JOURNAL
OF THE
SCIENCE OF FOOD AND AGRICULTURE
(INCLUDING ABSTRACTS)

Published by the Society of Chemical Industry

Volume 16 | No. 7 | July, 1965

SOCIETY OF CHEMICAL INDUSTRY

FOUNDED IN 1881 INCORPORATED BY ROYAL CHARTER 1907

Offices of the Society: 14 Belgrave Square, S.W.1
Telephone: BELgravia 3681/5

Annual Subscription to the *Journal of the Science of Food and Agriculture*
£15 post free, single copies £1 17s. 6d. post free

THE EFFECT OF HEAT IN DRYING ON THE NUTRITIVE VALUE OF WHEAT FOR ANIMAL FEED

By C. K. MILNER* and J. WOODFORDE†

Samples of Koga II wheat, harvested at 22% moisture and dried in streams of air heated to 180° F for 37 min., 220° F for 26 min. or 220° F for 120 min., were incorporated into two series of diets for young growing chicks. No significant differences were found between the samples, either when fed as sole protein source or when fed as the major energy source in completely adequate rations. None of the samples was significantly different from a control sample of wheat dried at 80° F.

Introduction

A considerable amount of work has been done on the effect of heat on the nutritive value of foodstuffs, and it is well known that excessive heat during processing can lead to the lowering of the biological value of proteins,[1,2] the destruction of heat labile vitamins, and poorer palatability.

These effects have been observed with all types of food materials and as a result, any processes involving the application of heat are generally carefully controlled to minimise heat damage, and in cases where no precise information is available recommendations tend naturally to err on the safe side. This is partly the situation with the artificial drying of cereals which have been harvested under wet conditions. Work has been published on the effect of too-high drying temperatures on the baking quality of milling wheat,[3] and the reduction in germinating power of wheat by comparatively low temperatures is well known. Workers in the U.S.A. have published a number of papers on the effect of high drying temperatures on the nutritive value of maize,[4–10] but to the authors' knowledge no similar information is available for wheat intended for use in animal feedstuffs, although Cashmore[11] has already stated that temperatures up to 220° F could be used without harm when drying grain for this purpose. The economic and practical advantages of being able to operate at temperatures exceeding the currently recommended maximum of 180°F[12] are quite clear and the aim of this investigation was therefore to fill this gap in our knowledge and possibly to provide an experimental basis for using higher temperatures than are at present used in grain-drying.

Experimental

Materials

Wheat of the variety Koga II (selected on account of its high protein content) was combine-harvested at the National Institute of Agricultural Engineering on 16 September, 1963, at a moisture content of 22%. It was allowed to dry a little by exposing it to the atmosphere and remixing intermittently.

Drying procedure

A control sample was prepared by drying about 10 kg. of this grain in a shallow tray with warm air passing through it at a temperature of about 80° F for a period of 30 h. after which the moisture content had been reduced to 13·8%.

The apparatus employed for drying the wheat at inlet air temperatures of 180° F and 220° F was generally the same as that described by Woodforde & Osborne[13] except for the grain container, which in the present work was a Perspex tray 12 in. × 12 in. in area and 7 in. deep. The inlet air temperature was controlled to within ±1° of the desired value by a thermostat in the plenum chamber beneath the tray. The airflow and humidity of the air were also closely controlled during the drying runs. The temperature in the grain bed was recorded continuously during each drying run by means of three sets of four thermocouples placed at the top, middle

* School of Agriculture, University of Cambridge
† National Institute of Agricultural Engineering, Silsoe, Beds.

and bottom of the grain and connected to a multipoint Honeywell Brown recording potentiometer. The top and bottom sets of thermocouples were positioned at $\frac{1}{2}$ in. from the surfaces of the grain.

The duration of drying runs Nos. I and II with air temperatures of 180° and 220° F was such that the final mean moisture content of the grain was reduced to about 15% as would often be the objective in farm practice. The drying time in Run III was extended to 120 min. to allow the mean final temperature to reach a considerably higher value than in Run II.

The same weight of damp grain (10 kg.) was used for each run and this gave an initial bed depth in the tray of approximately 6 in. The mean moisture content of the wheat at the end of the runs was calculated from the measured initial moisture content and loss of weight during drying. The air and grain conditions and the temperatures in the grain are summarised in Table I. Temperature curves for the bottom, middle and top of the bed for Runs II and III at 220° F are shown in Fig. 1.

Table I

Air and grain conditions

	Run no.		
	I	II	III
Inlet air temp., °F	180	220	220
Inlet air humidity, grains/lb.	60	60	60
Air flow, lb./h.	226·5	225·0	225·0
Drying time, min.	37	26	120
Grain moisture content, %, initial	21·3	21·2	21·0
on leaving dryer	14·8	15·0	5·8
Final temp. in grain, °F, top	121	119	213
middle	159	180	218
bottom	179	219	220
Mean temp. in grain for run, °F	126	137	192

After being dried, the materials were ground to pass 30 mesh sieves and spread out on sheets of paper to equilibrate with atmosphere moisture. The mean crude protein content of the four samples was 18·8% of dry matter.

Animals and management

Maxilay cockerels (250 day-old, from W. D. Evans, Market Harborough, Leics.) were debeaked on arrival and fed on a commercial starter mash for 10 days. They were weighed on the fifth and tenth days and 144 chicks showing the most uniform weight and rate of gain were selected and stratified by weight into six strata of 24 birds each. Within each stratum, three birds were allotted at random to each of eight cages and each of eight experimental diets was

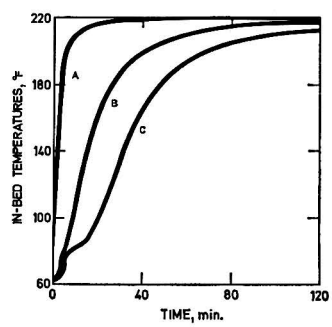


FIG. 1.—*Changes in temperature at three levels in the grain bed during drying*
(inlet air temperature 220° F)
A bottom B middle C top

allotted randomly to one cage in each stratum. The cage unit used and the general management of the birds have been described elsewhere.[14]

Diets

Two experiments each with four treatments were carried out simultaneously but they will be described separately for convenience.

Experiment 1 was designed to compare the four samples as the sole protein source for the chick. The basal diet had the percentage composition: 'Trex' (partially hydrogenated vegetable fat; J. Bibby & Sons Ltd., Liverpool) 5·0; mineral premix 6·0; vitamin premix 1·0; choline chloride 0·3; terramycin supplement (Pfizer Ltd., Folkestone, Kent) containing 2·2% oxytetracycline, 0·07. The mineral premix was that described by Fox & Briggs.[15] The vitamin premix supplied the following nutrients (mg./kg. diet): thiamine 10, riboflavin 10, pyridoxine 10, nicotinic acid 50, calcium pantothenate 30, biotin 1, folic acid 4, cyanocobalamin 0·01, menaphthone (vitamin K) 0·57, Rovimix A50 and D_3 (Roche Products Ltd., Welwyn Garden City; containing 50,000 I.U. of vitamin A and 12,500 I.U. of vitamin D_3 per g.) 176.

The four wheat samples (control and I, II and III) were included in diets A, B, C and D respectively at 87·6% contributing 14·8% crude protein. Maize starch was added to 100%.

Experiment 2 was intended to expose any effects that the drying conditions might have had on the wheat as a source of energy, or on its palatability. The four samples were included at a level of 61·8%, in diets E, F, G and H respectively, which were designed to be completely adequate in all respects for the young growing chick. The basal diet for this experiment had the following percentage composition: white fish meal 8·0, soyabean meal 8·5, decorticated extracted groundnut meal 8·1, DL-Methionine 0·2, ground oat husks 1·0, 'Trex' 5·0, mineral premix 6·0, vitamin premix 1·0, terramycin supplement 0·07. Maize starch was added to 100. The vitamin and mineral premixes supplied the same levels of nutrients as in Experiment 1.

Results and discussion

The mean weight gains and feed conversion efficiency (FCE) for the eight diets are shown in Table II. Each experiment was analysed separately and none of the recorded differences was

Table II

Growth, feed conversion efficiency and feed consumption of chicks receiving different dietary treatments for ten days

(Mean values for six groups each containing three birds)

	Diet	Sample	Weight gain, g.	Food eaten, g.	F.C.E., *
Expt. 1					
	A	1	47·5	275	0·174
	B	2	49·5	296	0·168
	C	3	49·7	282	0·175
	D	4	50·8	270	0·189
		Standard error	± 7·92	± 27·9	±0·020
Expt. 2					
	E	1	345·0	560	0·617
	F	2	339·5	564	0·603
	G	3	354·2	569	0·623
	H	4	332·5	549	0·607
		Standard error	± 20·6	± 27·8	±0·023

* Feed conversion efficiency is the weight gain, g./g. of food eaten

found to be significant. As anticipated, growth and FCE on diets A–D were poor since protein was the limiting factor. On diets E–H, however, growth and FCE were excellent and as high as the present authors have obtained with this type of bird. There were no significant differences between the amounts of the various diets consumed within each experiment.

These results demonstrate that the drying conditions used have had no adverse effect on the value of the wheat as an energy source, nor on its palatability. From the data of

de Man & Zwiep,[16] and the known amino-acid requirements of growing chicks,[17] lysine was calculated to have been the limiting amino-acid in the first experiment and since there were no significant differences between the performances of the chicks on diets A–D, it can be concluded that the availability of the lysine was not affected. No conclusions can be drawn for the other essential amino-acids. Cystine is known to be very sensitive to heat but this was not investigated here. Methionine is unlikely to have been affected since Milner[18] showed that a more drastic heat treatment than those used here had no adverse effect on the availability of methionine in wheat for chicks.

Experiments in the United States have shown that maize is also resistant to heat during drying. Emerick *et al.*[5] dried maize in shallow pans in a forced-draught oven at temperatures ranging from 50–350° F and found that these materials incorporated as sources of energy and supplementary protein into diets for chicks and rats supported equal growth. However, drying at 450° F for 2½ h. caused significant damage, leading to poorer weight gain, food conversion and food consumption. In that treatment all the grains were scorched to some extent, 30% of them severely.

Jensen *et al.*[4] fed maize dried under conditions very similar to the above, though with rather longer exposure to the hot air and found no effect on the growth of either two-week old pigs or pigs fed from 45 lb. to 190 lb. They also carried out vitamin assays and found that while riboflavin, carotene and nicotinic acid were unaffected, some pantothenic acid had been destroyed.

Experiments with cattle have given results in line with those from pigs and poultry. Clanton *et al.*[9] fed maize dried at 190° F to cattle and found no change in its digestibility. Albert & Neumann[10] found that maize dried from 30% to 16% moisture at 180° F had the same nutritive value for beef steers as field dried maize.

In contrast, Davis & Cabell[6] found that 'wet corn heated above 135° F showed a progressive loss in protein nutritive value for rats with increasing temperature.' The times taken for drying were extremely long, however, and in a later report, Cabell *et al.*[7] found that drying even at 240° F for 1·5 h. from 32% moisture had no effect on the nutritive value of maize for rats.

Hathaway *et al.*[8] used conditions similar to those of Davis & Cabell[6] and, like them, obtained results which conflict with the other literature on this subject. They dried maize from 27% to 14% moisture with forced-draught air at temperatures from 88°–240° F and found significantly depressed growth responses by rats to the materials dried at 140° F and above, when fed either as sole protein source or as an 'energy source' in rat rations containing 16% linseed oil meal. Their results were criticised by Emerick *et al.*[5] on the grounds that the rats were not growing optimally. Though one would not expect rats to grow optimally on 9·5% protein provided solely by maize, their criticism is valid for Hathaway's 'corn as source of energy' diets. It can be calculated from the data of de Man & Zwiep[16] and Bressani & Mertz[19] that lysine would be seriously deficient in this diet and so they would not have been evaluating corn as a source of energy but as a source of lysine.

The major difference between their treatments and the others in the literature is in the extremely long times taken for drying the grain. Thus 26 h. were required at 140° F, 13 h. at 160° F, or 9 h. at 180° F to bring their material to 14% moisture content. These conditions would probably encourage α-amylase activity leading to the production of reducing sugars. Miller *et al.*[20] have already shown with model systems of cod muscle and glucose that 20% of the lysine in cod protein can be made unavailable by heating at 70° C (157° F) for 9 h. Ericson & Larsson[21] have reported large losses of lysine during the baking of bread with a high content of reducing sugars. It is probable that Hathaway's conditions led to lysine binding and this would have been measured in both of his rations. The same criticism applies to the work of Davis & Cabell.[6]

Swahn & Thafvelin[22] have pointed out the danger of vitamin E destruction in heated and stored grain and this seems to be one of the most probable forms of damage to grain dried at high temperatures. With this qualification therefore, drying temperatures up to 220° F are unlikely to affect the nutritive value of wheat for farm livestock, though it would be advisable to make further investigations with animals other than poultry.

Acknowledgments

The authors wish to thank Mr. P. J. Lawton for preparing the dried grain. The work of one author (C. K. M.) was supported by a grant from the U.S. Dept. of Agriculture under Public Law 480.

School of Agriculture,
University of Cambridge
and
National Institute of Agricultural Engineering
Wrest Park
Silsoe

Received 4 December, 1964

References

1 Rice, E. E., & Beuk, J. F., *Adv. Fd Res.*, 1953, **4**, 233
2 Altschul, A. M., 'Processed Plant Protein Foodstuffs', 1958, p. 79 (New York: Academic Press Inc.)
3 Greer, E. N., *Agriculture*, 1963, August, **70**, 363
4 Jensen, A. M., Terrill, S. W., & Becker, D. E., *J. Anim. Sci.*, 1960, **19**, 629
5 Emerick, R. J., Carlson, C. W., & Winterfeld, H. L., *Poult. Sci.*, 1961, **40**, 991
6 Davis, R. E., & Cabell, C. A., *Feed Age*, 1957, **7**, 49
7 Cabell, C. A., Davis, R. E., & Saul, R. A., *J. Anim. Sci.*, 1958, **17**, 1204
8 Hathaway, I. L., Young, F. D., & Kiesselbach, T. A., *J. Anim. Sci.*, 1952, **11**, 430
9 Clanton, D. C., Hemstrom, M. L., & Matsushima, J., *J. Anim. Sci.*, 1960, **19**, 376
10 Albert, W. W., & Neumann, A. L., *Univ. Illinois Cattle Breeders Day Rep.*, 1955, No. A 5430h
11 Cashmore, W. H., *J. Minist. Agric.*, 1942, **49**, 144
12 'Farm Grain Drying and Storage', *Ministr. Agric. Fish Fd Bull.*, 1954, 1959, No. 149 (London: H.M.S.O.)
13 Woodforde, J., & Osborne, L. E., *J. agric. Engng Res.*, 1961, **6**, 300
14 Carpenter, K. J., March, B. E., Milner, C. K., & Campbell, R. C., *Brit. J. Nutr.*, 1963, **17**, 309
15 Fox, M. R. S., & Briggs, G. M., *J. Nutr.*, 1960, **72**, 243
16 De Man, T. J., & Zwiep, N., *Voeding*, 1955, **16**, 147
17 *Publ. nat. Res. Counc., Wash.*, 1960, No. 827
18 Milner, C. K., *Proc. 6th Int. Congr. Nutr.*, 1963, p. 491 (Edinburgh: Livingstone)
19 Bressani, R., & Mertz, E. T., *J. Nutr.*, 1958, **65**, 481
20 Miller, E. L., Carpenter, K. J., & Milner, C. K., in press
21 Ericson, L.-E., & Larsson, S., *Acta physiol. scand.*, 1962, **55**, 64
22 Swahn, O., & Thafvelin, O., *Vitams Horm.*, 1962, **20**, 645

INHIBITION OF SOYA LIPOXIDASE

By J. A. BLAIN and G. SHEARER

Certain long-chain polyacetylenic fatty acids prove to be more potent competitive inhibitors of lipoxidase than any of the compounds which have been examined previously. Inhibition by nordihydroguaiaretic acid under similar conditions is of the induction period type and is considered not to be competitive.

Introduction

The enzyme lipoxidase catalyses the oxidation of unsaturated fatty acids or esters containing the pentadiene group and has been the subject of a recent review by Tappel.[1] The association between lipoxidase and both oxidative rancidity of fats and carotene destruction has led to interest in compounds which might inhibit the enzyme. Metal-complexing agents have proved ineffective[2,3] as might be expected as the enzyme is apparently not a metalloprotein. Antioxidants have been shown to have an inhibitory effect but mostly at concentrations which are high in relation to the substrate.[4–6] Reports on the relative potencies of antioxidants are conflicting but agree on the superiority of nordihydroguaiaretic acid (NDGA).[1,4,6] It has been claimed by Siddiqui & Tappel that NDGA acts as a competitive inhibitor.[4]

A study on competitive inhibition was made by Holman & Elmer[7] who found that elaidolinolenic, conjugated linoleic, oleic and octanoic acids caused 50% inhibition at concentrations of 1·1, 1·3, 10 and 100 moles inhibitor per mole of linoleate substrate respectively.

In the work to be described a number of unsaturated fatty acids together with a number of other compounds were examined for inhibitory properties. Some were selected mainly to ascertain to what extent the surface properties might simulate inhibition by blocking water/lipid interfaces.

