarette smoked normally showed I and pyridine deriv. in the smoke in 30% concn. compared with the control. The results of g.c. of the pyrolytic products and t.g.a. and d.t.a. of I, N-hydroxyethyl-I, N'-hydroxyethyl-I, and/or their hydrochlorides are given. C. V.

Preservation of yeast. CANADIAN PATENTS & DEVELOPMENT LTD. (Br. Pat. 1,165,455, 9.6.67. Can., 18.6.66).—Yeast cells or spores having improved resistance to dehydration and improved storage properties are obtained by dispersing mature vegetative cells in an aq. soln. containing 0·1–0·5% (w/v) of AcOH (pH 5·5–6·5), aerating the suspension for ≮ 10 h and adding $MeCO_2H$ to keep the pH in the range 4·8–6. Optionally the cells are maintained in AcOH suspension until > 50% of the cells have formed spores, when the pH is adjusted to 4–5·5 with a non-toxic acid (H_2SO_4) and sporulation continued for 6–12 h. The spore-containing cells are separated and dried.   S. S. Chissick.

Treating tobacco. SERVICE D'EXPLOITATION INDUSTRIELLE DES TABACS ET DES ALLUMETTES (Br. Pat. 1,152,026, 27.6.66. Fr., 29.6.65).—To obtain tobaccos that have reduced harmful effects on the living tissues of the smoker, a comminuted blend of tobaccos is washed with water to remove the sol. harmful constituents, and the washed and dried product is sauced with aq. soln. of ingredients other than tobacco extracts (e.g., glycerin, sugars, humectants). In an example, up to 80% of alkaloids originally present are removed.   H. L. Whitehead.

Tobacco product additive. INTERNATIONAL FLAVORS & FRAGRANCES INC. (Br. Pat. 1,164,341, 4.1.67. U.S., 11.1 and 9.11.66).—A tobacco product with enhanced flavour and smoking characteristics comprises tobacco with 0·05–5 wt.-% of a lactic acid ester of an isoprenoid alcohol and, optionally, a polymer of lactic acid. The ester, containing 3–25 moles of lactic acid per mole of alcohol, has an acid content of 0–0·045, an alcohol content of 0·4–2·5 and an ester content of 0·3–7·5. The isoprenoid alcohol may be a terpene or sesquiterpene alcohol or an isoprenoid alcohol prep. by polymerising isoprene.   J. M. Jacobs.

[Tobacco] flavouring. R. J. REYNOLDS TOBACCO CO. (Inventor: D. L. ROBERTS) (Br. Pat. 1,164,050, 22.12.67).—A popcorn-like flavour is imparted to tobacco or foodstuffs by the incorporation of 0·00003–0·3 (0·005–0·05) wt.-% of 2-acetyl- or 2-acetyl-6- or -5-methylpyrazine.   J. M. Jacobs.

Improving the flavour of tobacco. NISSHIN SANGYO K.K., M. YAMADA and H. KOMOTA (Br. Pat. 1,167,404, 28.4.67. Jap., 28.4.66).—The gas given off during the fermentation of an alcoholic beverage is, e.g., adsorbed in a solvent and the soln. is added to the tobacco. In an example, molasses are fermented and the CO_2 and other gases given off are bubbled through an EtOH/glycerine mixture. Tobacco sprayed with this soln. prior to packing has an improved flavour.   S. S. Chissick.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Synopsis of the pesticide problem. N. W. MOORE (*Adv. ecol. Res.*, 1967, **4**, 75–129. 111 ref.).—Types of research and the main characteristics of the pesticides (toxicity, persistence and solubility, interaction) are discussed. Effects on a single species, on eco-systems and on evolution are examined.   C. V.

Barley diseases in Western Australia: distribution and pathogenicity. T. N. KHAN, W. J. R. BOYD and W. A. SHIPTON (*J. Proc. R. Soc. West. Aust.*, 1968, **51** (4), 123–128. 16 ref.).—The commercial significance of the major diseases is indicated together with their geographical distribution, variability of infection and pathogenicity. Differential hosts for each of these diseases are identified together with potential sources of host resistance.   C. V.

Effect of rain on plants, pests and pesticides. (*Chemy Ind.*, 1969, (42), 1495–1504. 31 ref.).—The following papers were read at the Soc. Chem. Ind. Symposium, 6 Feb. 1968, in London: **Interception of rainfall by plant cover.** F. B. THOMPSON. This includes interception loss, interception and storage efficiencies, and interception storage capacity; their calculation and use in predicting the interception behaviour of plant cover in different climates is discussed. **Factors controlling persistence of intercepted rainfall.** A. J. RUTTER. Factors affecting rate of evaporation of rain from leaf surfaces are outlined. **Effect of rain on plant diseases.** J. M. HIRST. The influence of raindrops, splash dispersal, leaf wetness, etc. on susceptible hosts and pathogens in an environment favouring infection is discussed. **Measurement of size of splash droplets.** P. H. GREGORY. Data obtained are discussed in respect of dispersion of microbial cells by splash. **Redistribution of fungicides by rain.** E. C. HISLOP. A review of the literature is given together with discussion of exptl. results on local and long range redistribution as affecting efficiency of protection of commercial fungicides. **Effects of rain on pesticide deposits.** R. J. COURSHEE. Qual. and semi-quant. treatment of pesticide loss due to wash-off or redistribution of sprays and wettable powders is described. **Effect of rain on efficiency of diquat and paraquat.** R. C. BRIAN. Results reported show that rain did not affect activity of paraquat spray on cocksfoot because initial uptake was so rapid that toxic concn. was attained before rain greatly decreased the amt. on the leaf. **Laboratory techniques for simulating rainfall.** J. B. BYASS. The problem of simulating rain is examined, considering methods of drop production and the N.I.A.E. rain simulator, together with their limitations.   W. J. Baker.

Review of methods for mercury [determination] in pesticide formulations. J. E. LAUNER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 764–767. 23 ref.).—The outlines are given of five methods for determining Hg that have been accepted by the Collaborative International Pesticides Analytical Committee of Europe. Further study is recommended of the method of Elmore (*ibid.*, 1946, **29**, 387) which involves the release of Hg with H_2SO_4–HNO_3 and determination by thiocyanate titration.   D. I. Rees.

Identification of pesticides in mixtures by high resolution mass spectrometry. R. E. LOVINS (*J. agric. Fd Chem.*, 1969, **17** (3), 663–667. 7 ref.).—Ions were identified in the spectra of each pesticide whose elemental compn. (accurate mass) is unique for that pesticide and can be used to identify the presence of the pesticide in the high resolution mass spectrum of a pesticide mixture.   I. Dickinson.

Use of vegetable oil as a source of peroxide in thin-layer plates for analysis of chlorinated pesticides. W. A. MOATS (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 871–872. 2 ref.).—The sensitivity of the method previously described (*Idem, ibid.*, 1966, **49**, 795) was improved five-fold by incorporating a rancid vegetable oil into the t.l.c. adsorbent. Al_2O_3 G was moistened with water and slurried with acetone containing $AgNO_3$ and rancid groundnut oil. The layers prepared from this slurry were air-dried. The pesticides were detected by exposing the chromatogram to short-λ u.v. light, steaming lightly and further exposing to the u.v. light.   D. I. Rees.

Versatility of Silicone Dow Corning 11 substrate for gas chromatography of pesticides. F. C. WRIGHT and J. C. RINER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 879. 4 ref.).—Retention data, using this non-polar stationary phase, are tabulated for several pesticides. A packed stainless steel column of 5% of the phase on 60–80 mesh Chromocarb W was used at 175, 200 or 210° with N_2 as carrier gas (57 ml/min) and flame ionisation detection.   D. I. Rees.

Herbicides

Studies on the pre-emergence treatment of weedicide Afalon on weeds in wheat. MANZOOR AHMAD, A. BALUCH, ZAFAR HUSAIN ABIDI, *et al.* (*W. Pakistan J. agric. Res.*, 1969, **7** (1) [(5)?], 8–12. Engl., 4 ref.).—A concn. of 2 kg/acre effectively controlled kanderi (*Argemone mexicana*) and sinjhi (*Melilotus alba*), whereas 2·5

kg/acre was necessary to control jhil (*Chenopodium album*) and naro (*Convolvulus arvensis*).　　　　　E. G. Brickell.

Effect of repeated annual use of atrazine on maize. O. C. BURN-SIDE, G. A. WICKS and C. R. FENSTER (*Agron. J.*, 1969, **61** (2), 297–299. 8 ref.).—When atrazine was applied pre-emergence at 2·2–4·5 kg/ha/yr over 3 yr, yields of maize and stover and seven other maize yield components were not affected. When applied at 9 kg/ha/yr for 3 yr, yields were decreased by 2–21% in the third yr but were not affected in the first 2 yr.　　　　　A. H. Cornfield.

Uptake and translocation of substituted aniline herbicides in groundnut seedlings. M. L. KETCHERSID, T. E. BOSWELL and M. G. MERKLE (*Agron. J.*, 1969, **61** (2), 185–187. 8 ref.).—Uptake of trifluralin (I) by groundnut seedlings was greater when seedlings were germinated in untreated soil and transplanted into treated soil than when they were germinated in treated soil. I moved acropetally and basipetally. Translocation decreased with increasing age of seedling. I accumulated in the cotyledons of the seedlings rather than the growing points. Basipetal movement of I was greater than that of benefin or nitralin.　　　　　A. H. Cornfield.

Adsorption of herbicides by roots. R. S. TAMES and R. J. HANCE (*Pl. Soil*, 1969, **30** (2), 221–226. 19 ref.).—The adsorptive capacities for five org. herbicides of the dry matter of roots of six plant species were lower than that of soil org. matter but were of a similar order of magnitude. In some cases, adsorption might provide a significant pathway by which herbicides are taken up by living plants, but it seems unlikely that root adsorption would have much effect on herbicide concn. in the soil soln.　　　　　A. H. Cornfield.

Tolerance of *Sorghum bicolor* (L.) Moench to several herbicides. F. R. MILLER and R. W. BOVEY (*Agron. J.*, 1969, **61** (2), 282–285. 10 ref.).—The tolerance of 40 cultivars of sorghum, grown in clay soil or sand treated with 3·2–28·8 ppm of active ingredient, decreased in the order propachlor > propazine > norea > GS-14260 (2-t-butylamino-4-ethylamino-6-methylthio-*s*-triazine). The 40 varieties, which were developed or originated in various countries, differed in the extent of their tolerance to herbicides. The best-yielding varieties in the absence of herbicides also grew best where herbicides were applied. Herbicide tolerance was more pronounced in sorghums from equatorial Africa than from areas where varieties had been developed through intensive breeding.　　　　　A. H. Cornfield.

Empirical relationship between chemical structure and the sorption of some herbicides by soils. R. J. HANCE (*J. agric. Fd Chem.*, 1969, **17** (3), 667–668. 6 ref.).—A factor given by (parachor − 45*N*), where *N* is the no. of sites in a mol. which can participate in the formation of a hydrogen bond, is shown to be correlated with log.(Freundlich *k* value) for the adsorption of 29 aromatic herbicides by two soils. This relationship may be useful for the prediction of the approx. extent of adsorption of such herbicides by soils in which the organic matter is the dominant adsorbing constituent.　　　　　I. Dickinson.

Titrimetric determination of diuron in herbicide formulations. S. H. YUEN and B. MILOSEVIC (*Analyst, Lond.*, 1969, **94** (1122), 820–822. 5 ref.).—The diuron was extracted into CHCl₃ from a dispersion of the sample in 4N-HCl and then, after removal of solvent, was hydrolysed in boiling 24N-H₂SO₄ for 30 min. The cool soln. was made alk. and the liberated Me₂NH was distilled into 2% H₃BO₃ and titrated with 0·05 N-HCl (methylene blue-methyl red indicator). The error was < 1% (commercial and prep. samples). Any component extractable by CHCl₃ and yielding a volatile base (e.g., 3,4-dichloroaniline) during acid hydrolysis, interferes giving a positive error.　　　　　W. J. Baker.

Infra-red analysis of Dacthal in formulations. L. A. WAPENSKY (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 874–875).—The sample (≡ 250–500 mg of dimethyl tetrachloroterephthalate) was Soxhlet extracted with benzene for 6 h, the benzene extracts were evaporated to dryness and the residue was dissolved in CS₂. The absorption of the 964 cm⁻¹ band was measured. Beer's law was obeyed over the 5–15 mg/ml concn. range.　　　　　D. I. Rees.

Comparison of competitive ability of certain common weed species. R. S. TRIPATHI (*Trop. Ecol.*, 1968, **9** (1), 37–41).—The competitive abilities (*CA*) of wheat (I) and grain (II) crops and four weed species were studied. *Anagallis arvensis* had the poorest *CA* in relation to I and II. *Asphodelus tenuifolius* was a strong competitor of I but with II it was far less. *Trichoderma indicum* had the highest *CA* in terms of shoot reduction of II but was a relatively poor competitor of I. *Euphorbia dracunculoides* in general had a higher *CA* with II than I.　　　　　C. V.

Fungicides

Studies on seed-borne microflora and the effect of the seed treatment of rice. GHULAM RASOOL SOLANGI, M. KAMAL and R. M. ABBASI (*W. Pakistan J. agric. Res.*, 1968, **6** (4), 92–98. Engl., 8 ref.).—*Xanthomonas* sp., *Cochliobolus spicifer*, *Trichoconis padwickii* and *Aspergillus* spp. were observed in the highest frequencies and were the most effective in reducing germination. Ceresan M was the best fungicide tested for improving germination, minimising disease incidence, and increasing yield.
　　　　　E. G. Brickell.

Fungicides in perspective. K. J. BENT (*Endeavour*, 1969, **28** (105), 129–134. 14 ref.).—Future developments in plant protection (esp. annual crops), will probably be in the field of systemic fungicides. These may assume the form of antibiotics which are selectively toxic to micro-organisms, or compd. which alter the metabolism of the plant, thus conferring disease resistance, e.g., growth-regulating substances such as the phenoxyacetic acids. Pyrimidine deriv., 1,4-oxathiins and benzimidazole deriv. are among such systemic compd. recently introduced.
　　　　　J. M. Jacobs.

Interaction of spore concentration and fungicide concentration on the control of pineapple disease of sugar-cane setts. B. C. PATIL-KULKARNI and J. E. C. ABERDEEN (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (39), 457–460. 3 ref.).—Setts were artificially inoculated with a const. high concn. of *Ceratocystitis paradoxa* (de Seynes) Moreau (I), and then dipped in various concn. of two mercuric fungicides. Infection decreased with increasing fungicide concn. Results indicated the presence of Hg-tolerant organisms as well as I.　　　　　M. T. Rawnsley.

Field evaluation of a new fungicide (Benomyl) for apple scab. W. J. MOLLER, T. J. WICKS and J. N. STEAD (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (39), 461–462. 3 ref.).—Benomyl [1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester] (I) as 50% wettable powder, and Melprex (R) (n-dodecylguanidine acetate) (II) were compared. I used at 4 oz/100 gal every 10–14 days was the most effective treatment, while at 2 oz/100 gal every 10–14 days, the same effects were obtained as for II at 8 oz/100 gal. Frogeye spot [leaf disease caused by *Physalospora obtusa* (Schw.) Cke.] was better controlled by II. Phytotoxicity was not apparent with I.　　　　　M. T. Rawnsley.

Studies on the chemical control of Alternaria fruit rot of chillies. M. S. PERWAIZ, SARDAR M. MOGHAL and M. KAMAL (*W. Pakistan J. agric. Res.*, 1968, **6** (4), 87–91. Engl., 4 ref.).—Best results were obtained with the fungicidal sprays Copper Sandoz and Bordeaux mixture. Dithane also produced good results.　　　　　E. G. Brickell.

Ethirimol—a new systemic fungicide for control of cereal powdery mildews. R. M. BEBBINGTON, D. H. BROOKS, M. J. GEOGHEGAN and B. K. SNELL (*Chemy Ind.*, 1969, (42), 1512–1513. 3 ref.).—Ethirimol (5-n-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine) is loosely bonded to soil colloids, absorbed by roots and moved in the xylem of the plant. Applied (1 lb/acre) as seed dressing or, preferably, coated onto fertiliser granules, it is effective on wheat, oats and barley; the yield of barley was increased by an av. 10·4% and a max. ~ 25%. No residues were detected in cereal grains, and the mammalian and avian toxicity is low.　　　　　W. J. Baker.

Insecticides and Others

Fumigation to control citrus nematode in sultana vineyards. M. R. SAUER (*J. Aust. Inst. agric. Sci.*, 1969, **25** (2), 128. 4 ref.).—Dibromochloropropane was found to be an effective control agent with a relatively long-term effect up to 3 yr.　　　　　E. G. Brickell.

Dieldrin uptake by maize as affected by soil properties. G. B. BEESTMAN, D. R. KEENEY and G. CHESTERS (*Agron. J.*, 1969, **61** (2), 247–250. 18 ref.).—Uptake of dieldrin, applied to soils at 1 or 5 ppm, by maize roots and shoots was inversely related to soil org. C content (0·4–7·7%), but was not related to cation-exchange capacity, free iron oxides or amount or type of clay minerals present. Dieldrin content in root tissue was 20–80 times greater than that in shoot tissue. Less than 3% of the dieldrin applied to the soils was translocated to the above-ground parts of the plant.
　　　　　A. H. Cornfield.

10-[*N*-(Dialkyl-phosphono　and　-thiophosphono)glycyl]phenothiazines. D. KH. YARMUKHAMETOVA, B. V. KUDRYAVTSEV and V. D. ERMAKOVA (*Izv. Akad. Nauk SSSR Ser. Khim.*, 1969, (1), 170–171. Russ., 5 ref.).—14 of these deriv. of glycylphenothiazine (I) were synthesised. I was synthesised from choracetylphenothiazine and urotropin, and was phosphorylated with phosphoric acid

chloranhydrides in the presence of bases such as NEt_3. The products obtained were crystalline, sol. in org. solvents and insol. in water. The compounds were as larvicidal as phenothiazine but half as effective 10-diethylphosphonoacetylphenothiazine. In the series, changing the acetyl group for glycyl reduced larvicidal activity.   L. A. Haddock.

Degradation and anticarboxylesterase activity of disulfoton and phorate after ^{60}Co gamma irradiation. D. L. GRANT, C. R. SHERWOOD and K. A. McCULLY (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 805–811. 11 ref.).—An aq., acetone or hexane soln., containing disulfoton and phorate, was irradiated at room or dry ice temp. with ^{60}Co γ-radiation at dose levels of 0–4 Mrad for 30 min/Mrad duration. The aq. soln. was extracted with $CHCl_3$, the $CHCl_3$ extracts were evaporated to dryness and the residue was dissolved in acetone. The parent compounds and their oxidation products, sulphoxide, sulphone, O analogue and the O analogue sulphoxide and sulphone, were analysed by t.l.c. using Kieselgel G-HR layers with acetone–hexane as solvent; the anticarboxylesterase inhibiting substances were detected by the method of Mendoza *et al.* (*Analyst, Lond.*, 1968, **93**, 34). The reaction products were also analysed by g.l.c. with use of a packed column containing either SE-30 + QF-1 or DC-200 + QF-1 with electron capture or phosphorus detection. The pesticides were degraded to the greatest extent in hexane or acetone soln. and less decomp. occurred at the lower temp.   D. I. Rees.

New gas chromatographic procedure for [determining] the total active ingredient content of Karathane Technical. C. P. KURTZ and H. BAUM (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 872–873).—A nitrobenzene soln. of the pesticide sample, containing a known amount of 4,6-dinitro-*o*-cresol, is analysed by g.l.c. on a glass column of QF-1 on Gas Chrom Q temp. programmed from 100 to 230° at 6°/min, with He as carrier gas and flame ionisation detection. The ingredients determined were octenes ($\sim 3\%$), crotonic acid ($\sim 2\%$), mononitro-octylphenols ($\sim 1\%$), dinitro-octylphenols ($\sim 5\%$) and dinitro-octylphenol crotonates ($\sim 75\%$).
                                                                  D. I. Rees.

Pesticidal granules. N. V. PHILIPS' GLOEILAMPENFABRIEKEN (Br. Pat. 1,164,709, 26.6.67. Neth., 28.6.66 and 6.6.67).—The granules, which are stable on prolonged storage, comprise a mixture of water ($\gg 5$ wt.-%), a pesticide volatile in the presence of water, a hydrophilic binder and a powdered, inert carrier. In an example, ground dolomite, Na sulphonated fatty alcohols and 2,6-dichlorobenzonitrile are mixed and water is atomised into the powder while stirring. The mixture is introduced into a compacter and then crumbled and sieved.   S. S. Chissick.

Treatment of malodorous organic thiophosphate pesticides. AMERICAN CYANAMID CO. (Br. Pat. 1,152,012, 14.9.66. U.S., 23.10.65).—Offensive odour (e.g. of malathion) is reduced by admixing with an aldehyde (Et glyoxylate, CH_3CHO, paraldehyde, PhCHO, furfuraldehyde, CH_2:CMe·CHO, *p*-C_6H_4Me·CHO, etc.) which is capable of forming a mercaptoaldehyde, b.p. $\ll 175°$, and which is at least slightly sol. in the pesticide.
                                                                  F. R. Basford.

Pesticidal preparations. CIBA LTD. (Br. Pat. 1,160,648, 24.5.67. Switz., 7.6.66).—Nitrazones Ph·NH·N: $C(NO_2)$·R^I or X·C_6H_4-N: N·$CH(NO_2)R^I$ and their salts with various cations are claimed, where X is H, halogen, 1–4C alkyl or alkoxy, NO_2, chlorophenoxy or CF_3 and R^I is 1–4C alkyl or X·C_6H_4. The compounds, which are prep. in the known manner, are suitable for use in herbicidal compositions and also exhibit activity against micro-organisms including fungi, insects, acarids, nematodes and molluscs, and their eggs.   S. S. Chissick.

Organotin compounds. U.S. BORAX & CHEMICAL CORP. (Br. Pat. 1,163,289, 10.11.66. U.S., 15.11.65, 11.8.66).—Compounds of the formula SnR_3·OCOR^I wherein R is alkyl, aralkyl or aryl, and R^I is alicyclyl, have herbicidal and pesticidal activity, and are prepared from the corresponding organo-Sn hydride, oxide or hydroxide. E.g., a mixture of $SnHBu_3$, adamantane-1-carbonyl chloride, and 3 : 1 vol. hexane–dioxan is boiled for 15 h, then distillation affords tributyltin-1-adamantane carboxylate, b.p. 175°/0·3 mm, m.p. 31–34°.   F. R. Basford.