Experimental

Reagents

Linoleic acid.—Hormel Institute linoleic acid re-ampouled *in vacuo* and stored at −20° was dissolved in ethanol and used within 24 h., being kept refrigerated when not in use. For screening experiments concentrates of 2·5 mg./ml. were used.

Unsaturated compounds screened as potential inhibitors.—The compounds listed in Table I were supplied by Roche Products Ltd., with the exception of nordihydroguaiaretic acid (NDGA) (L. Light & Co.). In general these were dissolved in ethanol to give concentrations of 0·5 mg./ml.

Buffer.—McIlvaine's citric acid/phosphate buffer pH 6·5 was used.[8]

Soya extracts.—Soya extracts were prepared by stirring 0·5 g. of finely ground defatted soya flour with 40 ml. of water for 20 min., followed by centrifuging.

Ethanol.—Ethanol was refluxed for 0·5 h. over aluminium foil and potassium hydroxide and distilled. This procedure was necessary to avoid high blanks in the thiocyanate assay.

Methods

For reaction mixtures, 1 ml. of linoleic acid solution, 0·25 ml. of inhibitor solution and a suitable volume of soya extract (0·5 ml. in the screening experiments) were added to 25 ml. of buffer with constant swirling. At suitable time intervals 1-ml. samples were taken into 10 ml. of ethanol or 60% ethanol to assay the hydroperoxide or conjugated diene content respectively.

Linoleate hydroperoxide was estimated by a modification of the thiocyanate assay of Koch *et al.*[9] Conjugated diene was measured as optical density at 232·5 mμ of the 60% ethanol samples, which were read against 'blank' reaction mixtures in which the soya extract had been inactivated by heat.

In the screening experiments, reaction rates were such that in the uninhibited controls about 10% of the substrate was converted to conjugated diene in 2 min., assuming a molar extinction coefficient of 28,000 for linoleate hydroperoxide.[10]

$$\text{Degree of inhibition is taken as } 100\left(1-\frac{\text{O.D. inhibited reaction at 2 min.}}{\text{O.D. control reaction at 2 min.}}\right).$$

(O.D. = optical density.)

Results

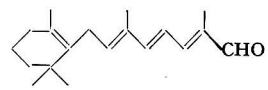
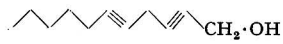
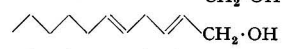
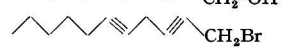
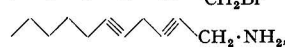
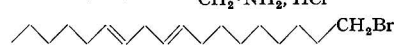
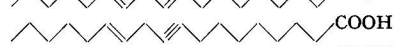
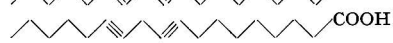
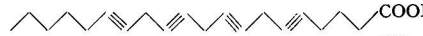
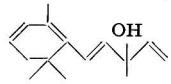
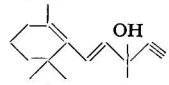
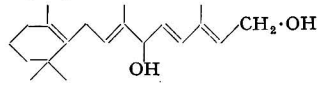
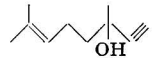
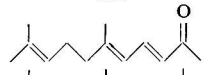
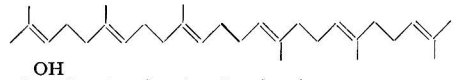
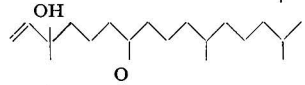
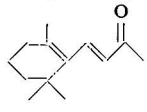
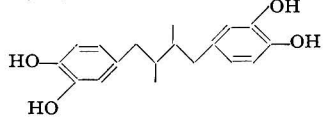
Table I shows the results of initial screening experiments. In these no significant differences were obtained between thiocyanate and conjugated diene assays. It can be seen that all the long chain structures having a methylene-interrupted unsaturated system show some inhibitory properties while the short chain compounds with similar unsaturated systems do not. There is no evidence that inhibition unrelated to the methylene-interrupted system occurs.

It was observed during these experiments that 1-bromo-octadeca-9,12-diene acted as a substrate, peroxide formation being of the same order as that for linoleic acid.

The time curves for inhibition were similar in all cases and only the most active compounds were studied further. In the subsequent experiments it was convenient to use reaction mixtures

Table I

Compounds screened as potential inhibitors of lipoxidase

Compound	Structure	% Inhibition
1. 8-(2′,6′,6′-trimethylcyclohex-1-enyl)-2,6-dimethyloctatri-2,4,6-en-1-al		0
2. Undecadi-2,5-yn-1-ol		0
3. Undecadi-2,5-en-1-ol		0
4. 1-Bromoundecadi-2,5-yne		0
5. 1-Aminoundeca-2,5-diyne hydrochloride		0
6. 1-Bromo-octadeca-9,12-ene		9
7. Octadeca-*cis*-12-en-9-ynoic acid		21
8. Octadeca-9,12-diynoic acid		17
9. Nonadeca-10,13-diynoic acid		30
10. Octadeca-6,9,12-triynoic acid		22
11. Eicosa-5,8,11,14-tetraynoic acid		68
12. Eicosa-5,8,11,14-tetrayn-1-ol		34
13. Vinyldehydro-β-ionol		0
14. Ethynyl–β–ionol		0
15. 1,6-Dihydroxy-3,7-dimethyl-9-(2′,6′-trimethylcyclohex-1-enyl)nona-2,4,7-tri-ene		0
16. *dl*-Linalool		0
17. 2,6-Dimethyl-6-hydroxyoct-2-en-7-yne (Dehydrolinalöol)		0
18. Pseudo-ionone		0
19. 2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene		0
20. Isophytol		0
21. β-Ionone		0
22. Nordihydroguaiaretic acid (NDGA)		100

containing substrate and inhibitor concentrations twice those used previously, 1 ml. volumes of soya extract being added as enzyme source. At this higher enzyme level, the mixtures were stirred to prevent limitation of reaction rate by disappearance of dissolved oxygen.[11] For spectrophotometry 1 ml. of reaction mixture was added to 25 ml. of 60% ethanol.

Table II shows that with eicosatetra-5,8,11,14-ynoic acid (tetraynoic acid) the degree of inhibition remains constant over a fourfold increase of enzyme concentration.

Table II

Effect of lipoxidase concentration on inhibition of linoleic acid oxidation by tetraynoic acid

	% Inhibition at 2 min.			
Soya strength	full strength	¾ strength	½ strength	¼ strength
Diene assay	72	71	66	71
Peroxide assay	73	71	73	75

The effect of altering the concentration of inhibitor, again using tetraynoic acid, is shown in Fig. 1. It can be seen that at $3 \cdot 1 \times 10^{-7}$ M, which is only 0·05% of the substrate, inhibition is still shown. Concentrations of more than $3 \cdot 1 \times 10^{-5}$ M fail to increase inhibition significantly.

To establish whether the effects observed conformed to the pattern of competitive inhibition, substrate concentrations were varied in the presence of three of the most effective inhibitors and reciprocals of reaction velocity (increase of optical density in two minutes) were plotted against those of substrate concentration. As can be seen in Fig. 2, straight lines having an intercept on the vertical axis identical to that of the uninhibited control are produced. This indicates that the inhibition is competitive in nature for the three compounds examined.[12]

The tetraynoic acid was also examined as inhibitor in a system in which the primary oxidation of linoleic acid gave rise to a secondary oxidation of vitamin A acetate. Details of this system have been described elsewhere.[6] While inhibition of the linoleate oxidation could be demonstrated, the coupled oxidation of vitamin A acetate was not significantly affected.

The tetraynoic acid was examined also as an inhibitor in an agar gel system containing methyl linoleate, β-carotene and soya lipoxidase. A description of this system has been published previously.[13] The rate of destruction of carotene in the gel is not diminished.

Under experimental conditions identical with those used to obtain the time curves shown in Fig. 1, an examination was made of the inhibitory action of NDGA, since this has been reported by Siddiqui & Tappel[4] to be a competitive inhibitor for soya lipoxidase. It can be seen from Fig. 3 that NDGA clearly causes an induction period which lengthens as the NDGA

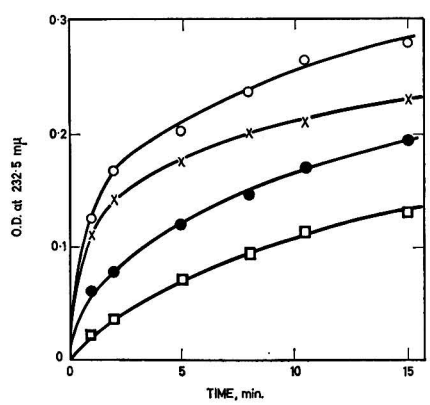


FIG. 1.—*Inhibitory activity of various concentrations of tetraynoic acid towards soya lipoxidase*

○ control × $3 \cdot 1 \times 10^{-7}$ M ● $3 \cdot 1 \times 10^{-6}$ M □ $3 \cdot 1 \times 10^{-5}$ M

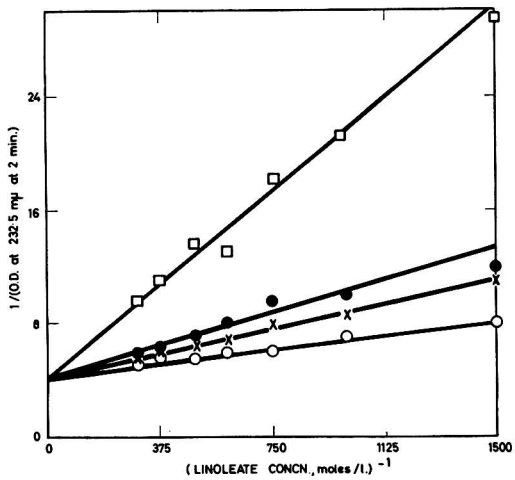


FIG. 2.—*Inhibitory activity of three compounds at different substrate concentrations*

□ eicosatetraynoic acid ● octadecatriynoic acid
× eicosatetraynol ○ control
Concentration of inhibitors $3 \cdot 1 \times 10^{-5}$ M

concentration is increased. During the reaction period there is development of a slight brown colour which is consistent with previous observations that NDGA is oxidised by lipoxidase-linoleate systems.[14]

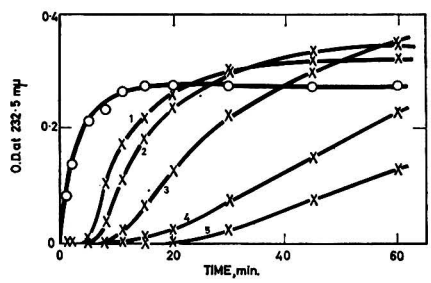


FIG. 3.—*Inhibitory activity of various concentrations of NDGA*

○ control curve 1 $1 \cdot 5 \times 10^{-5}$ M curve 2 $1 \cdot 8 \times 10^{-5}$ M
curve 3 $2 \cdot 1 \times 10^{-5}$ M curve 4 $2 \cdot 4 \times 10^{-5}$ M
curve 5 $3 \cdot 0 \times 10^{-5}$ M

Discussion

It is apparent from the results presented that the acetylenic compounds examined include very powerful competitive inhibitors. Eicosatetraynoic acid causes more than 50% inhibition at a concentration of 0·05 moles/mole substrate. The most powerful competitive inhibitor previously reported, elaidolinolenic acid, required 1·1 moles inhibitor/mole substrate for 50% inhibition.

Among the limited number of compounds examined, affinity for lipoxidase appears to be associated with chain lengths corresponding to those of lipoxidase substrates and with the presence of methylene-interrupted unsaturated centres. It has been pointed out by Holman & Elmer[7] that in lipoxidase substrates the position of the methylene-interrupted double bonds does not appear to be important since arachidonate is oxidised at the same rate as linoleate, a finding confirmed by Siddiqui & Tappel.[4] Other points which may be made are:

(1) Results on one pair of compounds, eicosatetraynoic acid and the corresponding alcohol suggest that the carboxyl group confers greater affinity for the enzyme than does the hydroxyl.
(2) Inhibition produced by octadeca-9,12-diynoic acid is little affected by the substitution of an acetylenic group by an ethylenic group as in octadeca-*cis*-12-en-9-ynoic acid.
(3) The 19-carbon nonadeca-10,13-diynoic acid, which differs only from octadeca-9,12-diynoic acid by the introduction of another methylenic group between the carboxyl and the unsaturated centre, gives more inhibition.
(4) The most effective inhibitor, eicosatetraynoic acid has a chain length of 20 carbons as well as added unsaturated centres and corresponds to the substrate arachidonate rather than linoleate.

From a study of the limited number of compounds available it is not possible to say whether inhibitory potency increases because of increased chain length from 18 to 20 C since additional unsaturated centres may be involved.

The pattern of inhibition by NDGA is obviously quite different from those of the acetylenic compounds and it is difficult to avoid the conclusion that it acts primarily in typical antioxidant fashion by producing an induction period. If Siddiqui & Tappel[4] are correct in stating that it is a competitive inhibitor, it is difficult to see how this may be established. These workers based their findings on a manometric technique, plotting reciprocal initial rates of oxygen uptake against reciprocal substrate concentrations. If the reaction period which they used fell within the induction period, it must be presumed that the oxygen uptake measured was substantially due to NDGA oxidation: if it was subsequent, then since NDGA is itself oxidised during its inhibition of linoleate oxidation,[11] competitive inhibition would be by oxidised NDGA.

At present it is not clear why the secondary oxidations of carotene and vitamin A are unaffected by the partial inhibition of lipoxidase activity by tetraynoic acid but the results obtained suggest that the competitive inhibitors examined are unlikely to be of value in affording protection of this nature. That NDGA does protect the secondary oxidant to some extent has been shown previously.[6]

Acknowledgment

The authors are indebted to Roche Products Ltd., Welwyn Garden City, for the supply of compounds tested and for financial support.

Dept. of Applied Microbiology & Biology
University of Strathclyde
Glasgow

Received 8 December, 1964

References

[1] Tappel, A. L., 'Autoxidation and Antioxidants', (Lundberg, W. O., Ed.), 1961, Vol. I, p. 325 (New York: Interscience Publishers)
[2] Holman, R. T., *Arch. Biochem.*, 1947, **15**, 403
[3] Boyd, D. H. J., & Adams, G. A., *Canad. J. Biochem. Physiol.*, 1955, **33**, 191
[4] Siddiqui, A. M., & Tappel, A. L., *J. Amer. Oil Chem. Soc.*, 1957, **34**, 529
[5] Holman, R. T., & Bergstrom, S., 'The Enzymes' (Sumner, J. B., & Myrback, K., Eds), 1951, Vol. II, Part I, p. 559 (New York: Academic Press)
[6] Blain, J. A., & Shearer, G., *Proc. Nutr. Soc.*, 1963, **22**, 162
[7] Holman, R. T., & Elmer, O., *J. Amer. Oil Chem. Soc.*, 1947, **24**, 127
[8] McIlvaine, T. C., *J. biol. Chem.*, 1921, **49**, 183
[9] Koch, R. B., Stern, B., & Ferrari, C. G., *Arch. Biochem. Biophys.*, 1958, **78**, 165
[10] Privett, O. S., Nickell, C., & Lundberg, W. O., *J. Amer. Oil Chem. Soc.*, 1955, **32**, 505
[11] Blain, J. A., & Barr, T., *Nature, Lond.*, 1961, **190**, 538
[12] Dixon, M., & Webb, E. C., 'Enzymes', 1957, p. 22 (London: Longmans, Green & Co.)
[13] Blain, J. A., & Shearer, G., *Chem. & Ind.*, 1962, p. 127
[14] Tappel, A. L., Boyer, P. D., & Lundberg, W. O., *J. biol. Chem.*, 1952, **199**, 267

SARCOMERE LENGTH OF FREE AND RESTRAINED BOVINE MUSCLES AT LOW TEMPERATURE AS RELATED TO TENDERNESS

By H. K. HERRING, R. G. CASSENS and E. J. BRISKEY

Tenderness of *semitendinosus* and *psoas major* bovine muscles was markedly affected by: (1) allowing the muscle to undergo rigor mortis and the associated contraction, following pre-rigor excision, or by (2) pre-rigor excision followed by restraint in a stretched state while the muscle undergoes rigor mortis. The extent of stretch or contraction induced by pre-rigor treatment was reflected by the sarcomere length. The average sarcomere length of the *semitendinosus* and *psoas major* muscles differed widely when samples were removed post-rigor from the carcass. The data indicate that the state of contraction (measured by sarcomere length), when altered in different portions of the same muscle by treatment, or when varying naturally in different muscles, was associated with tenderness.

Introduction

Numerous studies have been conducted during the past few years to determine the specific contribution of various factors to the tenderness of bovine muscle.[1–12] One of the factors reported has been the difference in contraction state of myofibrils in bovine muscles.[13] This worker postulated that the variations in sarcomere lengths among muscles, were due, in part, to the strains induced in the muscles when the carcass was vertically suspended, and also that since long sarcomeres occurred in muscles previously reported[14] to be generally tender, the state of contraction was considered a factor contributing to tenderness, where the effect of connective tissue was small.