Herbicidal diuracils. E. I. DU PONT DE NEMOURS & CO. (Br. Pat. 1,160,028, 4.4.67. U.S., 4.4.66, 13.3.67) (20 pp.).—The compounds (or isomers thereof), of formula ZY_2, are effective against *Digitaria* spp., *Echinocloa crusgalli*, *Chenopodium album*, *Helenium tenuifolium*, *Sorghum halepense*, *Poa* spp., *Allium vineale*, *Rumex* spp., *Lepidium virginicum* and *Rhus radicans* [Y is 3-R-5-X-

6-R^I-2,4-dioxo-1,2,3,4-tetrahydropyrimidyl; R is alkyl of 3–8C, Ph, CH_2Ph, or cycloalkyl of 5–8C optionally containing Me or OMe; R^I is alkyl of 1–3C; and X is Cl, Br, I, Me, or when R is cycloalkyl then X may be H; or X and R^I together represent $[CH_2]_{3\ or\ 4}$; and Z is 1,2,3,6-tetramethylphthalyl, CO, CO·CO, CHR^{II}·CO (R^{II} is H or alkyl of 1–4C), or CO·R^{III}·CO (R^{III} is alkylene or alkenylene of 1–12C, or is cyclohexane or benzene residue)]. In an example, $COCl_2$ (5) is added to a suspension in tetrahydrofuran (200) of the Na salt of 5-bromo-3-s-butyl-6-methyluracil (28), then after 16 h at room temp., solvent is removed/ < 1 atm, and the residue is stirred at 0° with NaOH (50 pt.) in water (1000 pt.), with separation of 1,1′-carbonylbis(5-bromo-6-methyl-3-s-butyluracil), m.p. 220°.   F. R. Basford.

Uramidobenzoate compounds and herbicidal compositions containing them. U.S. BORAX & CHEMICAL CORP. (Br. Pat. 1,156,866, 7.11.67. U.S., 15.11.66).—The title compounds of formula *o*-$C_6H_4R^1R^2$, where R^1 is an optionally halogen-substituted saturated or unsaturated aliphatic or aromatic ester group and R^2 is NH·CO·NH·$N(CH_2)_n$ where $n = 4$–7, are claimed together with a process for their prepn. and their use as the active constituents of herbicidal compositions. Prepn. is by reacting an appropriate ester of anthranilic acid with the corresponding *N*-aminoalkyleneimine in an inert solvent. In an example, a mixture of *N*-aminohexamethyleneimine, 2-methyl-2-propenyl 2-isocyanatobenzoate and C_6H_6 is refluxed for 3·25 h and worked up to yield 2-methyl-2-propenyl 2-(ω-hexamethyleneiminouramido)benzoate (88·7% yield). 22 examples of the herbicidal activity of compositions containing these compd. are presented. In general, pre-emergence treatment at 0·75–4 lb/acre gave complete kills of most weeds (e.g., ragweed, pigweed, ryegrass, crabgrass and Johnson grass) and, e.g., maize, millet, oats, peas, mustard, cucumbers and snap beans at 7·5 lb/acre, after 30–40 days; groundnuts, sorghum and safflower showed no injury.   S. S. Chissick.

Polymethyleneiminecarbothiolates. MONSANTO CO. (Inventor: J. J. D'AMICO) (Br. Pat. 1,167,084, 9.1.67).—Compd. with herbicidal properties have formula $PhCH_2$·S·CO·N$\overline{[CH_2]_n CH_2}$ wherein *n* is 6 or 7, and are esp. effective against barnyard grasses (esp. *Echinochloa crusgalli*) and bromegrass (*Bromus tectorum*) in wheat. In an example, 85% COS (97) is added during 70 min at 5–10° to a mixture of heptamethyleneimine (183), water (375) and 25% aq. NaOH (200), rapidly followed by $PhCH_2$Cl (158·3). After a further 1 h each at 5–10°, 10–15°, 15–20° and 20–25°, and 20 h at 25–30°, water (400 g) and ether (1000 ml) are charged, then 15 min later the washed org. layer is evaporated at 80–90°/1–2 mm, to give benzyl hexahydro-1(2*H*)-azocinecarbothiolate (99% yield).
                                                                  F. E. Basford.

Pyridazone derivatives. BADISCHE ANILIN- U. SODA-FABRIK A.-G. (Inventors: F. REICHENEDER and A. FISCHER) (Br. Pat. 1,163,835 and 1,163,837, [A] 29., [B] 30.12.66. Ger., [A] 5., [B] 7.1.65).—Compounds with herbicidal activity (e.g. against *Chenopodium album*, *Urtica urens*, *Sinapis arvensis*, *Lamium asplexicaule* and *Poa annua*) are listed, and examples are [A] 5-chloro-4-(2,2,2-trichloro-1-hydroxyethylamino)-1-*p*-fluorophenyl-6-oxo-1,6-dihydropyrid-azine, m.p. 228–229°, prepared by boiling a mixture of 5-chloro-4-amino-1-*p*-fluorophenyl-6-oxo-1,6-dihydropyridazine (5) and chloral (50 pt.) for 10 min, and [B] *O*-(5-chloro-1-phenyl-6-oxo-1,6-pyridazin-4-ylcarbamoyl)acetone oxime, m.p. 148–149°, prepared by boiling a mixture of 5-chloro-1-phenyl-6-oxo-1,6-dihydro-pyridazin-4-yl isocyanate (12), benzene (50) and Me_2C:NOH (3·6 pt.) for 10 min. Methods of application are described and results of biological tests with various plants, e.g. beet, maize, peas, onions, are tabulated, where the superiority of these compounds over known active ingredients, e.g., 5-chloro-4-(2,2,2-trichloro-1-hydroxyethylamino)-5-chloropyridazone, is shown.
                                                                  F. R. Basford.

Thiaimidazolidines [herbicides]. FARBENFABRIKEN BAYER A.-G. (Inventors: G. ZUMACH, L. EUE, W. WEISS, *et al.*) (Br. Pat. 1,162,472, 9.1.68. Ger., 27.1 and 10.8.67).—Compounds claimed have the formula $\overline{CO·NR·CO·N}$·$C_6H_{4-n}Cl_n$·CF_3 wherein *n* is 0 or 1 and R is an optionally halogenated aliphatic hydrocarbon radical of 1–4 C. They are prepared by condensing NHR·CO·NH·$C_6H_{4-n}Cl_n$·CF_3 (I) with COCl·SCl at 20–120°. E.g., a mixture of I (R = Me; *n* = 0) (22), COCl·SCl (14), and benzene 200 ml is boiled for 7 h to give 2-(*p*-trifluoromethylphenyl)-4-methyl-3,5-dioxo-1,2,4-thiaimidazolidine (16 g), m.p. 154° (MeOH). Its effect on *Echinochloa*, *Chenopodium*, *Sinapis*, etc., is described.
                                                                  F. R. Basford.

Substituted 1,2,4-triazoles. VEB LEUNA-WERKE 'WALTER ULBRICHT' (Inventors: H. BECKER and K. WEHNER) (Br. Pat. 1,157,256, 17.5.67).—The compounds are 3-R^1-5-R^2-1,2,4-triazoles where R^1 and R^2 are various substituted amino and mercapto and other substituents or R^2 is H, made by reacting 3-halo- or 3,5-dihalo-1,2,4-triazoles with a nucleophilic reagent, e.g., an alcohol, amine, mercaptan or acid, at 100–270°, optionally with a solvent, a catalyst and a weak base. In an example, 3-chloro-1,2,4-triazole is refluxed in $PhNH_2$ for 5 h and the mixture worked up to yield 3-anilino-1,2,4-triazole, m.p. 168–170°. The compds are generally suitable as weed-killers. S. S. Chissick.

N-Tetrahaloethylthiopyrazole and N-haloalkylthiobenzopyrazole pesticides. CHEVRON RESEARCH CO. (Inventor: J. G. E. FENYES) (Br. Pat. 1,151,623, 27.7.67. U.S., 22.8. and 15.9.66).—The title compounds have bactericidal and fungicidal properties (e.g., against the plant pathogens *Erwinia*, *Agrobacteria*, *Corynebacteria*, *Xanthomonas* and *Pseudomonas*). An example is 1-(tetrachloroethylthio)pyrazole, prep. by stirring an aq. mixture of pyrazole and $SCl \cdot CCl_2 \cdot CHCl_2$ for 19 h at room temp. F. R. Basford.

Dithiolphosphoric acid triesters. FARBENFABRIKEN BAYER A.-G. (Inventors: G. SCHRADER and H. SCHEINPFLUG) (Br. Pat. 1,162,740, 27.12.67. Ger., 1.2.67).—Esters of the formula $OR \cdot PO(SR^1)_2$, wherein R is cyclohexyl which may contain $\not>$ 3 alkyl of 1–6C and R^1 is Ph optionally containing 1–3 halogen, NO_2 or C_{1-4}-alkyl or -alkoxy, are fungicides especially effective against *Cochliobolus miyabeanus*, *Mycosphaerella*, *Cerospora*, *Alternaria*, *Corticium* and *Botrytis* spp., *Fusarium cubense*, *F. dianthi*, *Verticillium alboatrum* and *Phialophora cinerescens*. An example is *O*-cyclohexyl-*S*,*S*-diphenyldithiolphosphoric acid ester, m.p. 65°. F. R. Basford.

Oxetanes and their uses. UNION CARBIDE CORP. (Inventors: D. R. ARNOLD and A. A. SOUSA) (Br. Pat. 1,163,886, 1.9.66).—The title compounds, of which 4-pyridyl-4-phenyl-3-oxatricyclo-[4.2.1.0$^{2.5}$]nonanes are examples, are fungicidal (and especially antimildew) agents, and are prepared by condensing appropriate ketones with olefinic compounds in presence of irradiation at 2000–4000 Å. E.g., a mixture of 3-benzoylpyridine (0·05), norbornene (0·05 mole) and benzene (200 ml) is irradiated for 24 h, then solvent is removed/< 1 atm. The residue is continuously extracted with hexane, then material recovered from the extract is recrystallised from ether, to give 4-(pyrid-3-yl)-4-phenyl-3-oxatricyclo-[4.2.1.0$^{2.5}$]nonane (53% yield). Biological tests against *Erysiphe polygoni*, *Podosphaera leucotricha*, *Alternaria solani* and *Colletotrichum lagenarium* are described and comparative results with Karathane, Zn ethylene bisdithiocarbamate and Mn bisdithiocarbamate are tabulated. The oxetanes are generally effective at levels of 4–500 (20–100) ppm. F. R. Basford.

Novel oxetanes and their uses. UNION CARBIDE CORP. (Inventors: D. R. ARNOLD and A. A. SOUSA) (Br. Pat. 1,164,471, 10.9.66).—Oxetanes with fungicidal and mildewicidal action (as in preceding abstr.) have formula $CR^IR^{II} \cdot CR^{III} \cdot R^{IV} \cdot CR^VR^{VI}$, wherein R^I–R^{IV} are H, heterocyclyl or hydrocarbon radicals optionally substituted by halogen, NH_2, alkoxy or aminoalkoxy; and R^V–R^{VI} are the same (other than H) or may be hydrocarbon radicals substituted by CN or heterocyclyl, or $CONH_2$ optionally substituted by alkyl, cycloalkyl, or heterocyclyl. Other possibilities are listed. In an example, a soln. of Ph_2CO (18·2 g) in benzene (150 ml) is purged with N_2, $Me_2C:CH_2$ is added to 200 ml, then after 24 h in presence of light, solvent is evaporated. Chromatographic purification of the residue (22·1 g) gives 2,2-diphenyl-3,3-dimethyloxetane, m.p. 88·5–90°. Biological tests against *Erysiphe polygoni* and *Podosphaera leucotricha* are described. F. R. Basford.

Preparation of phosphoric, phosphonic, and thiono-phosphoric and -phosphonic acid esters. FARBENFABRIKEN BAYER A.-G. (Inventors: W. LORENZ, G. UNTERSTENHOFER and I. HAMMAN) (Br. Pat. 1,162,088, 13.2.68. Ger., 1.3.67).—Esters p-$C_6H_4[C(CN):N \cdot O \cdot PX(OR)OR^1]_2$ wherein X is O or S; R is alkyl of 1–4 C; and R^1 is Ph, OPh, cyclohexyl, cyclohexoxy, or C_{1-4}-alkyl or -alkoxy, are insecticides and acaricides of high activity. In an example, $(OEt)_2PSCl$ (113) is added with cooling to a mixture of p-C_6H_4-$[C(CN):N \cdot OH]_2$ (77), acetone 300 ml, and 95% NEt_3 (77), then 1 h later the batch is poured into water. Ppt. recrystallised from 1 : 1 ether–light petroleum affords bis[a-(O,O-diethylthiono-phosphoryl)oximino]phenylene-1,4-bisacetonitrile (150 g), m.p. 78°. Tests against *Phaedon cochleariae* larvae, *Tetranychus telarius* and *Plutella maculipennis* are described. F. R. Basford.

Preparation of phenyltrichloroacetoimidochlorides. FARBENFABRIKEN BAYER A.-G. (Inventors: H, G, SCHMELZER, E, DEGENER, H,

TARNOW, *et al.*) (Br. Pat. 1,151,627, 19.10.67. Ger., 28.10.66, 16.6.67).—The title compd., showing acaricidal properties, are prep. in high yield by chlorination of $(CF_3)_aC_6H_{5-a-m-n}R_nX_m \cdot N$: $CH \cdot CCl_3$ at 80–250° (X is halogen; R is alkyl, chloroalkyl, and/or CN; a is 0–2; m is 0–5; and n is 0–2). E.g., Cl_2 is passed at 100° into $C_6Cl_5 \cdot N$: $CH \cdot CCl_3$ for 3 h, temp. rising to 180° after 1 h, to give pentachlorophenyltrichloroacetoimidochloride, m.p. 131–133°. F. R. Basford.

Derivatives of $\triangle^1$**-pyrroline.** PRODUITS CHIMIQUES PECHINEY ST.-GOBAIN (Inventors: A. ETIENNE and Y. CORREIA) (Br. Pat. 1,167,809, 2.11.67. Addn. to Br. Pat. 1,080,737, 19.8.64).—Compd. with insecticidal activity (against *Calandra* and *Ephestia Kuehniella*) comprise $\triangle^1$-2-R^1-pyrrolines wherein R^1 is alkyl containing NO_2, CN, CO_2H and/or carbalkoxy. They are prepared by reacting a 2-alkoxy-$\triangle^1$-pyrroline with R^1H at 70–200°. E.g., a mixture of 2-methoxy-$\triangle^1$-pyrroline (1) and $MeNO_2$ (2 mole) is boiled for 15 h, to give 2-nitromethyl-$\triangle^1$-pyrroline (40%), m.p. 108°. F. R. Basford.

Benzylidenemalonitriles. SUMITOMO CHEMICAL CO. LTD. (Br. Pat. 1,161,019, 5.12.67. Jap., 6.12.66).—The compounds, useful as the active constituents of pesticidal prep., e.g., for use against rice blast, *Cochliobolus miyabeanus*, plant hoppers, mosquito larvae, spider mites and root-knot nematodes of tomatoes, have formula p-$MeCO_2 \cdot (C_6H_2R^1R^2) \cdot CH$:$C(CN)_2$, where R^1 and R^2 are alkyl groups or one of them is H. They are prep. from the corresponding 4-hydroxy-alkyl- or -dialkyl-benzylidenemalonitrile by reaction with an acyl halide or $(MeCO)_2O$ in C_6H_6, Et_2O, etc., in the presence of excess Mg powder or excess base, e.g., pyridine. In an example, AcCl is added to a mixture of 3,5-di-t-butyl-4-hydroxy-benzylidenemalonitrile and pyridine in C_6H_6 and the mixture is refluxed for 10 h and worked up to yield 4-acetoxy-3,5-di-t-butyl-benzylidenemalonitrile. S. S. Chissick.

Pentachlorobenzylideneamine derivatives. SUMITOMO CHEMICAL CO. LTD. (Br. Pat. 1,164,556, 11.10.67. Jap., 13.10. and 7.11.66).—The compounds, useful as the active constituents of microbicidal and insecticidal compositions (e.g., against rice blast, stem borers and leaf hoppers), are prep. from C_6Cl_5CHO by reaction with NH_2OH or RNH_2 (R is alkyl of 1–16 C, hydroxyalkyl of 2–3 C, allyl, Ph, cyclohexyl, halophenyl or methylphenyl) in boiling benzene. S. S. Chissick.

Diseases and Pests in Livestock;
Veterinary Treatments

Control of Exogenous Pests

Comparison of cattle tick control by pasture spelling, planned dipping and tick-resistant cattle. R. H. WHARTON, K. L. S. HARLEY, P. R. WILKINSON, *et al.* (*Aust. J. agric. Res.*, 1969, **20** (4), 783–797. 14 ref.).—Results of earlier expt. are confirmed, viz., adequate spelling of pastures, or repeated dipping of cattle at 21-day intervals, leads to a great improvement in cattle tick control. Assessment of the tick resistance of Zebu × British cattle showed that a mean survival to maturity of female ticks was 1·3 and 1·8% for herds requiring 4 and 10 dippings, resp., compared with 4·4 and 5·2% for 'resistant' Australian Illawarra Shorthorn which required 0 and 1 dipping, resp., and 8·9 and 14·0% for 'susceptible' herds which required 5 and 6 dippings, resp. E. G. Brickell.

Other Treatments

Evaluation of seven procedures for detection of abnormal milk due to mastitis. R. B. READ, JUN., A. L. REYES, J. G. BRADSHAW and J. T. PEELER (*J. Dairy Sci.*, 1969, **52** (9), 1359–1367. 11 ref.).—The results of an evaluation of five screening tests (California mastitis, catalase, milk quality, modified Whiteside, and Wisconsin mastitis tests) for detecting mastitis milk indicated that the Wisconsin test reflects, more closely than the other tests, somatic cell numbers as measured by the direct microscopic somatic cell count. The electronic somatic cell count was more precise than the direct microscopic cell count as a confirmatory procedure. M. O'Leary.

Genetic resistance to leukosis caused by the JM virus in the fowl. FAN-SIONG HAN, J. R. SMYTH, JUN., M. SEVOIAN and F. N. DICKINSON (*Poult. Sci.*, 1969, **48** (1), 76–87. 27 ref.).—The responses of various stocks to inoculation with the JM leukosis isolate were studied. In addition to pure line comparisons, reciprocal cross-bred progeny were compared in order to assess the genetic bases for resistance and susceptibility to this disease agent. A. H. Cornfield.

Anticoccidial activity of sulphadimethoxine potentiated mixture (Ro 5–0013) in chickens. M. MITROVIC, E. G. SCHILDKNECHT and G. FUSIEK (*Poult. Sci.*, 1969, **48** (1), 210–216. 11 ref.).—In battery and floor pen trials, addition of 0·01–0·02% of Ro 5-0013 [sulphadimethoxine potentiated with 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl)pyrimidine] to the feed was very effective for control of coccidiosis in broiler and replacement trials.

A. H. Cornfield.

Safety and compatability of sulphadimethoxine potentiated mixture (Ro 5-0013), a new broad spectrum coccidiostat–antibacterial, in chickens. W. L. MARUSICH, E. OGRINZ, M. BRAND and M. MITROVIC (*Poult. Sci.*, 1969, **48** (1), 217–222. 12 ref.).—8-week feeding trials with sulphadimethoxine at levels up to 0·1% and the potentiator [2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl)pyrimidine] at up to 0·05%, or combinations of the two (Ro 5-0013) at up to 0·08% (four times the normal dosage), did not affect feed efficiency, mortality, haematology or gross pathology of chickens. Continuous feeding of 0·08% of Ro 5-0013 for 20 weeks did not affect subsequent egg production, fertility, hatchability or quality of eggs. Ro 5-0013 was compatable with arsanilic acid, 3-nitro-4-hydroxyphenylarsonic acid, Zn-bacitracin, procaine penicillin, and penicillin + streptomycin.

A. H. Cornfield.

Complexes of benzimidazoles with thiobisphenols and sulphinylbisphenols. MERCK & CO. INC. (Inventor: R. J. LAPIERRE) (Br. Pat. 1,157,293, 6.2.67. U.S., 18.2.66. Addn. to Br. Pat. 1,056,022, 2.4.64).—The complexes, effective against a wide variety of helminths (roundworm, tapeworm, flukes in, e.g., sheep, cattle, goats, horses, mules), are formed from $1-R^I-2-R-5-R^{II}-6-R^{III}$-benzimidazole and $R^V[C_6HR^{IV}R^{VI}Cl(OH)-1,3,5,6,2]_2$ wherein R is thiazolyl, isothiazolyl, thiadiazolyl, pyrryl, furyl, thienyl, or Ph; R^I-R^{III} are H, C_{1-5}-alkyl, or R^I is alkenyl or $R^{II}-R^{III}$ are CF_3 or alkoxy of 1–5 C; R^{IV} is halogen or NO_2; R^V is S or SO; and R^{VI} is H, Cl, or alkyl of 1–5 C (but is H when R^V is SO). E.g., a mixture of $SO(C_6HCl_3 \cdot OH-3,5,6,2)_2$ (7·9), 2-(thiazol-4-yl)benzimidazole (I) (2·69) and MeOH 250 ml is boiled for ~ 16 h, then unchanged I (1·17) is removed. Filtrate is concentrated to ~ 50 ml, cooled to 0° and the pptd. complex (2·7 g), m.p. 191°, is collected.

F. R. Basford.

Substituted benzamides. SALSBURY LABORATORIES (Br. Pat. 1,166,793, 1.4.68. U.S., 14.8.67).—Benzamides of formula $NO_2 \cdot C_6H_3(CF_3) \cdot CXNR^IR^{II}$ are effective in the treatment of coccidiosis (X is O or S; R^I and R^{II} are H or alkyl or alkenyl, optionally substituted by halogen or alkoxy of 1–8 C). In an example, a mixture of $5,3,1-NO_2 \cdot C_6H_2(CF_3)CO_2H$ (50) and $SOCl_2$ (72 g) is boiled for 4 h, then $SOCl_2$ is removed/< 1 atm. The residue is quenched in 30% aq. NH_3 (1000 ml), with separation of 5-nitro-3-(trifluoromethyl)benzamide (37·4 g), m.p. 139–140°, in 76% yield. Veterinary trials with broiler-type heavy-breed birds and hybrid Leghorn chicks are described and extensive results are presented in 11 tables.

F. R. Basford.

Sulphathiadiazole compounds and compositions containing them. MERCK & CO. INC. (Br. Pat. 1,161,094, 13.10.66. U.S., 15.10.65, 1.8.66).—Compounds p-$NHR^{II} \cdot C_6H_4 \cdot SO_2NR^IR^{III}$ and alkali metal, alkaline-earth metal, and Mg salts thereof are useful in treatment and prevention of coccidiosis in poultry (especially against *Eimeria brunetti*) (R^{III} is 4-OR-1,2,5-thiadiazol-3-yl; R is C_{3-5}-alkyl or C_{2-5}-alkenyl or -alkynyl, R^I is H, alkyl, and R^{II} is H or acyl). In an example, a mixture of 3-chloro-4-allyloxy-1,2,5-thiadiazole (15·6), p-$NH_2 \cdot C_6H_4 \cdot SO_2NH_2$ (50·3), K_2CO_3 (40·4), and $AcNH_2$ (15) is heated at 145° for 25 min, then at 100° water (200) is charged. The mixture is distilled up to 100°, then aq. HCl is added at room temp. to pH 8·8 and solid is filtered off. The aq. filtrate is adjusted to pH 4 and ppt. is recrystallised from 50% aq. Pr^IOH, to give 4-(p-aminobenzenesulphonamido)-3-allyloxy-1,2,5-thiadiazole (15·3g), m.p. 153–155°.