Herring & Briskey[5] noted that portions of bovine *semitendinosus* muscle, excised pre-rigor and stretched during cooking, were tender, while similar portions from the same muscle, excised and cooked in the free unrestrained state, shortened markedly and were tough.

The present study was conducted in order to expand on the above general problem of tenderness and specifically to investigate further the report of Locker[13] which implicated muscular contraction in the problem of tenderness. Studies were made on different portions of the same muscle which were allowed to undergo rigor mortis under different experimental conditions in order to determine the relationships among contraction state, sarcomere length and tenderness.

Materials and methods

The *psoas major* and *semitendinosus* muscles were excised pre-rigor (45 min. post-mortem) from one side of each of six randomly-selected bovine carcasses of U.S. Choice grade. The muscles were measured prior to excision to determine the amount of shortening due to muscle removal. The time course of rigor mortis was determined on muscle strips with the use of a 'rigorometer' apparatus as described by Briskey *et al.*[15]

Each muscle was freed of external fat and connective tissue and was divided longitudinally into two identical 18 cm.-long portions (weighing about 450 g.) of approximately parallel fibres. One portion of each muscle was stretched with a force of 15 kg./kg. of muscle weight in an apparatus as shown in Fig. 1, and held in the stretched state for 48 h. The other portion of each muscle was allowed to contract, the extent of shortening being measured at various intervals after the 18 cm. portion was prepared for experimentation. Three replicates of each muscle (free and stretched-restrained, enclosed in plastic film) were placed at each of 5° and 1° for 48 h. Changes in pH were determined at various periods in portions of these same muscles held unrestrained at 5° and 1°.

'Post-rigor excised muscles' were excised 48-h. post-mortem from the opposite sides of the vertically suspended carcasses and were used as controls in these studies.

Unfixed samples (48-h. post-mortem) were blended for 1 min. in 0·08 M-potassium chloride in a chilled blender.[13] The suspension of myofibrils was examined directly in a phase contrast microscope with the sarcomere length being determined as an average from 25 myofibrils of each of the stretched-restrained, free (contracted) and post-rigor excised (control) muscles.

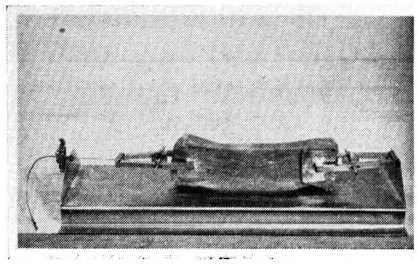


FIG. 1.—*Apparatus for stretching and restraining muscle portions*

All portions (stretched-restrained, free and post-rigor excised) were prepared for evaluation of tenderness 48-h. post-mortem by roasting at 177° to an internal temperature of 66° as individually measured with thermocouples. The organoleptic evaluations were conducted by a six-member laboratory panel with the use of the chew count[16] and hedonic scale,[17] ranging from 1 (very tough) to 9 (very tender). Objective values for tenderness were obtained on cores 2 cm. in diameter 24-h. post-cooking with the use of a Warner–Bratzler shear device.

Results

These experiments utilised a muscle considered to be tender (*psoas major*) and one considered to be somewhat tough (*semitendinosus*), which is probably, in part, a reflection of the connective tissue content.[10,18] This difference was noticeable when muscle sections were stretched as described above; most *semitendinosus* muscle sections were stretched beyond their resting length, while attempts to stretch the *psoas major* resulted in tearing with general failure to regain initial length.

The use of two temperatures (5° and 1°) for holding excised portions revealed that the delay phase of rigor mortis in the *semitendinosus* was decreased from 475 min. at 5° to 198 min. at 1° which is in agreement with the work of Cassens & Newbold.[19]

Effect of excision and handling on shortening

When the *semitendinosus* and *psoas major* muscles were excised shortly after death, they quickly shortened by 20–25% and 10–20% (of initial length) respectively, from their natural positions in the vertically suspended carcasses. It is not known whether this difference was due to degree of stretch in the carcass, elastic connective tissue content, chemical composition or irritability. Subsequent placement of the excised free muscle samples in cold environments caused a greater shortening effect at 1° than at 5° as has been reported by Locker & Hagyard,[20] although the shortening observed here was not as great as they reported.

Sarcomere length of free and restrained muscles

Fig. 2 shows sarcomere lengths of myofibrils representative of the range observed in these studies. The control *psoas major* had a sarcomere length of 3·8 μ (Fig. 2A) compared with 2·4 μ (Fig. 2D) for the control *semitendinosus*. The long sarcomere length of post-rigor excised *psoas major* bovine muscle has been previously reported by Locker,[7,13] and in view of the recognised tenderness of the *psoas major* muscle, this factor appears to merit further investigation. Even though the *psoas major* is low in connective tissue content, the extremely long sarcomeres may be particularly significant in relation to ultimate tenderness in view of the structural changes which may be involved.

Table I reveals the effects of physical handling and temperature treatments on the sarcomere lengths of the *semitendinosus* muscle. Sarcomere lengths were shortened as a result of pre-rigor excision and subsequent shortening especially when compared to the stretched-restrained samples, regardless of temperature. Mean sarcomere length of the 'free' samples was 1·7 μ at 1° and slightly longer at 5° (2·0 μ).

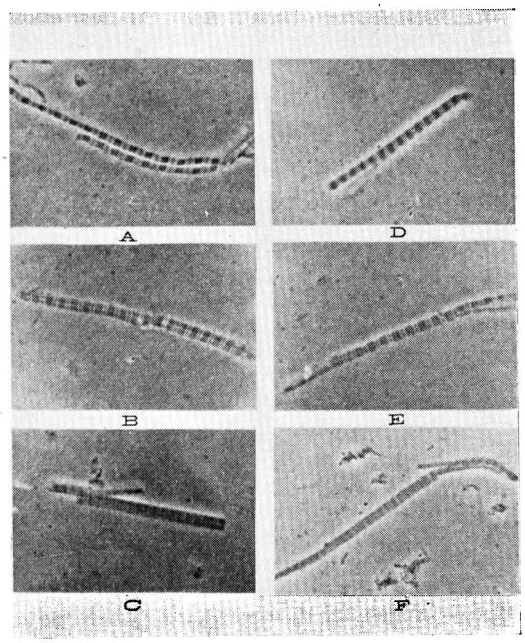


FIG. 2.—*Phase contrast micrographs of myofibrils of* semitendinosus *and* psoas major *bovine muscles*

A. *Psoas major* post-rigor excised (3·8 μ)
B. *Psoas major* stretched-restrained (2·4 μ)
C. *Psoas major* excised pre-rigor, free (1·9 μ)
D. *Semitendinosus* post-rigor excised (2·4 μ)
E. *Semitendinosus* stretched-restrained (2·7 μ)
F. *Semitendinosus* excised pre-rigor, free (1·6 μ)

Pre-rigor excised, stretched-restrained *semitendinosus* muscles exhibited longer sarcomeres than the control samples, in all but one instance. This is an indication that it is relatively easy to stretch *semitendinosus* muscles beyond their 'control-carcass' sarcomere length and that when these muscles are restrained in the stretched position, they do not undergo extensive localised sarcomere shortening, or at least this phenomenon was not detectable in the present experiment.

Table II shows the mean sarcomere lengths of the *psoas major* muscles. At both temperature treatments, excised 'free' muscles had sarcomere lengths of approximately 50% of the

Table I

Effect of temperature treatment on the sarcomere length of free and restrained semitendinosus *muscle*

Temperature treatment		Length of sarcomeres, μ		
		Pre-rigor excised[a] (free)[c]	Pre-rigor excised (stretched-restrained)[d]	Post-rigor excised[b]
5°	1	2·0	2·9	2·4
	2	1·9	3·0	2·4
	3	2·1	3·2	2·2
	Average	2·0	3·0	2·3
1°	1	1·7	2·4	2·6
	2	1·8	2·7	2·4
	3	1·6	2·7	2·7
	Average	1·7	2·6	2·6

[a] Approx. 45 min. post-mortem
[b] Approx. 48 h. post-mortem

Table II

Effect of temperature treatment on the sarcomere length of free and restrained psoas *muscle*

Temperature of treatment		Sarcomere length, μ		
		Pre-rigor-excised[a] (free)[c]	Pre-rigor excised (stretched-restrained)[d]	Post-rigor excised[b]
5°	1	1·8	2·1	3·3
	2	2·0	2·1	3·4
	3	1·7	2·0	3·7
	Average	1·8	2·1	3·5
1°	1	1·9	2·6	3·4
	2	1·8	2·4	3·4
	3	1·8	2·1	3·6
	Average	1·8	2·4	3·5

[c] Free to contract
[d] Stretched and held in apparatus as shown in Fig. 1

control post-rigor excised muscles (1·8 μ versus 3·5 μ). Pre-rigor excised samples which were stretched-restrained had sarcomere lengths of 2·1–2·4 μ. Upon excision, the muscle fibres shortened considerably and the initial muscle length and sarcomere length of the *psoas major* could not be achieved by stretching. The muscle fibres were torn or broken easily when stretching was attempted.

Organoleptic evaluation of free and restrained muscles

Pre-rigor excised *semitendinosus* muscle portions, left free to contract at the indicated temperatures for 48-h. post-mortem, were the least tender of all samples (Table III). These values were much higher than those for the pre-rigor excised, stretched-restrained samples from the same muscle. Pre-rigor excised muscles were especially tough in the 1° treatment group, having shear values of 37·2 lb. compared with 19·8 lb. in the stretched-restrained portions.

Slightly more shortening appeared to take place in the stretched-restrained samples at 1°, as indicated by sarcomere length, than at 5°. It is possible that some contraction due to cold-shortening occurred at this temperature. In any event, the samples which were stretched-restrained at 1° were less tender than those at 5°. The post-rigor excised samples of this muscle were more tender than either the free or stretched-restrained portions as shown in Table III.

Similar results were obtained for the *psoas major* muscle (Table IV). Pre-rigor excision decreased the palatability at both temperature treatments, with shear force and number of chews increasing and score value decreasing as a result of pre-rigor excision of this normally tender muscle, which has a low content of connective-tissue. Decreasing the temperature from 5° to 1° decreased the tenderness value of the *psoas major* muscle of both excised free and excised-restrained samples. The difference in tenderness between control and stretched-restrained samples can be explained by the inability to be stretched as reflected in the markedly reduced sarcomere lengths.

Discussion

It is clear from the present work that a positive association exists between organoleptic tenderness and sarcomere length. This is in support of the work of Locker,[13] who concluded that long sarcomeres occurred in muscles previously reported[14] to be generally tender. The state of contraction was, therefore, considered to be a factor contributing to tenderness, where the effect of connective tissue was small.

However, tenderness can probably not be best explained by a single factor such as amount or kind of connective tissue, amount of fat or marbling, or sarcomere length. Sarcomere length, a measure of contraction state, is probably only a gross indication of the molecular changes occurring in the actin and myosin of muscle. Molecular alterations associated with a strong contraction may well account for a large share of the variations in tenderness observed in this experiment.

Table III

Tenderness of free and restrained Semitendinosus *muscles*

Temperature treatment		Pre-rigor excised[a] (free)[c]			Pre-rigor excised (stretched-restrained)[d]			Post-rigor excised[b]		
			Panel			Panel			Panel	
		Shear[e]	Chews	Score[f]	Shear	Chews	Score	Shear	Chews	Score
5°	1	20·9	42	4·8	12·7	31	5·5	14·6	21	7·3
	2	37·2	55	2·0	19·4	29	5·8	13·1	26	6·5
	3	33·9	52	3·7	18·8	38	4·5	11·7	16	7·0
	Average	30·7	50	3·5	16·9	32	5·3	13·1	21	6·9
1°	1	38·2	93	1·2	17·0	47	4·0	11·6	26	6·2
	2	31·9	61	2·0	16·5	48	3·2	13·5	37	4·6
	3	41·5	57	2·6	25·8	37	4·0	16·0	26	5·8
	Average	37·2	72	1·9	19·8	44	3·7	13·7	29	5·5

[a] Approximately 45 min. post-mortem
[b] Approximately 48 h. post-mortem
[c] Free to contract
[d] Stretched and held in apparatus as shown in Fig. 1
[e] Pounds of shear force
[f] Panel score based on hedonic scale

Table IV

Tenderness of free and restrained psoas major *muscles*

Temperature treatment		Pre-rigor excised[a] (free)[c]			Pre-rigor excised (stretched-restrained)[d]			Post-rigor excised[b]		
			Panel			Panel			Panel	
		Shear[e]	Chews	Score[f]	Shear	Chews	Score	Shear	Chews	Score
5°	1	11·1	23	7·0	6·6	17	8·2	11·9	15	7·8
	2	18·3	29	6·2	12·1	21	7·8	11·1	14	8·1
	3	18·5	34	4·8	11·7	22	6·8	7·6	11	7·8
	Average	16·0	29	6·0	10·1	20	7·6	10·2	13	7·9
1°	1	14·5	29	5·5	13·2	24	6·0	8·0	12	8·0
	2	11·1	37	4·4	7·4	26	6·4	8·6	15	8·2
	3	41·7	59	2·6	16·8	28	4·8	11·5	10	7·8
	Average	22·4	42	4·2	12·47	26	5·7	9·37	12	8·0

[a] Approximately 45 min. post-mortem
[b] Approximately 48 h. post-mortem
[c] Free to contract
[d] Stretched and held in apparatus as shown in Fig. 1
[e] Pounds of shear force
[f] Panel score based on hedonic scale

The postulation that post-mortem tenderisation may be caused by a dissociation of actomyosin to actin and myosin[12] has been suggested and, more recently, studies have been reported concerning the relationship between tenderness and the solubility of different bovine muscle protein fractions.[21, 22]

Further evidence that contraction affects tenderness has been offered by Herring *et al.*[23] in a study dealing with thaw rigor contraction of large pieces of muscle similar in size to those reported in this paper. Samples which had undergone thaw rigor and associated contraction were markedly less tender than their respective controls or samples which had been allowed to undergo thaw rigor in a stretched-restrained condition.

Fibre diameter has been previously reported to be associated with tenderness,[24, 25] but in view of the possible role of contraction in tenderness it is important to establish the relationship between change in fibre diameter with change in contraction state of muscle; this aspect is being investigated.

No attempt was made in the present experiments to examine the effect of ageing, but rather the immediate effect of contraction state on tenderness. The contractions which were observed were a result of excision and removal of the muscle from a hot carcass, the contraction associated with the onset of rigor mortis and possibly the contraction associated with the cold-shortening effect. It appears that, under the conditions used, contraction markedly affected tenderness, and therefore the extent of contraction that may occur in the intact carcass may be an important factor. Locker[13] has suggested that certain muscles are obviously under more tension than others in the suspended carcass. Muscles under less tension could shorten generally along their length while those held under more tension could not shorten as much or could perhaps shorten only in localised areas.

The effect of contraction must be considered with all other factors affecting tenderness. The molecular changes associated with contraction appear to be a fruitful area for further research.

Acknowledgments

This paper is published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

This investigation was supported in part by a research grant from the Campbell Soup. Co., Camden, New Jersey, and by a research grant from the National Livestock and Meat Board. Acknowledgment is also made of the use of equipment purchased through Public Health Service Research Grant EF-81 (C6), from the Division of Environmental Engineering and Food Protection.

Dept. of Meat & Animal Science
College of Agriculture
University of Wisconsin
Madison 6, Wisconsin, U.S.A.