F. R. Basford.

[A] The antibiotic methobottromycin. [C] The antibiotic amethobottromycin. [B] Derivatives of methobottromycin and amethobottromycin. MERCK & CO. (Br. Pat. 1,150,474—6, 9.8.66. U.S., 16.8.65).—[A, B] Suitable strains of *Streptomyces canadensis* ATCC 17776 (or mutants thereof) are grown in aq. nutrient medium, then the fermentation broth is extracted for the recovery of [A] methobottromycin (I), m.p. 166–167°, C 59·5, H 7·52, N 13·5, S 3·9, O 15·58%, [c] amethobottromycin (II), m.p. 154–163°, C 58, H 7·44, N 13·42, S 4·77, O 15·97%. Both products are especially useful for treating chronic respiratory diseases in chickens and infectious sinusitis in turkeys. [B] Similarly useful products comprise amides of I and II, (e.g., I-methylamide), prep. by inter-action of esters thereof with $NHRR^I$ wherein $R–R^I$ are H, alkyl, hydroxyalkyl, cycloalkyl, aryl, etc., or NRR^I is morpholino, 2-aminobenzimidazolyl or imidazolyl.

F. R. Basford.

Household Pests, Sanitation, Food Hygiene

General Sanitation

Insecticidal properties of various pyrethrum clones compared to the synthetic pyrethroids: P I, P II and cinerin I. D. J. BROADBENT and J. D. HAGARTY (*Pyrethrum Post*, 1969, **10** (1), 17–20, 40. Engl., 11 ref.).—For housefly knockdown, synthetic P II is approx. 2·5 times more effective than P I; of the natural materials, clones 2525 and 1708 showed the greatest effect. Increased mortality is dependent on (*a*) an increase in concn. of pyrethrin I and (*b*) a decrease in total concn. of cinerin. Similar toxicity relationships were shown for the German roach.

E. G. Brickell.

Biological activity of mosquito coils based on pyrethrum and coils based on other active ingredients. R. WINNEY (*Pyrethrum Post*, 1969, **10** (1), 3–6. Engl., 13 ref.).—The review covers (*i*) activity of pyrethrum (*P*)-based coils and (*ii*) factors which influence the activity of *P* coils and (*iii*) use of other active ingredients in mosquito coils. Pyrethrum was found to be the most effective for knockdown and repellency.

E. G. Brickell.

Biological activity of mosquito coils of different 'pyrethrins' composition. R. WINNEY and D. J. WEBLEY (*Pyrethrum Post*, 1969, **10** (1), 44–48. Engl., 5 ref.).—An analysis of coils, prep. from 7 powders from clones of different composition, is presented. Knockdown is directly related to Pyrethrin I : Pyrethrin II ratio and to Pyrethrin I content. Activity is also improved by high cinerin content.

E. G. Brickell.

Rapid colorimetric assay for pyrethrins. D. FURMANEC, F. A. E. SCHILLING and B. B. BROWN (*Pyrethrum Post*, 1969, **10** (1), 21–23. Engl., 3 ref.).—An adequate separation of the insecticidal constituents of Pyrethrin I and Pyrethrin II from the inert side-products was effected on Silica G-coated plates, using hexane/EtOAc (75/25) with vapour saturation. Direct treatment of the scraped SiO_2 zone with H_3PO_4/EtOAc, followed by centrifugation, then provides an adequately stable coloured soln. for assay by the Williams method (*J. Ass. off. analyt. Chem.*, **39**, 872–879).

E. G. Brickell.

Effect of methyl phenyldiazenecarboxylate (azoester) on feeding behaviour of blood-sucking invertebrates. R. GALUN, E. M. KOSOWER and N. S. KOSOWER (*Nature, Lond.*, 1969, **224** (5215), 181–182. 18 ref.).—The following effects of azoester (I) (10^{-3}–10^{-4} M) were established: (*i*) I inhibited the response of ticks (*Ornithoderus tholozani*) to chem. stimuli which induce sucking, without affecting the response to stimuli which cause probing (this inhibition is readily reversible); (*ii*) starved leeches (*Hirudo medicinalis*) refused to taste soln. of L-arginine contg. I; (*iii*) tsetse fly (*Glossina austeni*) were not prevented from probing by I, but their sucking response to chem. stimulus (e.g., ATP) was slowly inhibited; (*iv*) I had a very strong repellent effect on mosquitoes (*Aedes aegypti*), both when feeding on saline soln. contg. ATP or on a living host, many being killed. Action of I on the neuronal receptor, with involvement of thiols (possibly glutathione), is postulated. As an anti-feeding agent, I should protect people from attack by haematophagous organisms.

W. J. Baker.

Systems for food effluent treatment. F. H. SLADES (*Fd Process. Ind.*, 1969, **38** (456), 37–40.)—Processes suitable for partial or complete treatment are reviewed. Activated sludge and percolating filter methods are discussed, emphasising the importance of O_2 transfer (esp. Lubeck process). Incineration in multiple hearth and fluidised bed furnaces is also considered.

J. B. Woof.

Effective developments for treating waste products and effluents. J. V. BURGESS (*Fd Process. Ind.*, 1969, **38** (456), 43–46).—The review covers: primary filtration, including sedimentation and flotation; secondary treatment, for which the activated sludge process has definite advantages; the disposal of solids by incineration.

J. B. Woof.

Food Hygiene

Isolation and preliminary identification of enteropathogenic serotypes of *Escherichia coli*. W. H. EWING (*Publ. Hlth Lab.*, 1969, **27** (1), 19–30. 13 ref.).—

C. V.

Biochemical reactions of *Salmonella* with emphasis on differentiation of this genus and the genera *Arizona* and *Citrobacter*. W. J. MARTIN, W. H. EWING, A. C. McWHORTER and M. M. BALL (*Publ. Hlth Lab.*, 1969, **27** (2), 61–78. 16 refs.).—　　　C. V.

Effect of processing on recovery of polio virus from inoculated foods. N. D. HEIDELBAUGH and D. J. GIRON (*J. Fd Sci.*, 1969, **34** (3), 239–241. 15 ref.).—11 foods (pH 2·9–6·1), each inoculated with polio virus, were either freeze-dehydrated, ^{60}Co γ-irradiated or stored at 4 or 20°. Following DEAE Sephadex chromatog., virus contents of the eluates were determined. Recovery was significant in all cases except in highly acid foods (e.g., orange juice). Reasons for this are discussed.　　　M. T. Rawnsley.

Gram-negative bacteria associated with sloughing, a softening of California ripe olives. R. H. VAUGHN, A. D. KING, C. W. NAGEL, *et al.* (*J. Fd Sci.*, 1969, **34** (3), 224–227. 17 ref.).—Spoilage occurs during washing processes for removal of NaOH, remaining after lye treatments and oxidn. for darkening olives. The Gram-negative bacteria, associated with the spoilage, were identified as *Aerobacter aerogenes*, *A. cloacae*, *Escherichia intermedia*, and *Paracolobactrum aerogenoides*. Pseudomonads were also identified and allocated to the genus *Aeromonas*. Possible mechanisms are considered.　　　M. T. Rawnsley.

Influence of temperature on some biochemical characteristics of *Pseudomonas* associated with spoilage of chicken. C. R. REY, A. A. KRAFT, R. G. SEALS and E. M. BIRD (*J. Fd Sci.*, 1969, **34** (3), 279–283. 25 ref.).—Four *Pseudomonas* cultures, isolated from frozen chicken, were used. Growth, lipolytic and proteolytic activities, and pyoverdine production were the properties used in detg. the metabolic activity. The cultures were incubated at 5, 15, −18, and −29°. It was found that freezing impaired the ability of the cultures to produce pigment, but proteinase and lipase were not affected by prolonged freezing. Above 0° survival was better; growth and enzyme activity was more extensive at 5° than at 15°. Below freezing, survival of organisms was proportional to metabolic activity. These results may explain the reason for spoilage after defrosting.　　　M. T. Rawnsley.

Incidence and growth of some health-related bacteria in commercial freshwater crayfish (genus *Procambarus*). R. T. LOVELL and J. A. BARKATE (*J. Fd Sci.*, 1969, **34** (3), 268–271. 14 ref.).—Louisiana crayfish, both raw and cooked, were used. Coliforms, *Escherichia coli*, faecal streptococci, coagulase-positive staphylococci, *Salmonella* and *Clostridium botulinum* were found in 100, 90·6, 94·1, 3·0, 3·0, and 0%, resp., of the samples analysed. *Streptococcus faecalis* grew slowly in raw flesh, but strongly in cooked flesh. *Staphylococcus aureus* and *Salmonella typhimurium* grew well at 25 and 37°, but none grew at 5°. *C. botulinum* produced toxin within 72 h at 30°, but not in ice. At pH > 8·0, the toxin became inactive.　　　M. T. Rawnsley.

Protecting our foods from environmental intrusion. II. Bacterial toxins in food. M. S. BERGDOLL (*Fd Technol.*, Champaign, 1969, **23** (4), 530–533. 10 ref.).—Problems of detection and contamination are discussed for (*i*) enterotoxins which are proteins produced by the staphylococci under certain conditions in foods and culture media, (*ii*) botulism which is due to toxins formed by the growth of botulinum micro-organisms in underprocessed food and (*iii*) *Clostridium perfringens* which is common and present in soil, water, milk, dust, sewage and in the intestinal tract of man and animals.　　　I. Dickinson.