Received 28 Sept., 1964; amended manuscript 18 Jan., 1965

References

[1] Bate-Smith, E. C., *Adv. Fd Res.*, 1948, **1**, 1
[2] Blumer, T. N., *J. Anim. Sci.*, 1963, **22**, 771
[3] Brady, D. E., *Proc. 30th Annu. Mtg Amer. Soc. Animal Prod.*, 1937, 246
[4] Goll, D. E., *J. Fd Sci.*, 1963, **28**, 503
[5] Herring, H. K., & Briskey, E. J., unpublished data, 1963
[6] Howard, A., & Lawrie, R. A., *Tech. Pap. Div. Fd Preserv. Transp., C.S.I.R.O. Aust.*, 1956, No. 2
[7] Locker, R. H., *J. biophys. biochem. Cytol.*, 1959, **6**, 419
[8] Naumann, H. P., Braschler, C., Mangel, M., & Rhodes, V. J., *Missouri Res. Bull.*, 1961, 777
[9] Ramsbottom, J. M., Strandine, E. J., & Koonz, C. H., *Fd Res.*, 1945, **1**, 497
[10] Strandine, E. J., Koonz, C. H., & Ramsbottom, J. M., *J. Anim. Sci.*, 1949, **8**, 483
[11] Wilson, G. D., Bray, R. W., & Phillips, P. H., *J. Anim. Sci.*, 1954, **13**, 826
[12] Wierbicki, E., Kunkle, L. E., Cahill, V. R., & Deatherage, F. E., *Fd Technol.*, 1956, **10**, 80
[13] Locker, R. H., *Fd Res.*, 1960, **25**, 304
[14] Ramsbottom, J. M., & Strandine, E. J., *J. Anim. Sci.*, 1949, **8**, 398
[15] Briskey, E. J., Sayre, R. N., & Cassens, R. G., *J. Fd Sci.*, 1962, **27**, 560
[16] Cover, S., *Fd Res.*, 1959, **24**, 564
[17] Peryam, D. R., & Pilgrim, F. J., *Fd Technol.*, 1957, **11**, 9
[18] Venable, J. H., *Proc. Meat Tenderness Symposium* (Campbell Soup Co.), 1963, p. 7
[19] Cassens, R. G., & Newbold, R. P., in preparation
[20] Locker, R. H., & Hagyard, C. J., *J. Sci. Fd Agric.*, 1963, **14**, 787
[21] Hegarty, G. R., Bratzler, L. J., & Pearson, A. M., *J. Fd Sci.*, 1963, **28**, 525
[22] Goll, D. E., Henderson, D. W., & Kline, E. A., *J. Fd Sci.*, 1964, **29**, 590
[23] Herring, H. K., Cassens, R. G., & Briskey, E. J., *Biodynamica*, 1964, **9**, 257
[24] Hiner, R. L., & Yao, T. S., *J. Anim. Sci.*, 1954, **13**, 970
[25] Tuma, H. J., Venable, J. H., Wuthier, P. R., & Henrickson, R. L., *J. Anim. Sci.*, 1962, **21**, 33

FUSEL OILS IN CIDERS AND PERRIES

By A. POLLARD, MARGARET E. KIESER, PAULINE M. STEVENS and O. G. TUCKNOTT

The amounts of higher aliphatic alcohols in the ciders and perries studied ranged from 116 to 255 p.p.m.; certain samples contained in addition about 100 p.p.m. of 2-phenylethanol. The production of these compounds in ciders and perries is discussed. Estimates of fusel oil content by gas chromatography were compared with those given by a colorimetric method. Where distillates contained mainly butanols and pentanols, the two methods were in good agreement. The higher values given by some samples in the colorimetric estimation were attributed to the presence of other components: 2-phenylethanol and tyrosol were found to be responsible for some of the discrepancy.

Introduction

The application of gas–liquid chromatography in recent years has led to a re-examination of the fusel oils of fermented beverages, the term 'fusel oil' being taken to mean the higher alcohol fractions of distillates. The literature up to 1961 has been comprehensively reviewed by Pfenninger,[1–3] but although there are numerous later references to beer[4–10] and to wines,[11–14] there are few to ciders.[3, 5, 15] Earlier estimates of fusel oil content have been obtained by colorimetric methods based on the Komarowski reaction or by the fractionation and analysis of distillates prepared on the large scale. Gas chromatography has been used to give more detailed, but qualitative, surveys of the compounds present: there have been few direct comparisons of the colorimetric and chromatographic methods for quantitative determination.[16, 17]

In earlier work at Long Ashton the higher alcohols found to predominate in cider distillates were isobutanol (2-methylpropan-1-ol) and isopentanol (3-methylbutan-1-ol). The quantitative determination of these and the associated components by gas chromatography was found to be in rough agreement with the fusel oil content determined by colorimetry, but the correspondence was poor for some samples.[18] The work has now been extended in greater detail to a wider range of ciders and perries.

Experimental

The ciders and perries studied were taken either from a series of small-scale experimental fermentations of single varieties of fruit or from bulks of blended juice fermented under semi-commercial conditions at the Research Station. In the small-scale fermentations the juices were normally sulphited and yeasted with a sulphite-trained wine yeast, fermented to a specific gravity of 1·005, and the ciders filtered and pasteurised in bottle. On the larger scale the fermentation, after sulphiting, was brought about by residual members of the natural yeast flora. These ciders and perries were fermented to 'dryness,' i.e., completion, filtered, and sampled after a period of bulk storage in vats or after subsequent sterile bottling.

Preparation of volatile fractions

The fractions containing the fusel oils were prepared by (*a*) vapour cycling through cold traps, or (*b*) distillation at atmospheric pressure.

(*a*) The vapour cycling method was based on that of Nawar & Fagerson.[19] A flask of 100 ml. capacity was connected through a peristaltic or pulsating pump to two cold traps at −70° in series, with a return connection dipping into the liquid in the flask. The system was filled with nitrogen, the sample (50 ml.) introduced, the flask immersed in a water bath at 45°, and the gas circulated through the system for 4 h. The cold traps were then isolated from the system, the condensates collected and rinsed out with distilled water to a total volume of 10 ml. Cycling was then continued for 2 h. at 65° and the condensate was collected as before. When applied to standard solutions of aliphatic alcohols similar to cider in composition, about 90% of the fusel oil fraction was recovered by cycling at 45°, the remaining 10% passing over at 65°.

(*b*) Samples of cider were distilled without reflux after the addition of one-third volume of water and a volume of distillate collected equal to that of the sample.[12]

Colorimetric determination of fusel oils

Aliquots of distillate prepared by method (*b*) were purified and saponified by the method of Guymon & Heitz,[11] to remove aldehydes and hydrolyse esters; the colour was developed by the method of Boruff,[20] but optical densities were measured at 520 mμ.[11] Calibration curves were prepared daily using standard solutions containing commercial isobutanol and 'isoamyl' alcohol in the ratio of 1 : 4.

Analysis by gas chromatography

The analyses for aliphatic alcohols were carried out with equipment built in this laboratory with a hydrogen flame ionisation detector and Kent Recorder with 1 mV full scale. The carrier gas was a 1 : 1 mixture of nitrogen and hydrogen with a flow rate of approximately 15 ml. per min. The glass column 122 cm. × 4·5 mm. i.d. was packed with 60–80 mesh C 22 fire-brick: the stationary phase, 10% polyethyleneglycol 400, and was run isothermally at 80°.

To avoid the injection of undesirable amounts of water that appeared to cause some instability of the column, the vapour head-space technique was used. An aliquot of the sample was pipetted into a test tube of capacity such that the ratio of head-space to liquid was 5 : 1. The tubes, tightly capped with aluminium foil, were immersed in a water bath at 65° for 5 min., with gentle shaking at intervals. The needle of a 0·5-ml. syringe was inserted through the cap, the syringe filled and emptied four times and 0·5 ml. of vapour immediately injected.

The chromatograms given by samples prepared by methods (*a*) and (*b*) were compared with those given by standards of similar and known composition run daily. Measurement of peak height was found to give a satisfactory estimate of quantity for the components present and the results were reproducible to within 5%. The alcohols present were identified by a comparison of their retention times with those of known compounds run on columns with different stationary phases.

Results

Comparison of analytical methods

The aliphatic alcohol contents found for a number of ciders and perries are shown in Table I. The values given by the colorimetric method are either compared with those found by gas

Table I

Higher aliphatic alcohols in ciders and perries

No.	Product	Preparation of injection sample	Components present (p.p.m.) by gas–liquid chromatography (GLC)				Total by GLC	Total by colorimetry
			Propan-1-ol	2-methyl-propan-1-ol	Butan-1-ol	2-Methyl-butan-1-ol + 3-Methyl-butan-1-ol		
	Ciders							
1	Michelin	(a)	9	60	8	126	203	210
2	Michelin ($-SO_2$)	(a)	9	18	12	117	156	158
3	Langworthy A	(a)	9	16	21	93	139	140
4	Langworthy A ($-SO_2$)	(a)	10	35	13	90	148	135
5	Bulmer's Norman	(a)	11	74	18	120	223	235
6	Bulmer's Norman ($-SO_2$)	(a)	10	71	18	100	199	227
7	Morgan Sweet (A)	(b)	15	82	16	142	255	275
8	Morgan Sweet (B)	(b)	15	41	17	75	148	160
9	Reine des Hatives	(b)	15	37	32	68	152	152
10	Langworthy B	(b)	9	21	12	74	116	135
11	Blend (Vat 2)	(b)	5	21	9	117	152	213
12	Blend (Vat 5)	(b)	9	16	10	106	141	170
13	Blend (Bottled)	(b)	20	14	20	95	149	172
	Perries							
14	Blend I (Bottled)	(b)	10	36	7	86	139	232
15	Blend II (Bottled)	(b)	8	38	6	95	147	218
16	Blend (Vat I)	(b)	8	43	6	104	161	227

($-SO_2$) juice not sulphited before yeasting; (a) vapour cycling method; (b) distillation method

chromatography of the same purified distillates (Nos. 7–14), or of fractions obtained independently by vapour cycling (Nos. 1–6). The values found by the two methods are in general agreement for the ciders made by small-scale fermentation (Nos. 1–10), whereas the large-scale products (Nos. 11–16) show wide discrepancies, the colorimetric method giving higher values.

An exact correspondence between the two methods of analysis would not be expected, and the very close agreement found for some samples may be fortuitous. The intensity of colour given by the different alcohols varies. The presence of n-propanol, that gives a weak colour,[16] would tend to lower the colorimetric figure; it was, however, present only in traces. The degree of correspondence found for the ciders Nos. 1–10 was similar to that found between the two methods for a range of potable spirits.[17]

No large peaks due to esters or other unidentified components were noted on the chromatograms, but small amounts of such compounds could emerge with the alcohol peaks and give rise to error in measurement of peak heights. Esters would not interfere in the gas chromatography of the purified distillates, for they would be determined as alcohols after saponification in both methods of estimation. To test this further, a number of distillates were chromatographed both before and after the saponification procedure. As shown in Table II, the estimates of total alcohols agreed to within the experimental error except in two samples (Nos. 8 and 15), where the difference exceeded 10%. It was therefore assumed that the amounts of esters or aldehydes present caused little interference.

The presence of other components

The results for ciders fermented on the small-scale with yeast addition are in contrast to those for ciders and perries made on the larger scale using only members of the natural microflora (Nos. 11–16). In the latter series, the colorimetric method gave much higher values for higher alcohols than did gas chromatography. It appeared possible that other components were present which contributed to the colorimetric estimation, but were undetected on the gas chromatograms. These ciders and perries and their distillates were subsequently found to contain 2-phenylethanol in considerable amount (100 p.p.m.), whereas the small-scale ciders contained only 7–15 p.p.m.[21] 2-Phenylethanol gave no peak with the column used for the gas chromatography of the aliphatic alcohols and is thus not included in the estimate of fusel oils by that method. It was considered unlikely that this compound would be included in the estimate by colorimetry in view of the negative findings of other workers who used *p*-dimethylaminobenzaldehyde[9] or 4-hydroxybenzaldehyde-3-sulphonic acid.[6] Later tests showed, however, that the former reagent under our conditions gives a red colour with

Table II

Analysis of distillates by G.L.C. before and after saponification

No.	Higher aliphatic alcohols in ciders and perries (p.p.m.)	
	Before saponification	After saponification
7	254	255
8	171	148
10	113	116
11	148	152
12	152	148
14	147	139
15	168	147
16	160	161

2-phenylethanol which can contribute to the measurement of total fusel oils. There is no well-defined peak of absorption over the range 480–520 mμ and the colour produced is much less than that given by the aliphatic alcohols (2-methylpropan-1-ol + 3-methylbutan-1-ol 1 : 4) and falls off with increasing concentration. At low concentrations of 2-phenylethanol the colour development was about 10% of that given by these aliphatic alcohols and at higher concentrations was about 5%.

In the small-scale ciders where the level of 2-phenylethanol was below 20 p.p.m. (Nos. 7–10), there would be little contribution to the total fusel oil figure given by colorimetry, but in those containing about 100 p.p.m. (e.g. Nos. 10–13), 2-phenylethanol would increase the total figure by about 5 p.p.m. This would not suffice to account for the discrepancy between the colorimetric and chromatographic determinations, and it appeared possible that other compounds were present and contributing to the former. The presence of tyrosol (*p*-hydroxyphenylethanol) was therefore considered as it is commonly present in fermented beverages, and has been tentatively identified in ciders by Burroughs[22] using paper chromatography as described by Sentheshanmuganathan & Elsden.[23]

Further examination of cider No. 13 showed that a concentrate prepared by continuous ether extraction contained a substance with the R_F value of tyrosol in amount, as estimated by spot size and intensity, corresponding to about 20 p.p.m. in the cider. Tyrosol has been found to react in the colorimetric procedure and to give a colour equivalent to about 20% of that given by the standard solution of aliphatic alcohols on a weight basis, when measured at 480 or 520 mμ. Although fractions prepared from cider distillates give a characteristic tyrosol reaction with the diazo reagent,[23] only traces would be expected to distil with the fusel oils in view of its high boiling point (310°). When a solution containing 200 p.p.m. of tyrosol was subjected to the purification and distillation treatments used for the colorimetric fusel oil determinations, less than 10% of this passed into the distillate.

The contribution of tyrosol to the total colour measured in the fusel oil determinations must therefore be small and represent no more than a few p.p.m. Although the presence of tyrosol and of 2-phenylethanol may account for some of the discrepancy between the colorimetric and gas chromatographic determinations, it would not be sufficient to account for the wide differences noted for some samples. Other unidentified components may be present and contribute to the colorimetric estimate of fusel oil. Thus, although this method of determination appears satisfactory where the main components of distillates are the butanols and pentanols, it gives erroneous estimates where their composition is more complex.

Discussion

The fusel oil content of ciders and perries

The amounts of aliphatic higher alcohols reported in beers and wines vary widely according to the type of product. Lager beers may contain no more than 30–80 p.p.m., whereas top fermentation beers or others of higher alcohol content may contain 200 p.p.m. or more.[4, 5, 10] The fusel oil content of wine is usually higher and may range from 150 to 500 p.p.m.,[11, 12] while a figure of 98 p.p.m. has been reported for a cider.[5] The ciders and perries tested in the present

work gave values ranging from 116 to 255 p.p.m. as estimated by gas chromatography, and they correspond to the higher values found for beers. This would be expected from the general relationship found between ethanol content and fusel oils in fermented beverages, for the ciders and perries, with alcohol contents between 5 and 7·5%, would correspond to higher gravity beers rather than wines.

As found in other fermentation products, 2-methylpropan-1-ol and the pentanols are main components in the higher alcohol fractions of ciders and perries. Propan-1-ol is present in small amounts and butan-1-ol may reach 20 p.p.m. in some samples. Similar results for two ciders were found by Pfenninger,[3] but butan-1-ol occurred in smaller proportions and traces of butan-2-ol were also noted; the latter was not identified in the Long Ashton samples. Since butan-1-ol is a main component in the aroma fraction of some apple juices,[24] some of that found in ciders may be derived from the original fruit, as will some small proportion of other fusel oil components. The methylbutanols were not differentiated by the column used in this work, but later investigations have shown that both 3-methylbutan-1-ol and 2-methylbutan-1-ol are present and occur in the ratio of about 6 : 1. In the two ciders studied by Pfenninger a ratio of about 7 : 1 was noted.[3]

Concentrations up to 50 p.p.m. of 2-phenylethanol have been found in beers,[8, 9, 25, 26] whereas in wines the amount may exceed 50 p.p.m.[27] and may represent an appreciable proportion of the total fusel oil fraction.[13, 14] In ciders and perries the amounts vary widely, from less than 20 p.p.m. to 100 p.p.m., and at the higher level 2-phenylethanol may represent a third of the total fusel oil fraction. The acetate has been reported in very small amounts in beers[26, 28] but has not yet been identified in ciders or perries.

The production of fusel oil in ciders

The nitrogen status of worts has been considered adequate for the production of fusel oil components in beer by the Ehrlich degradation of amino-acids[6] although this may not be the sole mechanism operating. In contrast, the lower amino-acid content of grape musts suggests that in wines much of the fusel oil production must proceed by alternative routes leading to amino acid synthesis by yeasts as shown by the work of Peynaud & Guimberteau[29] and others.[1, 30] The amino-acid content of apple and pear juices is even lower than that of grape musts.[31] The juices used for the small-scale fermentations in the present work had soluble nitrogen contents varying from 50 to 280 p.p.m., and of this the greater portion would be asparagine and aspartic and glutamic acids.[31] The level of isoleucine found in apple juices has not exceeded 10 p.p.m. and leucine has only been found in traces. These amounts would not suffice for the production of the pentanols from amino-acids, more especially as 3-methylbutan-1-ol, corresponding to leucine, is the predominating component. The production of 2-methylpropan-1-ol from valine would also be insufficient to account for the amounts present, for this amino-acid is a very minor constituent in apple juices.

Similarly, the formation of 2-phenylethanol and tyrosol cannot be attributed to the direct transformation of the aromatic amino-acids, for these occur only in traces in apple and pear juices. They are detectable towards the end of fermentation after yeast autolysis, but only in small amounts. It must be assumed that the aromatic alcohols arise by other metabolic pathways through shikimic acid leading to the synthesis of the aromatic ring. If shikimic acid itself can enter the reaction sequence, the amounts present in some apple juices could contribute appreciably to the total level of aromatic alcohols.[32]

The production of the aliphatic alcohols was not found consistently to be related to the nitrogen content of the juices or to their original specific gravities. Many other factors can influence their production as, for example, the type of yeast, the nutritional status of the medium, pH and temperature.[6, 29, 33] Cider apple juices are relatively low in many yeast nutrients and particular deficiencies would be expected to have a considerable influence on the formation of secondary fermentation products. The ciders Nos. 1–10 in Table I, that were all fermented with the same yeast and under the same conditions, do indeed show a wide variation in higher alcohol content according to the variety or origin of the fruit. The differences in the amounts of 2-phenylethanol found may, on the other hand, be also related to the microflora

or to the conditions of fermentation. High values were found only in the large-scale fermentations carried out by yeast present in the original juices. These fermentations were more protracted and the ciders and perries were higher in alcohol, about 7–8% as against 5–7% in the small-scale fermentations.