Symposium on natural food toxicants. (*J. agric. Fd Chem.*, 1969, **17** (3), 413–538).—19 papers, including the following:—**Shellfish poisons.** E. J. SCHANTZ (413–416. 31 ref.).—Poison in shellfish originates in certain dinoflagellates. It has been isolated from the hepatopancreas and siphon of shellfish, and is a heat-stable deriv. of a purine base ($C_{10}H_{17}O_4N_7.2HCl$). Paralysis and death occur quickly through inhibition of the Na influx associated with a nerve impulse. No known antidote. **Toxins from eggs of fishes and amphibia.** F. A. FUHRMAN, G. J. FUHRMAN, D. L. DULL and H. S. MOSHER (417–424. 50 ref.). **Use of Chlorella in mycotoxin and phycotoxin research.** M. IKAWA, D. S. MA, G. B. MEEKER and R. P. DAVIS (425–429. 7 ref.).—The growth of *Chlorella* in a liquid system was measured turbidimetrically and the concn. of toxin giving half-maximum growth was detd. Aflatoxin B$_1$ was active at 4 μg. By inoculating *Chlorella* agar plates with mould cultures, the production of toxic substances could be demonstrated by the appearance of zones of inhibition preceding the mycelial growth. Toxic substances in mushrooms or in algae could be demonstrated by placing fresh specimens directly on the agar

surface. **Biologically active compounds from field fungi.** P. M. SCOTT and E. SOMERS (430–436. 135 ref.).—Biologically active metabolites from field fungi are reviewed, with particular reference to the genera *Alternaria*, *Fusarium*, *Helminthosporium*, *Phytophthora* and *Stemphylium*. **Mycotoxins as a possible cause of fescue toxicity.** S. G. YATES, H. L. TOOKEY, J. J. ELLIS, *et al.* (437–442. 41 ref.).—Of 200 fungal isolates from toxic fescue or from nearby pastures, almost all the toxin-producing moulds belong to the genus *Fusarium*. The specific relationship, if any, of *Fusarium* toxins to fescue foot remains to be detd. by tests in cattle. **Toxins from mouldy cereals.** J. R. BAMBURG, F. M. STRONG and E. B. SMALLEY (443–450. 81 ref.).—Mouldy maize has caused poisoning of poultry, swine and cattle. Some of the fungi present and their toxic metabolites include the rubratoxins from *Penicillium rubrum*, zearalenone from *Fusarium graminarum*, and several trichothecanes, produced by *F. tricinctum*. **Destruction of aflatoxins in peanuts [groundnuts] during dry and oil roasting.** L. S. LEE, A. F. CUCULLU, A. O. FRANZ, JUN. and W. A. PONS, JUN. (451–453. 7 ref.).—Blanched whole groundnut kernels, inoculated with a toxigenic strain of *Aspergillus flavus*, were subjected to continuous shaking during incubation for 40, 46, 64 and 70 h. This produced groundnuts virtually free from visible mould and containing 4 graded levels of total aflatoxins, 130 260, 2560 and 6300 ppb (μg/kg). There was an overall reduction of 65% in B$_1$ and 62% in G$_1$ for oil roasting, and 69% in B$_1$ and 67% in G$_1$ for dry roasting. The degree of reduction in aflatoxin content was max. at the highest contamination levels, for both oil- and dry-roasted groundnuts. **Relation of aflatoxins in cotton seeds at harvest to fluorescence in the fibre.** P. B. MARSH, M. E. SIMPSON, R. J. FERRITTI, *et al.* (462–467. 16 ref.).—In lab. culture, 49 out of 50 isolates of *Aspergillus flavus* produced aflatoxin B$_1$. The fibre fluorescence could be used for screening to locate seeds with high level aflatoxins at harvest. **Mechanism of formation of a fluorescence in cotton fibre associated with aflatoxins in the seeds at harvest.** P. B. MARSH, M. E. SIMPSON, R. J. FERRETTI, *et al.* (468–472. 13 ref.).—Mechanically injured plant tissues were incubated with *Aspergillus flavus*. Results indicate that the fungus forms kojic acid which is converted to the fluorescing substance under the influence of peroxidase in the plant. **Toxic and teratogenic alkaloids of Western Range plants.** R. F. KEELER (473–482. 71 ref.).—The teratogenic potential of three range genera, *Veratrum*, *Lupinus* and *Astragalus*, is established. Three teratogens, all steroidal alkaloids, were isolated and structurally elucidated from *Veratrum*, and shown to be responsible for the natural cyclopic effects. **Natural glucosinolates (thioglucosides) in foods and feeds.** C. H. VANETTEN, M. E. DAXENBLICHLER and I. A. WOLFF (483–491. 107 ref.).—High concn. of glucosinolates in oilseed meals such as rape and crambe, limit the amt. of the seed meal that can be used in animal feeds. Under many conditions of autolysis (endogenous enzyme hydrolysis) of meal from the rapeseed *Brassica napus* and of crambe seed meal, the major glucosinolates form previously unrecognised organic nitriles instead of the 5-vinyloxazolidine-2-thiones (goitrins). **Toxic peptides and amino acids in foods and feeds.** J. W. HYLIN (492–496. 48 ref.).—Compd. reviewed include peptides from poisonous mushrooms, lathyrogenic amino acids, hypoglycin A, mimosine and selenoamino acids. **Toxicity and related physiological activity of phenolic substances of plant origin.** V. L. SINGLETON and F. H. KRATZER (497–512. 176 ref.).—Phenols appear generally toxic if natural barriers or detoxification mechanisms are overloaded, by amt., circumvented by mode of administration or foiled by uncommon compd. such as methylene diethers or isoprenoid structures. The toxicity of various plant phenols is reviewed. **Review of polycyclic aromatic hydrocarbons in foods.** J. W. HOWARD and T. FAZIO (527–531. 58 ref.).—Benzo[a]pyrene and other polycyclic hydrocarbons were isolated from smoked ham and smoked fish samples at ppb levels and were also found in some vegetable oils and total diet samples. The methodology for, and occurrence of such hydrocarbons in food are reviewed. **Natural toxic background in the food of man and his animals.** D. G. CROSBY (532–538. 92 ref.).—Natural toxicants may be intrinsic to foods, arise from microbial infestation, or be formed from less toxic precursors by enzymatic action or food processing. Present concern over traces of man-made chemicals in food must be considered in relation to possible toxic effects of long-term exposure to minor food constituents of natural origin.　　　I. Dickinson.

Aflatoxins: improved resolution by thin-layer chromatography. R. D. STUBBLEFIELD, G. M. SHANNON and O. L. SHOTWELL (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 669–672. 7 ref.).—Best separations of aflatoxins B$_2$ and G$_1$ were obtained on silica gel layers with H_2O-Me$_2$CO-CHCl$_3$ (1·5 : 12 : 88) and H_2O-MeOH-Et$_2$O (1 : 3 : 96) as solvents.　　　D. I. Rees,

Acetonitrile as an extracting solvent for aflatoxins. L. YIN (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 880. 2 ref.).—Groundnut, Brazil nut or cottonseed meal was mixed in a high speed blender for 3 min, or by shaking for 30 min, with MeCN–H₂O and hexane. The mixture was filtered and an aliquot of the MeCN layer was evaporated to dryness, the residue dissolved in benzene and an aliquot (≡ ∼ 0·1 g of sample) was spotted directly on a silica gel t.l.c. plate for analysis. D. I. Rees.

Fumigation–the final result. H. K. HESELTINE (*Chemy Ind.*, 1969, (40), 1405–1408. 8 ref.).—The need for fumigation (penetration of vapour of fumigant into material and its subsequent diffusion away) of bulk grain stored in bins or on floors, is emphasised. The mode of application, safety, residues, efficiency and cost of halogenated hydrocarbons and of PH₃ (as Al phosphide) are compared. MeBr is not normally used in Britain, except for disinfestation of malting barley for export. In general, PH₃ is cheaper and more effective (even at low dosage and long after application) than liquid CCl₄, C₂H₂Cl₂ and C₂H₂Br₂. W. J. Baker.

Control of insects in home-grown grain. J. A. FREEMAN (*Chemy Ind.*, 1969, (40), 1401–1404. 33 ref.).—Ways in which infestation by insects and mites can cause damage and hence loss to farmers are reviewed. Preventive measures, essential for small- or large-scale storage and utilisation of cereals produced annually in Britain, are strongly recommended. Costs of such measures (chem. or phys.) are less than those required to control an outbreak, in which, moreover, material can be lost or become unsaleable. The question of making prophylatic measures mandatory is also raised. W. J. Baker.

Insect and mite pests [in home-grown grain]. C. WAYMAN (*Chemy Ind.*, 1969, (41), 1445–1447. 14 ref.).—Discussion covers environmental factors which favour establishment of these pests in grain stores, and which lead either to acute infestation and damage by some cereal-feeding species (e.g., *Sitophilus granarius*, *Oryzaephilus surinamensis* and *Cryptolestes ferrugineus*), or to financial loss due to rejection of grain infected by non-cereal-feeding fungus beetles. Temp. is the critical factor for insects, and humidity for mites, e.g. *Acarus siro* thrives only in cool, moist grain under high ambient r.h. Control measures are briefly outlined. W. J. Baker.

Pyrethrins–piperonyl butoxide applied as a fog in an empty grain bin. F. L. WATTERS (*Pyrethrum Post*, 1969, **10** (1), 7–11. Engl., 7 ref.).—The inside surfaces of the bin were treated with a 0·13% pyrethrins–1·27% piperonyl butoxide mixture at 0·49 l per 1000 ft³ (applied with a mechanical fog generator). Floor targets received more insecticide than wall targets (some variation in distribution was due to air currents). Plywood floor targets and grooved plywood wall targets remained effective for 11 months when stored at 23 ± 3° and 35–50% r.h. E. G. Brickell.

New [grain] storage systems in relation to infestation. M. B. HYDE (*Chemy Ind.*, 1969, (41), 1448–1451. 16 ref.).—Non-chem. ways of controlling infestation by insects, mites and moulds include (i) drying, (ii) aeration to hold the H₂O content at 16–18% and the temp. at ⊁ 17° (very effective against insects), (iii) airtight storage in silos or plastic lined bins, and (iv) chilling with refrigerated air, usually from mobile units. Drying to low H₂O content is obligatory against moulds and mites if grain is to be used for all purposes. Damp grain is best treated by (iii) but is then suitable only for stock feed (i.e., barley having 25% H₂O max.), or by (iv) in which case the grain is suitable for all purposes if chilled to < 5–10° (otherwise the max. H₂O content must be limited to ∼ 20%). Costs are briefly considered. W. J. Baker.

Use of insecticides [in grain stores]. A. A. GREEN (*Chemy Ind.*, 1969, (41), 1452–1454. 18 ref.).—Treatment of storage buildings to reduce infestation and migration of insects is described together with measures for disinfestation of infected grain and protection of clean grain. Recent exptl. work by Tyler and Rowlands, on distribution of insecticide among grain, is summarised., viz., the effectiveness of continuous application of dil. material vs. intermittent application of high concn. to only a pre-detd. proportion of grains. The uptake, concn. and final fate of the insecticide are briefly examined in relation to residue problems. W. J. Baker.

Use of microanalytical methods in studies of the control of insect pests in stored products. D. F. HORLER (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 139–141. 3 ref.).—Organophosphorus insecticides, e.g., malathion, (range 0–10 ppm) were examined, (i) to determine effective residual life of insecticide, (ii) to examine degradation products and (iii) to examine biochem. aspects of insecticidal action

(resistance and synergism). Techniques used included g.l.c. and radiotracers as well as conventional chem. analysis. S. S. Chissick.

Correlation of uric acid content with fragment counts in insect-infested flours and wheat grains. N. P. SEN and A. W. VAZQUEZ (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 833–836. 7 ref.).—The flour and grain samples were analysed for insect fragment counts by means of the AOAC methods 36·027 and 36·035, resp., and uric acid contents by the method described previously (Sen, *ibid.*, 1968, **51**, 785). The grain samples were also examined for % agric. damage by the method of Harris *et al.* (*ibid.*, 1952, **35**, 115) and insect damage by the X-ray technique of Nicholson *et al.* (*ibid.*, 1953, **36**, 156). For wheat grains, the correlation between uric acid contents and X-ray data was found to be the best between the six possible intercomparisons. A good correlation was found between the uric acid contents and the insect fragment counts. D. I. Rees.

Substituted norbornene-2,3-dicarboximides and their use as raticides. RENTOKIL LABORATORIES LTD. (Inventor: J. L. NEUMEYER) (Br. Pat. 1,164,124, 12.10.65).—The dicarboximides contain :CRᴵᴵᴵRᴵⱽ in the 7-position, CRᴵRᴵᴵ·OH in the 5-position, and are substituted at N by [CR₂]ₙC:CX or [CR₂]ₙCR:CHX, wherein R is H, Me or Et; n is 1–4; Rᴵ and Rᴵᴵᴵ are Ph; and Rᴵᴵ and Rᴵⱽ are pyrid-2-yl. An example is the N-allyl deriv. of 5-(1-hydroxy-1-pyrid-2-yl-1-phenylmethyl)-7-(1-pyrid-2-yl-1-phenylmethylene)-norborn-5-ene-2,3-dicarbonimide. Raticidal compositions comprise the compounds with a bait, e.g., meat, cereal grain, bran, fruit or vegetable, or with a carrier such as starch, kaolin or diatomaceous earth, in a form such as solution, paste, powder or tablet. The active compd. may be present as addn. salts with org. or inorg. acids. F. R. Basford.