The presence of the higher alcohols in ciders and perries is not necessarily deleterious, they are essential components of the aroma and flavour and contribute to the fruit character of the products. An excess of certain components may, however, throw the flavour out of balance. The relation between fusel oil components upon quality and the factors governing their production are under further investigation.

Acknowledgment

The authors are indebted to Dr. L. F. Burroughs for the estimation of tyrosol in cider.

Dept. of Agriculture & Horticulture,
University of Bristol,
Long Ashton Research Station,
Bristol.

Received 11 December, 1964; amended manuscript 2 February, 1965

References

[1] Pfenninger, H., *Z. LebensmUntersuch. Forsch.*, 1963, **119,** 401
[2] Pfenninger, H., *Z. LebensmUntersuch. Forsch.*, 1963, **120,** 100
[3] Pfenninger, H., *Z. LebensmUntersuch. Forsch.*, 1963, **120,** 117
[4] Stevens, R., *J. Inst. Brew.*, 1960, **66,** 453
[5] Hudson, J. R., & Stevens, R., *J. Inst. Brew.*, 1960, **66,** 471
[6] Hough, J. S., & Stevens, R., *J. Inst. Brew*, 1961, **67,** 488
[7] Stevens, R., *J. Inst. Brew.*, 1961, **67,** 329
[8] Äyräpää, T., *J. Inst. Brew.*, 1961, **67,** 262
[9] Sihto, E., & Arkima, V., *J. Inst. Brew.*, 1963, **69,** 20
[10] Harold, F. V., Hildebrand, R. P., Morieson, A. S., & Murray, P. J., *J. Inst. Brew.*, 1961, **67,** 161
[11] Guymon, J. F., & Heitz, J. E., *Food Technol.*, 1952, **6,** 359
[12] Ribéreau-Gayon, P., & Peynaud, E., 'Traité d'Oenologie', 1961, II, p. 108 (Paris, Liege: Béranger)
[13] Webb, A. D., Ribéreau-Gayon, P., & Boidron, J. N., *C.R. Acad. Agric. Fr.*, 1963, **49,** 115
[14] Webb, A. D., Ribéreau-Gayon, P., & Boidron, J. N., *Bull. Soc. chim. Fr.*, 1964, p. 1415
[15] Matthews, J. S., Sugisawa, H., & MacGregor, D. R., *J. Fd Sci.*, 1962, **27,** 355
[16] Bouthilet, R. J., & Lowry, W., *J. Ass. off. agric. Chem., Wash.*, 1959, **42,** 634
[17] *Annu. Rep. Govmt Chemist for* 1963, p. 16 (London: H.M.S.O.)
[18] *Annu. Rep. Long Ashton Res. Sta. for 1963*, 1964, p. 40
[19] Nawar, W. W., & Fagerson, I. S., *Analyt. Chem.*, 1960, **32,** 1534
[20] Boruff, C. S., *J. Ass. off. agric. Chem., Wash.*, 1961, **44,** 383
[21] Kieser, Margaret E., Pollard, A., Stevens, Pauline M., & Tucknott, O. G., *Nature, Lond.*, 1964, **204,** 877
[22] Burroughs, L. F., unpublished
[23] Sentheshanmuganathan, S., & Elsden, S. R., *Biochem. J.*, 1958, **69,** 210
[24] Kieser, Margaret E., & Pollard, A., *Proc. Symp. Volatile Fruit Flavours*, 1962, p.249 (Berne, Zürich: Juris-Verlag)
[25] Shigematsu, N., *Bull. Brew. Sci. Tokyo*, 1962, **8,** 44 (*J. Inst. Brew.*, 1963, **69,** 277)
[26] Strating, J., & Venema, A., *J. Inst. Brew*, 1961, **67,** 525
[27] Äyräpää, T., *Nature, Lond.*, 1962, **194,** 472
[28] Kepner, R. E., Strating, J., & Weurman, C., *J. Inst. Brew.*, 1963, **69,** 399
[29] Peynaud, E., & Guimberteau, G., *Ann. Technol. agric.*, 1962, **11** (2), 85
[30] Äyräpää, T., *Proc. Europ. Brewery Conv. Congr.*, 1963, **9,** 276 (*Chem. Abstr.*, 1964, **61,** 8860)
[31] Burroughs, L. F., *J. Sci. Fd Agric.*, 1957, **8,** 122
[32] Whiting, G. C., & Coggins, R. A., *J. Sci. Fd Agric.*, 1960, **11,** 337
[33] Harris, G., *J. Inst. Brew.*, 1964, **70,** 193

FURTHER STUDIES ON THE LIMITING AMINO-ACIDS IN DIFFERENTLY PROCESSED GROUNDNUT MEALS*

By HANS FISHER

Different samples of groundnut meal were fed to growing chicks and supplemented factorially with the three amino-acids lysine, methionine and threonine. There were appreciable differences among the samples in terms of their growth-promoting properties and the order of amino-acid limitation. In a comparison of differently processed groundnuts from the same fresh supply, it was found that a solvent-extracted preparation was equally limiting in all three amino-acids and responded only to the triple-supplementation. An expeller pressed meal, as well as an expeller and solvent-extracted preparation was first limiting in lysine but responded differently to other combinations of the three amino-acids. Amino-acid analysis of the different groundnut samples indicated differences, particularly a lower sulphur-containing amino-acid content for solvent-extracted meals. All of the groundnut meals tested had a lower lysine content than those reported in the literature.

Introduction

Pieterse[1] and Fisher[2] have recently drawn attention to the contradictory results that have been published concerning the limiting amino-acids in groundnut protein. Whereas Grau,[3] Pieterse,[1] and Milner & Carpenter[4] reported methionine to be the first limiting amino-acid in studies with both chicks and rats, Fisher[2] and Douglas & Harms[5] found the most limiting amino-acid to be lysine. Pieterse[1] also observed that lysine supplementation caused a marked growth depression when added in the absence of methionine. McOsker,[6] who studied toasted and untoasted groundnut preparations, observed that in blanched but unroasted groundnuts the amino-acids lysine, threonine and methionine were equally limiting, whereas for properly toasted groundnuts the sequence of amino-acid limitation was lysine, threonine and then methionine. The present studies were undertaken in an attempt to find possible reasons for these contradictory observations.

Experimental

White Rock or crossbred Vantress male chicks were raised from 1 day to 3 weeks on the experimental diets. They were housed in electrically heated battery brooders and food and water was available to them at all times. The composition of the experimental basal diet was as follows (in %): groundnut meal, sufficient to provide 12·0% crude protein (N × 6·25); starch, 15·0; dextrin, 5·0; mineral mix,[7] 4·9; fibre, 3·0; maize oil, 3·0; vitamins, 0·25;[7] choline chloride (70% concentrate), 0·3; glucose to 100. The levels of amino-acid supplementation were based on those used by Milner & Carpenter,[4] which were found satisfactory in an earlier study.[2]

Amino-acid analyses were carried out by ion-exchange column chromatography on all samples of groundnut meal used in these studies by means of a Phoenix amino-acid analyser with columns prepared according to the procedures of Moore and his colleagues.[8,9] No corrections were made for possible losses of amino-acids during hydrolysis.

For the first experiment, all but one groundnut meal was obtained from commercial sources, and it was known only whether they had been prepared by an expeller- or solvent-extraction-type process. The one meal with a known processing history (sample 4) was an expeller-type meal that had been cooked for 45 min. at a final temperature (maintained for about 15 min.) of 105°. According to best available information, the commercial meals had also undergone some heat treatment.[10]

The meals used in the second experiment all came from the same supply of fresh Virginia Bunch 67 groundnuts and had the following processing history: sample 4, as indicated above for the first experiment; sample 6, a batch of sample 4 meal which was additionally extracted with commercial hexane, then air-dried to remove solvent; sample 7, fresh groundnuts which were cracked, flaked, solvent-extracted *without cooking*, and then air dried.

* Paper of the Journal Series, New Jersey Agricultural Experiment Station

Results

In the first experiment (Table I) three solvent-extracted and two expeller-pressed samples of groundnut meal were compared. Chicks receiving sample 1 did not respond to lysine supplementation, showed a slight growth response to addition of methionine, but a considerable response to the combination of lysine plus methionine. Maximum nutritive value, however, was not obtained unless the sample was supplemented with threonine in addition to methionine and lysine. Since all the chicks receiving the four other meals responded to lysine supplementation (Table I), lysine appeared to be the first limiting amino-acid. Sample 2 responded by promoting markedly better growth when supplemented with the combination of lysine plus methionine; no similarly effective response was noted for samples 3 or 4. Sample 5 gave a highly significant response to lysine plus threonine supplementation, and a significant growth depression was noted when methionine was added together with lysine (in comparison with the single lysine supplementation). The triple amino-acid supplementation of sample 3 produced the least improvement in growth response of the five groundnut meals compared in this experiment.

Table I

Effect of amino-acid supplementation of different samples of groundnut meal on chick growth
(meals mixed into the diet to provide 12% of crude protein)
(G/F = gain/feed ratio)

Amino-acid supplement,*** %	Groundnut meal 1*		Groundnut meal 2*		Groundnut meal 3*		Groundnut meal 4†		Groundnut meal 5†	
	3-Week		3-Week		3-Week		3-Week		3-Week	
	Weights, g.	G/F	Weights, g.	G/F	Weights, g.	G/F	Weights, g.	G/F	Weights, g.	G/F
None	78 ± 4**	0·24	68 ± 2	0·22	69 ± 3	0·22	89 ± 5	0·27	75 ± 2	0·25
0·4 Lysine	75 ± 5	0·21	75 ± 6	0·23	78 ± 6	0·24	94 ± 7	0·29	100 ± 10	0·31
0·2 Threonine	68 ± 4	0·21	69 ± 4	0·22	68 ± 3	0·22	72 ± 3	0·24	70 ± 4	0·23
0·3 Methionine	87 ± 3	0·27	64 ± 4	0·20	73 ± 4	0·23	70 ± 3	0·23	72 ± 5	0·22
0·4 Lysine + 0·2 threonine	75 ± 5	0·21	80 ± 5	0·24	77 ± 6	0·25	88 ± 4	0·26	121 ± 14	0·33
0·4 Lysine + 0·3 methionine	96 ± 9	0·28	102 ± 11	0·33	82 ± 7	0·26	83 ± 6	0·26	87 ± 9	0·27
0·2 Threonine + 0·3 methionine	79 ± 4	0·23	68 ± 3	0·26	65 ± 1	0·23	78 ± 4	0·26	69 ± 2	0·22
0·4 Lysine + 0·2 threonine + 0·3 methionine	220 ± 11	0·45	208 ± 15	0·44	150 ± 11	0·38	184 ± 11	0·43	201 ± 16	0·48

* A solvent-extracted meal † An expeller-pressed meal
** Mean with standard error for duplicate groups of 8 White Rock males per treatment
*** Added as L-lysine hydrochloride, DL-methionine and L-threonine respectively

Table II gives the amino-acid patterns for groundnut meals 1–5; the patterns are presented as ratios of threonine, with threonine considered to be unity. The absolute values for threonine are also given in Table II, so that individual amino-acid values can be calculated. Distinct pattern differences can be noted, the most interesting being the lower cystine and methionine content of the solvent-extracted meals in comparison with the expeller-pressed meals. Another important observation was that the lysine content of all meals was lower than that reported in the literature.[10,11]

A second study was carried out with three differently prepared meals from the same supply of fresh groundnuts (see Experimental section). The growth and feed utilisation values resulting from amino-acid supplementation of these meals are presented in Table III. Of the three differently processed meals, sample 6 supported the best growth when supplemented with the three limiting amino-acids methionine, threonine, and lysine. This same meal showed a growth response to supplementation with lysine plus methionine, whereas no such response was observed with sample 4. Both samples 4 and 6 showed some response to lysine plus threonine supplementation, this being more marked than the lysine plus methionine effect just noted for sample 6. There was no response to any but the triple combination of amino-acids for sample 7, suggesting that all three amino-acids were equally limiting and had to be supplied simultaneously for proper expression of the nutritive value of this meal. It will be noted that there are some differences in the growth response for groundnut meal 4 between Expts. 1 and 2 (Tables I and III). These differences are ascribed primarily to the difference in growth potential between the breeds of chickens used: Vantress for experiment 2 versus White Rocks in experiment 1.

Table II

Amino-acid pattern (threonine = 1) of different groundnut meal samples

Amino-acid	Groundnut meal				
	1*	2*	3*	4†	5†
Aspartic acid	4·1	4·3	4·4	4·1	4·8
Serine	1·7	1·9	1·9	1·8	2·0
Glutamic acid	6·4	7·0	7·3	6·9	7·7
Proline	1·4	1·6	1·6	1·8	1·7
Glycine	2·1	2·1	2·1	2·2	2·5
Alanine	1·4	1·4	1·4	1·4	1·5
½ Cystine	0·34	0·33	0·34	0·42	0·38
Valine	1·4	1·5	1·3	1·4	1·4
Methionine	0·28	0·31	0·31	0·41	0·45
Isoleucine	1·2	1·2	1·3	1·2	1·3
Leucine	2·1	2·3	2·3	2·3	2·4
Tyrosine	1·0	1·5	1·4	1·4	1·4
Phenylalanine	1·5	1·8	1·8	1·9	1·8
Lysine	1·3	1·2	1·3	1·1	1·1
Histidine	0·76	0·82	0·91	0·72	0·83
Ammonia	0·69	0·76	0·72	0·67	0·74
Arginine	3·8	4·3	4·3	4·0	4·5
Threonine**	2·3	2·7	2·4	2·4	2·7

* A solvent-extracted meal † An expeller-pressed meal ** Absolute values in g./16 g. N

Table III

Effect of amino-acid supplementation of different samples of groundnut meal on chick growth

(G/F = Gain/feed ratio)

Amino-acid supplement,*** %	Groundnut meal 4*		Groundnut meal 6†		Groundnut meal 7**	
	3-Week		3-Week		3-Week	
	Weights, g.	G/F	Weights, g.	G/F	Weights, g.	G/F
None	63 ± 2††	0·25	72 ± 2	0·32	63 ± 2	0·24
0·4 Lysine	73 ± 4	0·27	82 ± 7	0·36	66 ± 4	0·26
0·3 Methionine	59 ± 2	0·24	68 ± 3	0·24	64 ± 3	0·24
0·4 Lysine + 0·3 methionine	62 ± 3	0·26	83 ± 7	0·36	66 ± 3	0·24
0·4 Lysine + 0·2 threonine	71 ± 6	0·26	94 ± 7	0·34	69 ± 2	0·26
0·2 Threonine + 0·3 methionine	66 ± 3	0·26	68 ± 3	0·27	67 ± 3	0·24
0·4 Lysine + 0·2 threonine + 0·3 methionine	127 ± 10	0·46	216 ± 8	0·60	138 ± 7	0·44

* Same meal as used in previous experiment; expeller-pressed (see Experimental section for details)
† Prepared by solvent-extraction of a batch of groundnut meal 4
** Raw, solvent-extracted groundnut meal prepared from the same supply as meal 4
†† Mean with standard error for duplicate groups of 8 Vantress-cross males per treatment
*** Added as in Table I

Table IV gives the content of the limiting amino-acids, lysine, threonine, and the sulphur amino-acids—cystine and methionine—for the groundnut meals tested in Expt. 2. There were differences, with sample 7 (unheated, solvent-extracted) having lower concentrations for all the limiting amino-acids than either of the other two samples; both solvent-extracted meals (samples 6 and 7) had a lower content of sulphur-amino-acids than the expeller-pressed meal (sample 4).

Discussion

The results of this study indicate that processing may have a marked effect on amino-acid availability and therefore on nutritive quality of groundnut meals. Solvent-extracted meals had a lower concentration of the sulphur-containing amino-acids, although this did not necessarily express itself in a deficiency in which the sulphur-amino-acids would become the first limiting amino-acids. All the meals used in these studies had a much lower lysine content than the average values for groundnut meal reported by others.[10, 11] The extent to which such differences

Table IV

Sulphur-amino-acid, threonine and lysine contents of differently processed groundnut meals
(results as g./16 g. N)

Amino-acid***	Groundnut meal*		
	4†	6**	7††
Threonine	2·21	2·19	2·04
½ Cystine	1·00	0·98	0·94
Methionine	0·88	0·80	0·79
Lysine	2·94	2·86	2·78

* All meals were prepared from the same supply of fresh groundnuts
† An expeller-pressed meal
** Prepared by solvent-extraction of a batch of meal 4
†† Raw, solvent-extracted from the same supply of fresh groundnuts as meal 4
*** Added as in Table I

are due to variation among types and varieties of groundnuts remains to be determined; according to Pieterse,[1] these factors may account for the different biological responses that have been reported in the literature for amino-acid supplementation.

It is of interest to note that a raw, solvent-extracted meal (sample 7, Table III) was equally limiting in lysine, methionine and threonine, an observation in close agreement with that reported by McOsker[6] in rat-feeding trials, in which a blanched but untoasted groundnut paste was used. This suggests that a certain amount of heat is necessary for optimal availability and utilisation of the amino-acids in groundnut protein. It is, however, difficult from the present results to draw definitive conclusions as to optimal processing conditions. On one hand, solvent-extracted meals were lower in the sulphur-amino-acids; on the other, solvent-extraction improved an expeller-pressed meal (sample 4 vs. sample 6, Table III). At the moment it can only be concluded that fat-extraction and cooking processes are important factors in the nutritive value of groundnut meal and they should receive further careful attention.

Acknowledgment

The author would like to express his appreciation to Dr. Leo A. Goldblatt, Southern Regional Utilization Laboratory, U.S. Dept. of Agriculture, New Orleans, Louisiana, for supplying the groundnut samples used in these studies.

This work was supported in part by a grant-in-aid from the Central Jersey Farmers Cooperative Association, Hightstown, New Jersey.

Rutgers, The State University,
New Brunswick,
New Jersey, U.S.A.

Received 21 December, 1964

References

[1] Pieterse, P. J. S., *S. Afr. J. agric. Sci.*, 1963, **6**, 541
[2] Fisher, H., *J. Sci. Fd Agric.*, 1964, **15**, 539
[3] Grau, C. R., *J. Nutr.*, 1946, **32**, 303
[4] Milner, C. K., & Carpenter, K. S., *Proc. Nutr. Soc.*, 1963, **22**, xxi
[5] Douglas, C. R., & Harms, R. H., *Poultry Sci.*, 1959, **38**, 786
[6] McOsker, D. E., *J. Nutr.*, 1962, **76**, 453
[7] Summers, J. D., & Fisher, H., *J. Nutr.*, 1961, **75**, 435
[8] Moore, S., Sparkman, D. H., & Stein, W. H., *Analyt. Chem.*, 1958, **30**, 1185
[9] Sparkman, D. H., Stein, W. H., & Moore, S., *Analyt. Chem.*, 1958, **30**, 1190
[10] Altschul, A. M., 'Processed Plant Protein Foodstuffs', 1958 (New York: Academic Press Inc.)
[11] Block, R. J., & Weiss, K. W., 'Amino Acid Handbook', 1956 (Springfield, Illinois: C. C. Thomas)

FACTORS INFLUENCING THE PYRUVIC ACID CONTENT OF WINES

by B. C. RANKINE

The pyruvic acid content of 67 Australian commercial varietal unblended wines, determined by a rapid enzymic method (details given) ranged from 1 to 128 p.p.m., mean 29 p.p.m. Dessert wines from irrigated Muscat Gordo Blanco grapes were high in pyruvic acid and red table wines which had undergone malo-lactic fermentation were low. Otherwise grape varieties (13 examined), viticultural areas (10) and vintage years (6) had no obvious influence.

Yeast strain markedly influenced the pyruvic acid content of 100 experimental wines made on a laboratory scale, and appears to be the major factor affecting the pyruvic acid content of wine. The content was usually higher at 30° than 15° although yeasts varied somewhat in this respect.

Significant interaction between grape variety and vintage year occurred with 72 experimental white table wines on the pilot plant scale. The separate effects of soil type, grape variety and vintage year were not significant.

The significance of pyruvic acid on wine quality, and particularly on binding of sulphur dioxide, is discussed.

Introduction

Besides being an important intermediate in alcoholic fermentation, pyruvic acid is of significance in wine because of its ability to bind sulphur dioxide and its likely role in the malo-lactic fermentation. The germicidal power of sulphur dioxide is largely dependent on the free content, and any compound which binds sulphur dioxide reduces its effectiveness as a germicide. Pyruvic acid is next in affinity to acetaldehyde in this regard.

Little information has hitherto been available on the pyruvic acid content of wines because of analytical difficulties, and an enzymic method of analysis was developed to provide a suitable assay procedure. Whiting & Coggins[1] determined pyruvic acid in ciders by conversion to the 2,4-dinitrophenylhydrazone and identified it by catalytic hydrogenation to α-alanine, but the method is slow and involves considerable manipulations. Blouin & Peynaud[2] used an enzymic assay to determine pyruvic acid on 10 musts and 20 white Bordeaux wines but did not give details of the assay method used.

Accordingly, the method used in this work will be described since it may be of use to other workers in this field.

Experimental

Pyruvic acid assay

Pyruvic acid was determined by reduction to lactic acid with lactic dehydrogenase with corresponding oxidation of reduced diphosphopyridine nucleotide (NADH2) at pH 7·0. The reaction is quantitative and oxidation of NADH2, as measured by reduction in optical density at 340 mμ, is proportional to the amount of pyruvic acid present.

White wines could be assayed directly in the spectrophotometer cell but red wines required preliminary clarification to reduce the high background light absorbance at 340 mμ. A Shimadzu ultra-violet spectrophotometer was used and the results agreed closely with those obtained on an Eppendorf photometer (Netheler & Hinz G.m.b.H., Hamburg). The hydrogen-discharge ultra-violet light source was necessary, as poor results were obtained with the tungsten lamp, due to stray light transmitted by the wide slit-width required. A Bausch & Lomb Spectronic 20 spectrophotometer also gave poor results.

Procedure.—A suitable aliquot of filtered white wine, usually 0·5 ml., was pipetted into a quartz 1 cm. square spectrophotometer cell and phosphate buffer pH 7·0 was added to make the volume to 3·1 ml. Then 0·1 ml. of NADH2 0·2% solution (Boehringer) was added, the contents of the cell mixed by inversion and the optical density (O.D.) at 340 mμ measured against a phosphate buffer blank. Approximately 0·01-ml. of muscle lactic dehydrogenase

(5 mg. per ml., Boehringer) was then added with a fine glass rod to the test cell and the blank, the contents mixed and the O.D. measured at 2-min. intervals until a stable reading was obtained.

The corrected O.D. reduction ($\Delta E'_{340}$) was obtained by deducting the final from the initial O.D. and correcting for the volume of enzyme suspension added and, if necessary, for endogenous activity in absence of NADH2.

A $\Delta E'_{340}$ of 0·100 corresponds to 0·0161 μmoles of NADH2 per ml. or 1·417 μg of pyruvic acid per ml.

The volumes of wine and NADH2 need to be adjusted so that the O.D. reduction is between about 0·8 and 0·4, in the most sensitive range of the instrument. Excess NADH2 is required for reduction of all the pyruvate, and this was routinely checked by adding a known amount of pyruvic acid to the cell after a stable reading was obtained, and observing a further O.D. reduction.

Red wines were clarified by pipetting 5·0 ml. into a centrifuge tube, adding 2 ml. of saturated neutral lead acetate and 0·1 ml. of ammonia solution (sp. gr. 0·88), mixing and centrifuging. To remove any excess lead, 0·1 ml. of oxalic acid 10% solution was then added, mixed with the supernatant liquid by gentle inversion without disturbing the precipitate and centrifuging. The supernatant was transferred to another centrifuge tube and the precipitate shaken thoroughly with 2·8 ml. of distilled water and centrifuged. The supernatants were combined, centrifuged if necessary and an aliquot taken for the assay.

The usual precautions for enzymic test methods were observed as described in the Boehringer pamphlet 'General remarks on the performance of enzymic test methods'. Pyruvic acid for recovery tests was twice vacuum-distilled[3] and a mean recovery of 103% was obtained for both white and red wines. Low recovery occurred if the precipitate in the clarification of red wine was not washed to extract occluded pyruvic acid.

Accuracy.—The optical density readings of duplicate analyses usually agreed to 0·01 unit, and reproducability in p.p.m. thus depended on the level of pyruvic acid. As an example the duplicate analyses of 40 white and red wines with a mean level of 19 p.p.m. agreed to within 5%.

The following compounds were tested in aqueous solution in the approximate concentrations occurring in wine, but no O.D. reduction was observed: lactic acid, succinic acid, tartaric acid, citric acid, DL-malic acid, acetaldehyde, ethanol, α-ketoglutaric acid and sulphite.

It is known that β-hydroxybutyrate and glyoxylate are reduced at a similar rate to pyruvate, while α-ketobutyrate and α-keto-n-valerate are reduced at considerably slower rates,[4] but no reference could be found to the presence of these compounds in wine.

Results

(1) *Commercial wines*

A total of 67 commercial unblended wines derived from single grape varieties were analysed, and the range of values was from 1 to 128 p.p.m. with a mean of 29 p.p.m. Details are given in Table I. Dessert wines made from Muscat Gordo Blanco grapes grown under irrigation contained more pyruvic acid than other wines examined, the mean value of 7 such wines being 64 p.p.m., but otherwise the variety of grape, viticultural area and year had no obvious influence on pyruvic acid content. A direct comparison between dry white and dry red table wines was not possible because of the influence of malo-lactic fermentation on the pyruvic acid content of the latter, as shown later.

(2) *Experimental wines*

A total of 72 varietal white table wines were made under identical conditions in 30-gal. lots in the experimental winery of the Wine Research Institute from grapes grown on soils of known type. The pyruvic acid content of the wines is shown in Table II.

The range of values is from 8 to 34 p.p.m., mean 20 p.p.m. Interaction between grape variety and year was highly significant due to lower values for Semillon in 1962 compared with 1963 and 1964. Differences due to variety and soil and between years were not significant.

Table I

Pyruvic acid content (p.p.m.) of 67 commercial varietal wines in relation to grape variety, wine type, district and year

	No. of wines	Range of values	Mean
Grape variety			
Blanquette	1	44	—
Cabernet Sauvignon	3	9–41	19
Clare Riesling	1	18	—
Frontignac	4	4–128	40
Grenache	1	28	—
Muscat Gordo	7	9–108	64
Palomino	3	23–68	50
Pedro	8	5–77	39
Riesling	6	14–51	24
Semillon	9	14–31	25
Shiraz	18	1–36	14
Verdelho	4	24–33	28
White Hermitage	2	13, 40	27
Wine type			
Dry White	29	4–51	28
Dry Red	22	1–41	15
Dessert	10	9–128	71
Sherry (flor)	4	7–68	31
Champagne	2	26, 33	30
District			
Adelaide, South Australia	1	20	—
Barossa, South Australia	5	5–25	16
Clare, South Australia	5	18–33	26
Coonawarra, South Australia	5	1–9	4
Eden Valley, South Australia	6	5–51	19
River Murray, South Australia	11	9–128	71
Southern Vales, South Australia	18	2–77	29
Goulburn Valley, Victoria	3	4–13	9
Rutherglen, Victoria	2	1, 9	5
Hunter Valley, N.S.W.	11	10–44	29
Year*			
1964	35	4–128	33
1963	18	1–44	22
1962	2	3, 27	15
1961	1	1	—
1960	3	3–51	23
1959	2	3, 10	7
Overall result	67	1–128	29

* Four sherries and two dessert wines not included as blends of more than one year

Table II

Influence of grape variety, district and soil type on pyruvic acid content (p.p.m.) of experimental white table wines
(mean of triplicates)

Grape variety	Locality	Soil type	Year 1962	1963	1964
Riesling	Barossa	Red Brown Earth (R.B.E.)	23	19	18
		Solodized Solonetz (S.S.)	18	14	18
Riesling	Eden Valley	Grey Brown Podzolic (G.B.P.)	16	21	21
		Yellow Brown Podzolic (Y.B.P.)	16	18	25
Semillon	Barossa	R.B.E.	12	28	32
		S.S.	9	21	21
Semillon	Eden Valley	G.B.P.	10	18	18
Semillon	River Murray	Solodized Brown	8	33	34
		Mean	14	22	23
		Overall mean		20	

Least significant difference ($P < 0.01$) for comparison of means in body of Table = 8

(3) *Influence of malo-lactic fermentation*

The pyruvic acid content of 59 commercial and experimental dry red wines was examined in relation to the occurrence of malo-lactic fermentation in these wines. A total of 38 wines had undergone the fermentation, as determined by paper chromatography, and the mean pyruvic acid content was 14 p.p.m., compared with a mean of 36 p.p.m. for the 21 wines which had not undergone the fermentation.

These results show that malo-lactic fermentation reduces the pyruvic acid content of the wines. For this reason no red wines were included in Table II.

(4) *Influence of yeast strain*

Experimental wines (100) were made in the laboratory with 25 selected pure-culture yeasts in duplicate at two temperatures, using 250-ml. lots of filter-sterilised grape juice of the Doradillo variety 1964 vintage contained in 26 fl. oz. bottles. The containers were closed with lead acetate indicator tubes to measure hydrogen sulphide as part of another investigation.

The fermentations were allowed to proceed at 15° and 30° (±1°) until loss of weight measured at three-day intervals was constant. The wines were then maintained at fermentation temperature until analysed, and the results treated statistically. Examples of the results showing the range of values found are presented in Table III.

The pyruvic acid content of the wines made on laboratory scale was higher than the commercial wines and the experimental wines made on pilot winery scale, but it is apparent that it was influenced considerably by the yeast strain. Values for the 25 yeasts ranged from 51 to 190 p.p.m. and larger amounts were usually formed at 30° than at 15°.

The quantity of ethanol produced is included in the Table to indicate how yeasts differed in this regard, although no relationship was apparent between pyruvic acid and ethanol production.

Discussion

Whilst the range of commercial wines examined is not a complete cross-section of Australian wines, it should be sufficient to give a reliable indication of the general level of pyruvic acid in such wines. Collection of wines made from a single variety is difficult owing to the widespread practice of blending. The smaller quantity of pyruvic acid present in dry red wines which had undergone malo-lactic fermentation indicates that pyruvic acid is partially metabolised by the bacteria concerned. This aspect is being studied in more detail and will be reported separately. Results recently obtained by J. C. M. Fornachon showed that addition of up to 100 p.p.m. of pyruvic acid to three white table wines produced a more rapid malo-lactic fermentation.

Table III

Influence of yeast strain on pyruvic acid (p.p.m.) and ethanol (% v/v) content of wines fermented at 15° and 30° on laboratory scale

(means of duplicates)

	15°		30°	
Yeast strain	Pyruvic acid	Ethanol	Pyruvic acid	Ethanol
161	90	14·9	135	14·0
183	160	14·5	98	11·4
278	65	10·1	62	12·7
286	99	13·3	146	12·8
350	133	14·7	179	13·9
702	76	9·0	49	10·2
723	84	16·0	190	14·3
724	106	15·7	193	13·7
Mean of 24 yeasts	111	13·7	140	12·9

Significant differences ($P < 0.01$) between means in body of Table: pyruvic acid 36 p.p.m., ethanol 1·0% v/v

The pyruvic acid content of two wines before and after champagnisation by bottle fermentation showed increases of 22 to 26 and 25 to 33 p.p.m. respectively, indicating that the yeast produced, and not consumed, pyruvic acid during the secondary yeast fermentation.

Blouin & Peynaud[2] found values of 20–70 p.p.m. for 20 white Bordeaux wines, which is consistent with the results obtained in this work.

The higher pyruvic acid levels in laboratory wines as compared with experimental winery and commercial wines is due to the conditions of fermentation. The laboratory wines were made from grape juice thoroughly aerated by preclarification and sterile filtration and fermented in small containers with a large surface area-to-volume ratio. Pyruvic acid has been shown to be higher in fermentations under partially aerobic conditions[6] and aeration similarly increases the production of the related carbonyls acetoin and diacetyl.[5]

Whiting & Coggins[1] found that the pyruvic acid content of cider from 6 varieties of apples was between a trace to 130 p.p.m. for non-sulphited fermentation and 80 to 640 p.p.m. for sulphited fermentation. The content was higher during fermentation than at 'dryness'. Deibner[7] has reviewed what published results are available on the pyruvic acid content of wines and the results quoted are consistent with the present findings.

The amount of sulphur dioxide bound to pyruvic acid is in equilibrium with the free pyruvic acid and free sulphur dioxide[8] according to the dissociation constant k of pyruvate sulphonic acid $\frac{[\text{Free Pyruvate}]\,[\text{Free } SO_2]}{[SO_2 \text{ bound to pyruvate}]} = 4{\cdot}0 \times 10^{-4}$. The recommended free sulphur dioxide content in Australian wines depends on pH and ethanol content and varies from about 10 to 50 p.p.m. It is usually low in dry red table wines to encourage malo-lactic fermentation, and 30 p.p.m. is recommended for white table wines with pH values below about 3·6.

At 30 p.p.m. of free sulphur dioxide, 53% of the pyruvic acid is bound to sulphur dioxide and for the range of pyruvic acid in wines (1–128 p.p.m.), this corresponds to 0–49 p.p.m. of sulphur dioxide bound to pyruvic acid. Wines with 29 p.p.m. of pyruvic acid (the mean value) would have 11 p.p.m. of sulphur dioxide bound to pyruvic acid.

Since pyruvic acid is in equilibrium with the system it can bind more sulphur dioxide if this is added, in contrast to acetaldehyde which is almost completely bound whenever free sulphur dioxide is present. Burroughs & Whiting[8] found that all ciders which had a high pyruvic acid content also had a high sulphur dioxide-binding power. The same applies to wines and this will be reported separately.