Contamination by Pesticides

Soil, Air, Water

Dual column and derivative techniques for improved specificity of gas–liquid chromatographic identification of organochlorine insecticide residues in soils. H. B. PIONKE, G. CHESTERS and D. E. ARMSTRONG (*Analyst, Lond.*, 1969, **94** (1123), 900–903. 8 ref.).—Use of a 2-m column of 10% DC-200 on Gas Chrom Q and a 1-m column of 10% diethylene glycol succinate on Gas Chrom Q, in conjunction with the prepn. of KOH and HCl deriv. of the parent insecticides, provides improved Rₜ values (time for compd. to traverse the column) sufficiently different for correct identification of insecticide residues. Results are reported for *p,p′*-TDE, *p,p′*-methoxychlor, *o,p′*-DDT, *p,p′*-DDT, *p,p′*-DDE and dieldrin in untreated extracts of silty loam with indications of the respective sensitivities. W. J. Baker.

Adsorption of gamma-BHC from solutions on several selected adsorbents. A. C. MILLS and J. W. BIGGAR (*J. agric. Fd Chem.*, 1969, **17** (3), 604–608. 14 ref.).—Lindane (99–100% γ-BHC) (I), an insecticide relatively persistent in soils, was investigated. Decomp. during adsorption was assessed by liquid scintillation counting and electron-capture g.c. Adsorption from hexane and benzene was relatively low on peaty muck and greatest on SiO₂ gel and Venado clay. Decomp. was observed in benzene and hexane solvents (at 20°), but not in aq. and alcohol solvents (at 20°). Pesticide adsorption by the different surfaces was influenced by solvent differences and solubility of I. I. Dickinson.

Trapping of dieldrin lost from aqueous algae cultures. W. B. WHEELER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 760–764. 7 ref.).—The study described indicated loss of dieldrin by codistillation with water, 11% being lost by a 60 min standing period and 40% by autoclaving. A piece of tube, inserted in the rubber stopper of the flask containing the aq. soln., and containing a 1-cm plug of 5% of DC-200 on 80–100 mesh Gas Chrom Q, effectively trapped the pesticide and prevented atm. contamination. Analyses were carried out by g.l.c. with a packed column of the plug material with electron capture detection. D. I. Rees.

Crops, Livestock, Food

Use of ion-exchange resins in residue analysis. A. CALDERBANK (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 141–142. 8 ref.).—Ion-exchange resins can be used for the concn. of pesticides from crude extracts of plant or animal tissues, if the pesticides are ionic or can be converted into an ionic form. The aq. effluent is then analysed

directly or the purified pesticide may be extracted with an organic solvent first.   S. S. Chissick.

Analysis of grains for multiple residues of organic fumigants. B. MALONE (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 800–805. 13 ref.).—A codistillation procedure with toluene vapour, a steam distillation procedure and an acid reflux procedure for the isolation of the fumigant residues (CCl_4, CS_2, ethylene dichloride, ethylene dibromide, CH_2Br and $CHCl_3$) are described and the last method gave the most quant. recoveries. The method involves heating the grain (100 g) under reflux with acid, sweeping the fumigants with N_2 through a column containing Chromosorb to remove H_2O and collecting in toluene held at $-86°$. An aliquot of the toluene soln. is analysed by g.l.c. on a column of DC-200 on Gas Chrom Q at 100° with N_2 as carrier gas and electron capture detection. Down to $0 \cdot 1$ ppm of the fumigants could be detected except for ethylene dichloride (~ 4 ppm).   D. I. Rees.

Phygon. Fate of 2,3-dichloro-1,4-naphthoquinone in crop extracts. E. R. WHITE, W. W. KILGORE and G. MALLETT (*J. agric. Fd Chem.*, 1969, **17** (3), 585–588. 13 ref.).—A g.c. procedure for monitoring Phygon (org. fungicide) residues is described. The effect of storage and light on Phygon in soln., and the isolation, structure elucidation and synthesis of the corresponding photo-conversion products is examined.   I. Dickinson.

Routine quantitative residue determinations of S-[(2-methoxy-5-oxo- $\triangle^2$-1,3,4-thiadiazolin-4-yl)methyl] O,O-dimethylphosphorodithioate (Supracide) and its oxygen analogue in forage crops. A. M. MATTSON, R. A. KAHRS and R. T. MURPHY (*J. agric. Fd Chem.*, 1969, **17** (3), 565–570. 4 ref.).—The unchanged Supracide was detd. by microcoulometric g.c. (detectability $\not> 0 \cdot 05$ ppm). The sample was extracted with petroleum ether and cleaned up on Al_2O_3 columns. After extraction with acetone, the O-analogue was detected on t.l.c. plates by fly head cholinesterase inhibition (detectability $\not> 0 \cdot 01$ ppm). The efficacy of the extraction method for weathered residues is discussed.   I. Dickinson.

Gas chromatographic determination of residues of dalapon in several substrates. M. E. GETZENDANER (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 824–831. 7 ref.).—Aq. extracts obtained from fresh and dry plant tissues, and body fluids, were extracted with Et_2O and the residue (2,2-dichloropropionic acid) was analysed on a glass column of LAC-2R-446 plus H_3PO_4 on Gas Chrom S at 100° with N_2 as carrier gas and electron capture detection. Recoveries of $\sim 90\%$ were obtained of ~ 10 ppm of the residue in plant tissues and $0 \cdot 05$–10 ppm in body fluids.   D. I. Rees.

Azinphos-ethyl residues on field-grown tomatoes. J. T. HUGHES, K. G. TATE and P. D. WILSON (*N.Z. Jl agric. Res.*, 1969, **12** (2), 417–422, 3 ref.).—Gross residues, from sprayings of azinphos-ethyl at $1 \cdot 1$ lb active ingredient/acre, did not exceed 2 ppm. The bulk of these were external residues, removable by hexane extraction or by peeling the fruit. Internal residues did not exceed $0 \cdot 1$ ppm. The rate of residue dissipation was fairly slow; the mean decline in external levels over 2 weeks was 57% with a max. of 83% ($1 \cdot 97$–$0 \cdot 33$ ppm).   E. G. Brickell.

Distribution of arsenic residues by activation analysis. J. R. GEISMAN, W. E. CAREY, W. A. GOULD and E. K. ALBAN (*J. Fd Sci.*, 1969, **34** (3), 295–298. 7 ref.).—A simple activation analysis method has been developed which is sensitive to $0 \cdot 2$ ppm of As in < 1 g of material. Interfering elements (K and Na) can be removed by pre-irradiation chemistry. The analyses showed that As was concentrated in the roots of tomato plants. The activation technique could be used with precision and repeatability, and with simple sample prepn. The method can determine whether the As level is below official U.S. tolerance.   M. T. Rawnsley.

Spray residues of 2,4-D and 2,4,5-TP in 'Pineapple' orange peel. R. HENDRICKSON and W. R. MEAGHER (*J. agric. Fd Chem.*, 1969, **17** (3), 601–603. 7 ref.).—Electron capture g.c. was used to investigate the metabolites of the growth regulators 2,4-dichloro-phenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP). Max. residues were recovered from the peel approx. 5 weeks after field application of a dil. spray (20 ppm). A heat-labile fraction, not extracted with acetone, became the predominant metabolite in 3–5 weeks and showed the greatest persistence. The procedure for recovery of the growth regulators is described.   I. Dickinson.

Succinic acid 2,2-dimethylhydrazide (Alar) residues after application to apple trees. V. G. SHUTAK, C. E. OLNEY and T. W. KERR (*Proc. Am. Soc. hort. Sci.*, 1968, **62**, 63–66. 5 ref.).—The max. concn. of Alar residues in fruit were found when sprays were applied 60–90 days before harvest. The residues did not decrease

during 228 days in cold storage (0°). Residues were found in fruit harvested in the year following application of Alar.   A. H. Cornfield.

Extraction and clean-up for the t.l.c. analysis of neodecanoic acid in onions. L. E. ST. JOHN, JUN. and D. J. LISK (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 876–877. 3 ref.).—The method involves extraction with acetone, removal of interfering substances with a Celite–H_2SO_4 mixture and analysis by the t.l.c. method of Mizany (*J. Chromat.*, 1967, **31**, 96). The method is sensitive to 1 ppm of the defoliant in onions.   D. I. Rees.

Fate of S-[(2-methoxy-5-oxo-$\triangle$^2-1,3,4-thiadiazolin-4-yl)methyl] O,O-dimethylphosphorodithioate (Supracide) in a lactating cow. J. E. CASSIDY, R. T. MURPHY, A. M. MATTSON and R. A. KAHRS (*J. agric. Fd Chem.*, 1969, **17** (3), 571–575. 13 ref.).—A cow was fed ^{14}C-labelled Supracide (I) for 5 days at the rate of 1 mg/kg/day. No I or its O-analogue was found in the milk (sensitivity of the analytical method was $0 \cdot 01$ ppm). The total amt. of radioactivity found in the milk during the 15-day study represented only $0 \cdot 6\%$ of the oral dosage. Fractionation of the milk indicated extensive metabolism, as did the nature of the radioactivity recovered in the urine (24%) and faeces (34%). The highest level of radioactivity ($\equiv 0 \cdot 11$ ppm of I) in the tissues was found in the liver.   I. Dickinson.

Fate of carbon-14 trifluralin in artificial rumen fluid and in ruminant animals. R. GOLAB, R. J. HERBERG, E. W. DAY, *et al.* (*J. agric. Fd Chem.*, 1969, **17** (3), 576–580. 10 ref.).—In time-rate studies, ^{14}C-trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) (I) was degraded in 11 h in an artificial rumen fluid medium. In ruminant animals, 99% of the ingested radioactivity was recovered within 6 days in the urine ($17 \cdot 8\%$) and faeces ($81 \cdot 2\%$). Neither urine nor faeces contained any I, the radioactivity being excreted as nonidentifiable polar substances. Milk and blood contained no radioactivity.   I. Dickinson.

Evaluation of esterases from livers of beef, pig, sheep, monkey and chicken for detection of pesticides by thin-layer chromatographic-enzyme inhibition technique. C. E. MENDOZA, D. L. GRANT, B. BRACELAND and K. A. McCULLY (*Analyst, Lond.*, 1969, **94** (1122), 805–810. 9 ref.).—Results of specific pesticide–esterase interactions are given. Dichlorvos, ethion and dimethoxon, with or without bromination, were detected with the 2000- or 3000-g supernatants of the H_2O or tris-buffer extracts of the livers. After bromination, carbaryl (I), oxydemeton-methyl (II), demeton and the thiol isomer (III) of demeton sulphone were detected with beef- or sheep-liver extracts. II and III were detected with pig- or monkey-liver extracts after bromination, but demeton ($\ll 50$ ng) was detected with or without bromination. The detection of I, with or without bromination with the various extracts, is described fully. The 2000-g supernatants were generally higher in protein and gave more intense t.l.c. backgrounds than the 3000-g ones. Background intensities of aq. and tris-buffer extracts were comparable, but the former extract has a lower protein content.   W. J. Baker.

Determination of Nitroxynil [4-cyano-2-iodo-6-nitrophenol] in experimental animals and in meat, by polarography. M. PARNELL (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 143).—The defatted sample was macerated with satd. borax soln. (I), acidified with conc. HCl and shaken with benzene. The org. layer was shaken with I and the aq. soln. analysed polarographically. The concn. was detd. by the method of standard addition.   S. S. Chissick.