Acknowledgments

The author is indebted to Prof. M. R. Atkinson of the Waite Agricultural Research Institute for advice and assistance in developing the enzymic method of analysis, and to Mrs. M. J. Wilson for competent technical assistance. He is also indebted to Mr. J. C. M. Fornachon, Director of the Australian Wine Research Institute, for constructive criticism during the course of the investigation. Thanks are due to Penfolds Wines Pty. Ltd., Thomas Hardy & Sons Pty. Ltd. and to Walter Reynell & Sons Ltd. for supplying most of the commercial wines.

Australian Wine Research Institute,
Adelaide,
South Australia

Received 23 November, 1964; amended manuscript 12 January, 1965

References

1 Whiting, G. C., & Coggins, R. A., *J. Sci. Fd Agric.*, 1960, **11**, 705
2 Blouin, J., & Peynaud, E., *C.R. Acad. Sci. Paris*, 1963, **256**, 4521
3 Horecker, B. L., & Kornberg, A., *J. biol. Chem.*, 1948, **175**, 385
4 Bergmeyer, H. U., 'Methods of Enzymatic Analysis', 1963 (London: Academic Press)
5 Crowell, E. A., & Guymon, J. F., *Amer. J. Enol. Vitic.*, 1963, **14**, 214
6 Wang, C. H., Labbe, R. F., Christensen, B. E., & Cheldelin, V. H., *J. biol. Chem.*, 1952, **197**, 645
7 Deibner, L., *Ann. Technol. agric.*, 1963, **12**, 229
8 Burroughs, L. F., & Whiting, G. C., *Annu. Rep. Long Ashton, Bristol, for* 1960, p. 144

THE REACTION OF WHEAT PROTEINS WITH SULPHITE
I.—Sulphitolysis of the proteins of flour with cuprammonium sulphite

By E. E. McDERMOTT and J. PACE

The reaction of wheaten flour and of some flour protein fractions, with cuprammonium sulphite solutions at pH 10·2 has been investigated. Under these conditions, with a reaction time of 2–4 h. at room temperature, the thiol and disulphide groups of the flour proteins are completely converted to *S*-sulphocysteine residues and the proteins dissolve, together with some carbohydrate. The insoluble residue is a high quality starch. The effect of the sulphitolysis procedure on the amide groups of the proteins, in flours of widely different protein content, has been examined and there was no evidence that deamidation had occurred. Similarly the amino-acid composition of a flour protein fraction remained unchanged by the sulphitolysis procedure. In these respects the specificity of the reaction confirmed the original observations of Swan with keratin, but evidence obtained by prolonging the reaction time suggests that with flour proteins, some disaggregation or cleavage of their structure, other than that due to the splitting of disulphide bonds, may also occur and this possibility is being investigated in further work. Some general properties of the solubilised material are described.

Introduction

It was shown by Clarke[1] that cystine (CySSCy) reacts with sulphite to give an equilibrium mixture of cysteine (CySH) and *S*-sulphocysteine ($CySSO_3H$):—

$$CySSCy + SO_3^{2-} \rightleftharpoons CyS^- + CySSO_3^- \quad . \quad . \quad . \quad . \quad (1)$$

Kolthoff & Stricks[2] subsequently established that cysteine reacts with cupric ions and sulphite in ammoniacal solution to give *S*-sulphocysteine:—

$$CyS^- + 2\,Cu^{2+} + SO_3^{2-} \rightarrow CySSO_3^- + 2\,Cu^+ \quad . \quad . \quad . \quad (2)$$

Thus by the use of cuprammonium sulphite both cystine and cysteine may be quantitatively converted to *S*-sulphocysteine—the overall reaction for cystine being:—

$$CySSCy + 2\,Cu^{2+} + 2\,SO_3^{2+} \rightarrow 2RSSO_3^- + 2\,Cu^+ \quad . \quad . \quad . \quad (3)$$

Swan[3,4] was the first to examine the application of these reactions to the thiol and disulphide groups in proteins, by studying the interaction of cuprammonium sulphite with keratins. He found that the cystine disulphide bonds were broken symmetrically, according to Equation (3), that the reaction went to completion and that with the reaction media at pH values of 9–10·5 a large proportion of keratin became soluble in the form of *S*-sulpho-keratin. Thus, in a typical instance, 61% of a sample of wool was dissolved in 24 h. at 22°. Swan also provided evidence that amino-acids other than cystine and cysteine were unaffected by the ammoniacal cupric sulphite reagent. Swan's procedure thus provided a method for the disruption of disulphide bonds in proteins, which appeared to be specific and which, under appropriate conditions, could lead to the solubilisation of a protein largely insoluble in its native form.

Swan's method, with a number of variations in the detailed procedure, has been applied by other workers to other proteins. Pechere *et al.*[5,6] prepared the *S*-sulpho derivatives of trypsinogen and α-chymotrypsinogen. The reaction was carried out (for 1 h. at room temperature, in solution containing sulphite and urea, brought to, and maintained at, pH 10·2 with ammonia), by the step-wise addition of a solution of copper nitrate. It was shown that the cystinyl groups in the proteins were quantitatively converted to the corresponding *S*-sulpho groups. The *S*-sulphoproteins were soluble at pH values above 7·0 but lowering the pH and increasing the ionic strength induced precipitation. Henschen[7] prepared *S*-sulpho-fibrinogen and fibrin by reaction of the proteins, for 1 h. at room temperature, in 8 M-urea at pH 9·0 with sodium sulphite and cupric sulphate. Both the *S*-sulphoproteins were soluble, in low concentrations, in water but aggregated when the pH was lowered and the ionic strength increased.

Henschen showed that the reaction went to completion and obtained no evidence that other amino-acid residues were affected or that peptide bonds were broken during the sulphitolysis. Clegg & Bailey[8] used the procedure of Pechere *et al*[5] to cleave the disulphide bridges linking the three peptide chains of fibrin and also prepared *S*-sulpho-fibrinogen. The *S*-sulphoproteins were soluble at pH values greater than 7·0 but were strongly aggregated. Weil & Seibles[9] prepared *S*-sulpho-α-lactalbumin and-β-lactoglobulin by reaction at pH 9·0 for 2 h. at room temperature in the absence of urea. Both *S*-sulphoproteins were soluble in water at pH values greater than 6·0.

One of the main difficulties in studying the protein complex of wheat endosperm is the intractable nature of the main protein components in relation to solubility. It was therefore of interest to apply Swan's procedure to flour, to examine whether it would solubilise the proteins and, if this occurred, to investigate how the process modified the native proteins. Studies of the modification of disulphide and thiol groups of flour proteins have also a further interest arising from the primary importance of these groups in relation to the rheological behaviour of dough (cf. Frater *et al.*[10]). In preliminary work in this laboratory[11] it was found that a procedure similar to that used by Swan with keratin could be used to solubilise the protein of wheaten flour. The present paper gives details of this work and describes some of the properties of the solubilised material. Further work is continuing on the sulphitolysis of wheat proteins under a variety of experimental conditions and on the fractionation of the products. These observations will be published in subsequent papers.

Experimental

Materials and methods

(1) *Flour*

Flour was milled in the laboratory on a Buhler mill. The rate of extraction varied, with the type and variety of wheat, from 65% to 70%.

(2) *Gliadin*

Flour from Manitoba wheat was extracted at room temperature with light petroleum (b.p. 40–60°), and the extracted flour used to prepare gluten in the usual way by washing out the starch. Wet gluten, 50 g., was immediately extracted at room temperature first with 250 ml. of 68% (v/v) ethanol/water mixture and then twice with 250 ml. of 60% (v/v) ethanol/water. The extracts were combined, centrifuged and then filtered through Whatman No. 5 Filter paper using Celite filter aid. The clear extract was concentrated under reduced pressure and then held at 3° overnight. A white syrupy precipitate formed. The supernatant liquid was decanted off and discarded, and the precipitate taken up in 60% (v/v) ethanol/water. This solution was dialysed against 60% ethanol/water over three days with several changes of the outer liquid. A small amount of material in the extract precipitated out during dialysis. This was removed by centrifugation and the extract concentrated again by evaporation of solvent under reduced pressure. The concentrated extract was held at 3° and the precipitate which formed was separated off and dried with ethanol and ethyl ether. The preparation had an ash content of 0·04% and a nitrogen content of 17·62% (reckoned on a moisture- and ash-free basis).

(3) *Acetic acid extract of flour*

Flour (200 g.) was extracted with water-saturated butanol (600 ml.), centrifuged and then extracted again with water-saturated butanol (300 ml.). The residue was washed successively with absolute butanol, absolute ethanol and ethyl ether, and then air dried. The air dried residue was extracted three times with 1% acetic acid (600 ml., 300 ml., 300 ml.). The extracts were centrifuged, and the combined supernatant extracts filtered through glass wool and dialysed against several changes of distilled water at 0°. After dialysis the extract was freeze dried. In a typical example 200 g. of Manitoba flour after the solvent extraction gave 185 g. of residue

containing 2·38% of N at a moisture content of 11·8%. After the acetic acid extraction the residue contained 0·83% of N at a moisture content of 12·4%. Thus the acetic acid extracted about 65% of the flour protein.

(4) *Cuprammonium solution and its application to flour or flour protein*

This was essentially the same as used by Swan.[3] Copper sulphate pentahydrate/(0·625 g.) was dissolved in 25 ml. of distilled water and concentrated ammonia was added dropwise to this solution until the precipitate of hydroxide just dissolved to give a clear deep blue solution. Sodium sulphite heptahydrate (1·575 g.) was dissolved in distilled water (25 ml.) and added to the cuprammonium solution and the volume brought to 63 ml. with distilled water. Flour (10 g.), previously defatted by extraction with light petroleum (40–60°) or flour protein fraction (1 g.) was suspended in 0·1 M-aqueous ammonia (62 mls.), and the mixed cuprammonium sulphite reagent added. The pH of the mixture was 10·1–10·2.

(5) *Amide nitrogen*

Analytical procedures for the determination of amide nitrogen have been critically examined by Leach & Parkhill.[12] Conditions used for amino-acid analyses (6 N-hydrochloric acid at 100°) give high figures, even after correction for ammonia produced by deamination of serine and threonine. Leach & Parkhill found that 12 N-hydrochloric aicd at 37° produces no secondary breakdown but this is a time-consuming process—about 10 days may be required for the hydrolysis period. They found, however, that using 2 N-hydrochloric acid at about 100° was a rapid and convenient procedure and gave values closely similar to those obtained by the lengthier method.

The rate of release of amide nitrogen during hydrolysis differs with different proteins. In preliminary experiments the rate of release of amide-N from the proteins in flour was studied by hydrolysing flour for different periods of time according to the method of Leach & Parkhill, i.e. with 2 N-hydrochloric acid at 100°. It was found that hydrolysis for 2, 4 or 6 h. gave essentially similar results. Accordingly, a standard procedure of hydrolysing with 2 N-hydrochloric acid for 2 h. at 100° was adopted.

The hydrolysate (1 g. flour with 10 ml. of hydrochloric acid) was immediately cooled down, the pH cautiously adjusted to about 6·0–6·5 with 2 M-sodium hydroxide, made up to volume and a suitable aliquot transferred to a micro-Kjeldahl apparatus. It was then brought to pH 9·4 by the addition of borate buffer and the ammonia immediately steam-distilled into 1% boric acid and titrated with 0·01 N-hydrochloric acid.

(6) *Amino-acids*

Material to be analysed was hydrolysed with 6 N-hydrochloric acid, under reflux, for 24 h. Amino-acids were determined on the hydrolysates by ion-exchange chromatography using the methods of Moore & Stein[13] with operating conditions described previously.[14]

(7) *Carbohydrate* was determined by the orcinol method of Synge & Wood.[15]

(8) *Copper* was determined by the recommended method of the Analytical Methods Committee.[16]

(9) *Disulphide and thiol groups*

These were measured polarographically by the method of Leach,[17] using methyl mercuric iodide as the titrating agent.

Reaction of flour with the cuprammonium sulphite reagent

The mixture of flour and cuprammonium sulphite reagent was kept in a stoppered 250 ml. flask at room temperature for 3–4 h. and was shaken occasionally by hand. It was then centrifuged and the residue washed twice with 0·1 M-aqueous ammonia and separated each time by centrifuging. The extract and the supernatant from the ammonia washings were combined.

The clear deep blue solution so obtained was dialysed at 0–1° against frequent changes of distilled water to remove excess reagents. The solution remained blue, and some copper was bound by the dissolved protein to form a copper protein complex. Removal of this complexed copper by dialysis against solutions of other complexing agents such as ammoniacal EDTA was tried, but was found to require unduly prolonged periods of dialysis. Dialysis against dilute acid released the copper from the complex and was quicker and more efficient than the EDTA procedure. Accordingly the solution, after the preliminary dialysis against water, was dialysed overnight at 1° against 0·05 N-hydrochloric acid and then against several changes of distilled water. The dialysed extract was then freeze dried. In more recent work it has been found that the complexed copper may be removed by passing a solution of the copper–protein complex in ammoniacal EDTA over a column of Sephadex 50, but the present paper deals with material isolated by the dialysis procedure given above.

The residue of starch from the extraction process was washed with distilled water, acetic acid (0·1 M) and again with water. It was then dried either by washing with acetone or in a vacuum oven at 40°.

Results

(1) *Some general properties of the extracted material*

The material extracted from flour is first obtained, after dialysis to remove reagents, as a clear blue-purple solution. This contains virtually all the protein of the flour together with solubilised pentosans and complexed copper. The degree of extraction of nitrogen, from flours from a number of different wheat varieties is shown in Table I. The nitrogen contents were measured on the blue solution of the copper complex after prolonged dialysis, and on the dried residue from the extraction process.

Since the extraction process solubilises pentosan and hemicellulose material as well as protein, the residue is a high quality starch. This has been confirmed by Adkins,[18] who found that the method gave slightly higher yields of starch as compared with the conventional dough-wash material and the starch had pasting qualities which were superior to those of wheat starch prepared in the normal way.

The content of copper in the soluble complex is of the same order in extracts prepared from flours of different character and content of protein. After freeze-drying the solution the copper content of the dried material, on a dry weight basis, is about 4·0%. Thus the dried complex from a Manitoba flour had a copper content of 4·0% and that from a weak English flour, wheat variety Jufy, a content of 4·2%.

Solubilised material after removal of bound copper

The nitrogen contents of a representative series of preparations after removal of copper and then freeze-drying are given in Table II. These preparations were extracted from: (*a*) different flours, (*b*) the material extracted from flour by 1% acetic acid and then freeze-dried.

The carbohydrate content of the preparations from flour, expressed in terms of glucose equivalent, was 16–19%. The acetic acid extract from flour (6 in Table II) was freeze-dried, and the dried material was found to be completely soluble in the cupric-ammonium-sulphite reagent. The data of Table II show that the different types of flour behave similarly in the extraction and isolation process, yielding material which is similar in protein content.

Table I

Extraction of protein from flour by cuprammonium sulphite at pH 10·2

Wheat variety	N of extracted material, as % of original flour N	N content of extracted material, %	N content of residue, %
Manitoba (a)	95	13·4	0·03
Manitoba (b)	95	13·3	0·05
Hybrid 46	96	13·2	0·04
Bersee	94	13·6	0·06
Svenno	97	13·6	0·03

Table II

Nitrogen content of solubilised material after freeze-drying

Starting material	Nitrogen content, % dry weight basis
1. *Flour*, Manitoba, a	14·5
2. *Flour*, Manitoba, b	14·8
3. *Flour*, Goby	14·6
4. *Flour*, Roter Löwe	14·8
5. *Flour*, Svenno	14·5
6. *Acetic acid* (1%) *extract* of Manitoba flour	14·9

Extent of cleavage of disulphide bonds

The material isolated from flour was examined for its content of disulphide and thiol groups, using the polarographic methods of Leach.[17] No disulphide or thiol groups were found showing that the sulphitolysis reaction had gone to completion, and indicating that all the cystinyl residues in the native proteins had been converted to the *S*-sulpho derivative.

Solubility of the extracted material in relation to pH

(*a*) *Extracted material before removal of bound copper.*—The freeze-dried extract, containing the complexed copper, is dissolved by the addition of dilute ammonia to give a clear purplish-blue solution at pH 7·5–8·0. As the pH is lowered precipitation begins. This is illustrated by the data given in Table III.

Table III

Precipitation in relation to pH (extracted flour from Svenno wheat)

pH of precipitation*	Protein and carbohydrate (as % of that originally present in solution at pH 7·5)	
	Protein	Carbohydrate
4·0	14	8
5·0	17	8
6·0	44	22
7·0	28	18

* Precipitation at pH 4·0 and 5·0 in acetate buffer (0·01 M) and at pH 6·0 and 7·0 in phosphate buffer (0·01 M).

(*b*) *Extracted material after removal of bound copper.*—The freeze-dried extract, from which the bound copper has been removed, dissolves above pH 7·0. The material begins to precipitate out of solution at pH 7·0 and maximum precipitation occurs at about pH 4·0 where about 50% of the protein and 10% of the carbohydrate comes out of solution.

The precipitation behaviour at pH values from 2·2 to 7·0 is shown in Table IV, which includes values obtained with two extracts. One was from flour from a high-protein wheat, Svenno (N = 2·45%) and the other from flour from a low-protein wheat, Arletta (N = 1·15%).