Residues data—purpose and requirements. J. A. R. BATES (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 137–139. 5 ref.).—Analysis of foodstuffs for pesticide residues and evaluation of health risks are discussed with reference to: design of trials; sampling; treatment of samples; analysis of sample; precision (reproducibility); accuracy; and confirmation of identity of pesticide.   S. S. Chissick.

Colorimetric determination of 2,6-dichloro-4-nitroaniline (dichloran) residues in foods. J. A. HEAGY (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 797–799).—A modification is given of the method of Groves and Chough (*J. agric. Fd Chem.*, 1966, **14**, 668) involving changing the reagent concn., filtering extracts through Celite and clean-up on a Florisil column. Recoveries of 90–100% of the fungicide from various crops were obtained.   D. I. Rees.

Quantitative gas chromatographic determination of fungicide residues in grapes, must and wine after application of Ortho-Phaltan. E. LEMPERLE and E. KERNER (*Z. analyt. Chem.*, 1969, **247** (1–2), 49–52. Ger., 6 ref.).—The grapes (200 g), wine or must (100 g) are first extracted with acetone, then the fungicide residue is purified by

extraction with CHCl3 and column chromatog. on silica gel. Final detn. is by g.c. on Chromosorb W. Down to $0 \cdot 1$ µg of folpet, the active constituent of Ortho-Phaltan, can be determined; recoveries are 80–100%.   R. Waspe.

Determination of phosphorus in fruits and fruit products by a spectrophotometric molybdovanadate method and by the official gravimetric quinoline molybdate fertiliser method. B. ESTRIN and W. S. BRAMMELL (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 865–870. 8 ref.).—The spectrophotometric method described is similar to that of Brammell (*ibid.*, 1965, **48**, 859) and the gravimetric method is the AOAC 2·025b method. The two methods were compared with the AOAC volumetric method 20·031. They avoided the use of the hazardous HClO4 and gave results in good agreement with the volumetric method when applied to jams, preserves and jellies.
                                                        D. I. Rees.

Collaborative study of confirmative procedures by single sweep oscillographic polarography for the determination of organophosphorus pesticide residues in non-fatty foods. R. J. GAJAN (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 811–817. 12 ref.).—Parathion and methyl parathion were determined by the method of Gajan (*ibid.*, 1963, **46**, 216) and malathion by that of Jura (*Analyt. Chem.*, 1955, **27**, 525). Diazinon was determined in an aq. soln. containing NMe4Br and HOAc, the peak potential at -90V against a Hg pool or Ag wire reference electrode being measured. The pesticides were determined in extracts of apples and lettuce at the $0 \cdot 5$ and 2 ppm levels with > 96% recoveries.   D. I. Rees.

Rapid method for the extraction, clean-up and gas–liquid chromatographic determination of toxic residues of Temik. J. C. MAITLEN, L. M. McDONOUGH and M. BEROZA (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 786–789. 5 ref.).—The method is similar to that described previously (*Idem, Analyt. Chem.*, 1965, **37**, 291) except that the total content of Temik, its sulphoxide and its sulphone is determined. The residue obtained after extraction and evaporation is oxidised with H2O2–HOAc and the non-toxic oxime metabolites are removed by passage down a Florisil column. The Temik sulphone obtained is determined by g.l.c. with flame photometric detection. Recoveries of 72–114% were obtained from vegetables containing $0 \cdot 02$–$1 \cdot 50$ ppm of the pesticide.   D. I. Rees.

Comparison of ten methods for the analysis of milk for residues of chlorinated pesticides. J. H. LAWRENCE and J. A. BURKE (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 817–824. 19 ref.).—10 methods were studied for the determination of 1 ppm of p,p'-TDE in milk, and average recoveries of > 90% were obtained with six of them. The modified AOAC method which gave 89% recovery was the most rapid. None of the methods was suitable as a rapid, routine method giving precise results.   D. I. Rees.

Collaborative study of the sweep codistillation clean-up for chlorinated pesticide residues in edible fats and oils. B. MALONE and J. A. BURKE (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 790–797. 6 ref.).—The method described previously (Storherr *et al., ibid.*, 1967, **50**, 605) was slightly modified and the results of the collaborative study described showed recoveries of 86–120% of the pesticides added to butterfat and soyabean oil at the $0 \cdot 2$–2 ppm level.   D. I. Rees.

Extraction, separation and g.l.c. detection of silvex and its propylene glycol butyl ether ester in fish tissue. P. W. WILLIAMS and J. I. TEASLEY (*J. Ass. off. analyt. Chem.*, 1969, **52** (4), 782–785. 11 ref.).—The phenoxyalkyl carboxylic acid herbicide, silvex, and its propylene glycol butyl ether ester were extracted from the fish tissue with MeOH and analysed by g.l.c. with use of a glass column of DC-200 on Gas Chrom Q at 200° with N2 as carrier gas and electron capture detection. Recoveries of 80–98% of each herbicide at the $0 \cdot 002$–1 ppm level were obtained.   D. I. Rees.

Water-soluble arseno-organic compounds in marine fishes. G. LUNDE (*Nature, Lond.*, 1969, **224** (5215), 186–187. 5 ref.).—The exchange between ^{76}As and the ^{75}As originally present in the N-liquor from boiled mackerel, capelin and herring was studied. Results of ion-exchange detn. show that As (3–22 ppm) in this H2O-sol. phase is present mainly as one or more As-org. compd. in which the org. As does not exchange with inorg. As.   W. J. Baker.

Insecticide residues in cigarettes. T. J. SHEETS, J. W. SMITH and M. D. JACKSON (*Tob. Sci.*, 1968, **12** (1), 66–69).—In a 1966–67 survey, DDT was found in concn. of $3 \cdot 48$–$5 \cdot 78$ ppm (av. $5 \cdot 05$), and TDE $7 \cdot 14$–$14 \cdot 31$ ppm (av. $12 \cdot 17$). DDT + TDE in 1967 did not differ appreciably from the 1966 findings ($17 \cdot 22$ and $16 \cdot 98$ ppm, resp.). Endrin, corrected for 70% recovery, ranged from $0 \cdot 49$ to $1 \cdot 57$ (1966) and $0 \cdot 31$–$1 \cdot 20$ ppm (1967), this concn. being lower than any reported since 1956. Carbaryl was not detected in 1966.   C. V.

4.—MISCELLANEOUS

Microanalytical methods in agricultural chemistry. H. EGAN (*Proc. Soc. analyt. Chem.*, 1969, **6** (8), 131–137. 27 ref.).—General discussion is followed by review of specific areas, with special reference to some current problems, e.g., selection of representative samples, significance of trace materials, specificity and clean-up of initial extracts and positive identification.
                                                    S. S. Chissick.

T.l.c.–fluorometric analysis for atmospheric scopoletin. E. SAWICKI and C. GOLDEN (*Microchem. J.*, 1969, **14** (3), 437–447. 19 ref.).—An extract of the scopoletin (7-hydroxy-4-methoxycoumarin) dust particles was chromatographed on silica gel containing a fluorescent indicator, with PhMe–EtOAc–HCO2H (5 : 4 : 1, v/v) as solvent. The spots, detected under u.v. radiation, were extracted with MeOH containing 2% of a 40% aq. soln. of NEt4OH and determined fluorometrically in this soln. Recovery at the $0 \cdot 05$–$0 \cdot 175$ µg level averaged 97%. The method was applied to the determination of scopoletin in airborne particles, house dust and coffee roast effluents.   G. Russell.

Plants used against cancer: a survey. J. H. HARTWELL (*Lloydia*, 1967, **30** (4), 379–436; 1968, **31** (2), 71–170; 1969, **32** (1), 79–107; (2), 153–205. 912 ref.).—A very detailed listing. Botanical and common names are given, and the parts used and methods of prepn. An indication of the disease or condition is given together with comments as to country or source of information.   C. V.

Toxicity of several oil-spill removers to some species of fish and shellfish. J. E. PORTMANN and P. M. CONNOR (*Mar. Biol.*, 1968, **1** (4), 322–329. 13 ref.).—The toxicities of 12 commercially available products, were assessed using four different shellfish. In solvent emulsifiers, the solvent component was the most toxic but this was rapidly lost by evaporation. Larvae of *Cranyon cranyon* and *Carcinus maenas* were 10 times more susceptible than the adults to solvent emulsifiers.   C. V.

Marine algae carbohydrates. E. PERCIVAL (*Oceanogr. Mar. Biol. A. Rev.*, 1968, **6**, 137–161. 194 ref.).—A review.   C. V.

Yeasts of marine origin. E. O. MORRIS (*Oceanogr. Mar. Biol. A. Rev.*, 1968, **6**, 201–230. 82 ref.).—Some 130 have been isolated. The possibility of utilising such yeasts or of making use of the information to determine the migration patterns of fish is considered.   C. V.

5.—RECENT BOOKS AND JOURNALS

Ecological aspects of the mineral nutrition of plants. Ed. I. H. RORISON. 1969, 484 pp. (Oxford: Blackwell Scientific Publications).—Papers (31) read at a Symposium of the British Ecological Society, Sheffield, 1–5 April 1968.   C. V.

Crop technology. M. EDDOWES. 1969, 220 pp., 50/-. (London: Hutchinson Educational Ltd.).   S. C. H.

Freezing and irradiation of fish. Ed. R. KREUZER. 1969, 519 pp. (London: Fishing News (Books) Ltd.).—The techniques and equipment for freezing fish at sea and the factors affecting the quality are outlined; freezing media and thawing are discussed. The economics of production and marketing are presented and an assessment of quality is given, storage, packaging and distribution being covered. Preservation by irradiation is examined for cod, selected herring, whale and horse mackerel.   S. C. Haworth.

Practical canning. A. LOCK. 1969, 3rd edn. (revised & enlarged), 415 pp. (London: Food Trade Press Ltd.). S. C. H.

Chemicals for pest control. G. S. HARTLEY and T. F. WEST. 1969, 316 pp. (Oxford, etc.: Pergamon Press).   S. C. H.

Diseases of poultry. P. SENEVIRATNA. 1969, 2nd edn., 229 pp., 46/-. (Bristol: John Wright & Sons Ltd.).   S. C. H.

Food-borne infections and intoxications. Ed. H. RIEMANN. 1969, 698 pp. (New York, London: Academic Press).   S. C. H.

Grain storage: the rôle of fungi in quality loss. C. H. CHRISTENSEN and H. H. KAUFMANN. 1969, 153 pp. (Minneapolis: University of Minnesota Press).—The problem of loss is considered and the characteristics of field and storage fungi are examined. Moisture content is measured, heating and respirations determined and germinability, discoloration and fat acidity values are reviewed together with mycotoxins and the resulting grain quality. Based on these observations, the condition is evaluated and the storability

assessed. Drying, aeration and refrigeration techniques are outlined and the damage caused by insects, mites and rodents is summarised.   S. C. Haworth.

Principles of microbiology. A. L. SMITH. 1969, 6th edn., 669 pp. (Saint Louis: C. V. Mosby Co.).   S. C. H.

Pesticide Science. 1970, **1** (1), bimonthly. (London: Society of Chemical Industry).—A journal of international research and technology on crop protection and pest control, covering all aspects of the production and use of chemicals for control of pests and diseases of crops, herbicides, crop desiccants, plant growth regulators, and pesticides for veterinary and public health use. It also contains papers dealing with the ecological implications and economics of using such chemicals, and with regulation and suppression of pests, diseases and weeds by any method.

         P. C. W.

AUTHOR INDEX

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