Table IV

Precipitation in relation to pH

Protein and carbohydrate precipitated (% of that originally present in solution)

pH of precipitation	Svenno		Arletta	
	Protein	Carbohydrate	Protein	Carbohydrate
2·2	3·2	3·9	24·5	11·7
3·0	41·9	7·6	36·3	13·6
4·0	54·2	8·7	45·6	12·3
5·0	46·6	6·8	37·8	12·6
6·0	37·8	6·5	25·7	9·0
7·0	24·0	4·5	13·9	5·6

(Precipitation in citrate–HCl buffer, 0·01 M)

This solubility behaviour of the material isolated from flour in relation to pH is similar to that observed by Pechere *et al.*[5] with *S*-sulpho-trypsinogen and -chymotrypsinogen and by Clegg & Bailey[8] with *S*-sulpho-fibrinogen in that they found that the *S*-sulphoproteins they studied were only completely soluble at pH values greater than 7·0.

The results of Table IV also show that although there is some fractionation of protein from carbohydrate as the pH is lowered there is only a partial separation. There is clearly a strong association between the aggregated protein and the solubilised carbohydrate even in the pH region, 4·0–5·0, where the precipitate contains the highest proportion of protein. While some of the associated carbohydrate may be in the form of true glycoprotein, which Kündig *et al.*[19] have found in flour, most of it is not in such covalent linkage with the protein.

(2) *Effect of the solubilising process on the amino-acid residues of the protein*

Swan,[3,4] using amino-acids and a number of peptides, found that the cuprammonium sulphite reagent did not modify any amino-acids other than cystine and cysteine. Henschen[7] stated that the amino-acid composition of *S*-sulpho-fibrinogen and -fibrin 'seemed to be the same as those of the native proteins'. Weil & Seibles[9] found that in converting α-lactalbumin and β-lactoglobulin to the *S*-sulpho derivatives the tryptophan, tyrosine and phenylalanine contents were not affected. Thus there is substantial evidence for the specificity of the reaction. It cannot, however, be assumed that this necessarily would hold for other proteins. This *caveat* would appear to apply particularly to the flour protein complex with its 'exceptional' amino-acid composition having an unusual and characteristic high content of amide groups. Thus cupric ions might be complexed with the amide groups and catalyse their hydrolysis. It has been shown by Holme & Briggs[20] that partial deamidation of gliadin changes the solubility behaviour and the modified protein becomes soluble at pH 7·0.

The effect of the solubilising reagent on the amide groups and on the amino acid residues of flour proteins was therefore examined.

Amide nitrogen

Several groups of investigators[21–23] have determined amide nitrogen in protein fractions from flour,[22] but information in the literature on the content of amide nitrogen in a range of flours, as distinct from protein fraction derived from them, is sparse. This was therefore determined in a series of flours of different protein content, and the results are given in Table V.

There is a tendency for the flours with a protein content greater than about 13% to have a higher proportion of amide nitrogen in the protein than is found in the protein of the flours in the lower protein range of 7–10%. This may be explained by the observation of Pence *et al.*[22] that the proportion of 'soluble' (in neutral aqueous solution) protein in the total protein of flour increases as the protein content of the flour decreases. Since the soluble protein fraction

Table V

Content of amide N in flour

Wheat variety	Protein content of flour (% dry wt. basis) N × 5·7	Amide N (as % of total N)
Ayr Challenge, Red Soft	7·6	18·0
Cappelle Desprez (a), Red Soft	9·4	18·5
Cappelle Desprez (b), Red Soft	9·4	18·8
Prestige, Red Hard	9·5	19·0
Heines 65, Red Soft	9·7	18·2
Gaby, Red Soft	11·4	19·7
Elite Lepeuple, Red Hard	11·7	18·8
Roter Löwe, Red Hard	13·0	20·0
Atle, Red Hard	14·1	20·0
TB 93/26/24, Red Soft	14·1	20·0
TB 93/8/6, Red Soft	14·6	20·7
Svenno, Red Hard	14·7	20·9
Manitoba	15·4	21·0

contains significantly less amide nitrogen than the insoluble fraction the tendency to be observed in Table V, in the ratio amide N/total N, is consistent with the findings of Pence *et al.*[22] on the inverse correlation of the content of soluble protein in the protein with the total protein content of the flour.

Effect of the extraction process on the amide groups of the protein

Results obtained for several flours, and for the material extracted from them by the cupric-ammonium sulphite reagent, are given in Table VI.

Table VI

Content of amide N in S-sulpho-protein extracted from flour compared with that of parent flour

Wheat variety	Amide N as % of total N	
	Flour	Extracted protein
Roter Löwe	20·0	20·9
Svenno	20·9	21·9
TB 93/8/6	20·7	21·0
Gaby	19·7	20·9
Manitoba	21·2	21·7

These results provide no evidence that deamidation occurs during the extraction and isolation of the protein by the cupric-ammonium sulphite reagent. If deamidation has occurred then it has been masked by binding of ammonia by the modified protein. The ratio of amide N/total N is in fact slightly higher for the extracted protein than for the parent flours. But this may be because the total N of the flour includes non-protein N which is removed by dialysis in the isolation procedure.

The extraction process has also been used with acetic acid (1%) extracts of flour, after these have been freeze-dried, as the starting material. Amide nitrogen determinations have been made on the material before extraction with the cupric-ammonium sulphite reagent, on the copper complex as extracted and freeze-dried, and on the extracted material after removal of copper from the complex. Results obtained with an acetic acid extract from Manitoba flour are given in Table VII.

Table VII

Content of amide N in acetic acid soluble protein and its S-sulpho derivatives

Freeze-dried material	N content (%) of material as used (*not* on a dry wt. basis)	Amide N/ total N, %	Amide N, mg. per 100 mg. of protein
Acetic acid (1%), Extract (A)	13·96	22·9	3·2
Material extracted from (A) in form of copper complex (B)	13·34	23·8	3·2
Material after removal of copper from B	15·0	23·4	3·5

These results also give no indication of deamidation either during the extraction process or during the removal of copper. There is again, as with the extractions from flour, a slight increase in the amide N/total N ratio in the extracted material as compared with the starting material.

Effect of the extraction process on amino-acid composition

As is well-known, hydrolysis of protein in the presence of a large excess of carbohydrate, as in the case of flour, leads to the formation of relatively large quantities of humin and to some destruction of susceptible amino-acids. For this reason, it was considered preferable to examine the effect of the cupric-ammonium sulphite reagent on the amino-acid composition of extracts of flour which are predominantly protein. Experiments have therefore been carried out with acetic acid (1%) extracts of flour as the starting material. When this is solubilised with the

reagent the material subsequently isolated has approximately the same carbohydrate content as the initial material. The hydrolyses are therefore carried out with initial material and final material of about the same (gross) composition with respect to protein and carbohydrate.

The amino-acid composition of an acetic acid (1%) extract of flour and of the material obtained from it (after solubilisation with the reagent, isolation and freeze-drying) are given in Table VIII. The results for both materials are expressed as amino-acid N as % of total N. The total N refers to the N content of the solid material before hydrolysis. The results are the mean of analyses in triplicate.

Table VIII

Content of amino-acids

(Amino-acid N as % of total N)

Amino-acid	Acetic acid (1%) extract of flour (A)	Material isolated after treatment of (A) with solubilising reagent	Amino-acid	Acetic acid (1%) extract of flour (A)	Material isolated after treatment of (A) with solubilising reagent
Aspartic acid	1·93	1·90	Isoleucine	2·71	2·78
Threonine	1·74	1·73	Leucine	4·58	4·92
Serine	3·71	3·79	Tyrosine	1·45	1·47
Glutamic acid	22·67	22·91	Phenylalanine	2·98	3·09
Proline	10·80	11·10	Lysine	1·29	1·23
Glycine	3·02	3·02	Histidine	3·39	3·40
Alanine	2·25	2·27	Arginine	5·49	5·64
Valine	3·02	3·09	Ammonia	22·10	21·24
Methionine	0·98	0·99			

From these results it will be seen that the overall amino-acid composition of the protein is substantially the same after the solubilising and isolation procedure. No new or unusual peaks were observed on the ion-exchange chromatograms and there was no evidence that amino-acid residues, other than cystine or cysteine, were modified by the procedure. Thus in this respect the results are in accord with the observations of Swan and of Henschen.

The data presented above would not, however, indicate unequivocally whether or not the sulphitolysis procedure used had induced other changes in the native state of the proteins, such as disaggregation due to causes other than the splitting of disulphide bonds including, for example, the disruption of some peptide bonds. This possibility is difficult to establish with flour proteins, or fractions of flour proteins such as gliadin, since these are highly heterogeneous mixtures. Because of this heterogeneity, the determination of terminal amino acids in the peptide chains, before and after the sulphitolysis procedure, is unlikely to provide conclusive evidence. If, in fact, such other types of change are proceeding, they might however be indicated by changes in the properties of the isolated material as the reaction time of the sulphitolysis procedure is prolonged.

Some preliminary evidence on this question has been obtained by carrying out the sulphitolysis procedure with gliadin for much longer times than are required for the complete conversion of the disulphide groups to the *S*-sulpho derivative.

(3) *Effect of a prolonged reaction time on gliadin*

In this experiment 2 g. of gliadin were reacted with 200 ml. of the reagent solution. At known time intervals 20-ml. samples were removed and immediately dialysed against a large excess of water. After removal of the complexed copper by dialysis against dilute acid the protein was redissolved by addition of dilute NaOH to bring the pH to 7·5–8·0. The solution was made up to volume and its content of nitrogen determined. Results obtained are given in Table IX.

These results suggest that prolonged interaction with the reagent leads either to deamidation or to disruption or disaggregation of the gliadin complex into units of lower molecular weight, some of which are then lost by diffusion through the dialysis sack. Other evidence that the

state of the protein is radically changed, by prolonged interaction with the reagent, was obtained by examining, with each of the solutions obtained at the different reaction times, the proportion of the protein which was precipitated when the solution was brought to pH 4·0. These observations are given in Table X.

It is apparent that reaction times longer than 20 h. produce modifications in the protein complex which are dramatically reflected in changes in the solubility behaviour at pH 4·0. Whether the modification is due to deamidation or to some other disaggregating effect is at present uncertain, and this is being investigated.

Table IX

Effect of time on N recovered from gliadin treated with the cuprammonium sulphite reagent

Reaction time (h.)	1·5	20	65	97	168	240	336
N recovered (as % of original gliadin N)	90	97	92	87	80	77	62

Table X

Reaction time (of gliadin with reagent), h.	Precipitation behaviour at pH 4·0 of the protein obtained at the different reaction times (based on N determination)	
	% precipitated at pH 4·0	% soluble at pH 4·0
1·5	75	25
20	76	24
65	62	38
97	48	52
168	20	80
240	12	88
336	9	91

(Precipitation at pH 4·0 with acetate buffer. Precipitate centrifuged off at 15000*g*, washed, centrifuged and dissolved in dilute NaOH for N determination. Supernatant and washings combined for determination of soluble N.)

Discussion

The observations described in this paper show that when cuprammonium sulphite solutions are reacted with flour, under conditions similar to those used by Swan[3,4] with keratin, the proteins of the flour are dissolved. Cleavage of the disulphide bonds in the flour proteins is complete and the results also confirm Swan's observation that other amino acid residues in the protein remain unmodified by the procedure. In this sense the reaction with flour proteins is highly specific, as Swan and other workers have shown with a number of different proteins. But the results obtained with gliadin, using long reaction times, show that with this protein complex from flour, other disaggregating reactions, including the possibility of some splitting of peptide bonds, may occur with cuprammonium sulphite at pH 10·2. Whether or not this occurs to some degree during the short reaction times used in the present work is uncertain but it remains a possibility and this is currently being investigated. This possibility also raises the question of whether the conversion of the flour proteins to their *S*-sulpho forms is solely responsible for their solubilisation at pH 10·2, or whether other disaggregating or cleavage reactions are also involved. It may be pertinent to note, in this connexion, that Swan found that sulphitolysis of keratin at pH 7·0 did not lead to solubilisation. These problems are being investigated by carrying out the sulphitolysis reaction with only catalytic amounts of copper present, in the manner used by Leach & Swan with insulin,[24] and also with no added copper but with the proteins dispersed in strong urea solutions with an excess of sulphite and air as the oxidant. It has recently been observed that under these latter conditions, gliadin proteins may be completely converted to the *S*-sulpho forms. The properties of the material obtained in this way are being compared with those of the material produced by sulphitolysis of gliadin using the cuprammonium sulphite reagent. The results of these, together with other experiments on fractionation, will be reported in a subsequent paper.

Acknowledgments

This work is supported in part by a grant from the United States Dept. of Agriculture, under U.S. Public Law 480. The authors are grateful to Dr. H. D. C. Jones for help in the determination of amide nitrogen.

Research Association of British Flour-Millers,
Cereals Research Station,
Old London Road,
St. Albans, Herts.

Received 1 January, 1965

References

1 Clarke, H. T., *J. biol. Chem.*, 1932, **97**, 235
2 Kolthoff, I. M., & Stricks, W., *Analyt. Chem.*, 1951, **23**, 763
3 Swan, J. M., *Nature, Lond.*, 1957, **180**, 643
4 Swan, J. M., *Aust. J. Chem.*, 1961, **14**, 69
5 Pechere, J. F., Dixon, G. H., Maybury, R. H., & Neurath, H., *J. biol. Chem.*, 1959, **233**, 1364
6 Pechere, J. F., Dixon, G. H., Maybury, R. H., & Neurath, H., *Biochim. biophys. Acta*, 1959, **31**, 259
7 Henschen, A., *Acta chem. scand.*, 1962, **16**, 1037
8 Clegg, J. B., & Bailey, K., *Biochim. biophys. Acta*, 1962, **63**, 525
9 Weil, L., & Seibles, T. S., *Arch. Biochem. Biophys.*, 1959, **84**, 244
10 Frater, R., Hird, F. J. R., Moss, H. J., & Yates, J. R., *Nature, Lond.*, 1960, **186**, 451
11 McDermott, E. E., & Pace, J., *Nature, Lond.*, 1959, **184**, 546
12 Leach, S. J., & Parkhill, E. M. J., *Proc. Int. Wool Textile Res. Conf. (Australia)*, 1955, p. 92
13 Moore, S., & Stein, W. H., *J. biol. Chem.*, 1951, **192**, 663
14 McDermott, E. E., & Pace, J., *J. Sci. Fd Agric.*, 1960, **11**, 576
15 Synge, R. L. M., & Wood, J. C., *Biochem. J.*, 1958, **70**, 321
16 Analytical Methods Committee, *Analyst*, 1963, **88**, 253
17 Leach, S. J., *Aust. J. Chem.*, 1960, **13**, 520
18 Adkins, E. K., *Stärke*, 1962, **14**, 129
19 Kündig, W., Neukom, H., & Deuel, H., *Helv. chim. Acta*, 1961, **44**, 823
20 Holme, J., & Briggs, D. R., *Cereal Chem.*, 1959, **36**, 321
21 Mecham, D. K., & Olcott, H. S., *Industr. Engng Chem.*, 1947, **39**, 1023
22 Pence, J. W., Weinstein, N. E., & Mecham, D. K., *Cereal Chem.*, 1954, **31**, 29
23 Lee, J. W., Tracey, M. V., Winzor, D. J., Wrigley, C. W., & Zentner, H., *Aust. J. biol. Sci.*, 1963, **16**, 717
24 Leach, S. J., & Swan, J. M., *Aust. J. Chem.*, 1962, **15**, 365

QUANTITATIVE CHANGES IN THE POLYPHENOLS DURING THE PROCESSING OF TEA LEAF AND THEIR RELATION TO LIQUOR CHARACTERS OF MADE TEA

By I. S. BHATIA and M. R. ULLAH

Quantitative changes occurring in the polyphenolic make-up of tea shoots during manufacture of black tea are recorded. It has been shown that in addition to (−)-epigallocatechin and (−)-epigallocatechin gallate, significant amounts of (−)-epicatechin gallate and theogallin are also consumed during the fermentation process. The loss in (−)-epigallocatechin gallate has been used to calculate the extent of cell distortion incurred by leaf during rolling and cutting in different types of manufacturing machinery. The total colour produced per unit mass of the important catechins consumed in fermentation is highest for Legg-cut, less for C.T.C. and least for the conventional method of manufacture.

Introduction

The presence of a variety of polyphenols in the unprocessed tea shoots is now well established.[1–5] Important among these are the flavanols, which include (—)-epigallocatechin gallate (EGCG), (—)-epicatechin gallate (ECG), (—)-epigallocatechin (EGC) in comparatively

larger quantities and (+)-catechin, (−)-epicatechin and (+)-gallocatechin in rather small amounts. In addition, appreciable amounts of theogallin (TG), which has been tentatively identified as galloyl quinic acid,[6,7] are also present.

The manufacture of black tea from green leaf involves a series of processing steps, viz., withering, rolling, fermentation and drying. An extensive study of the changes occurring during fermentation has been made by Roberts.[8,9] On the basis of chromatographic analysis, a study of oxidation–reduction potentials and *in vitro* enzymic oxidations of isolated flavanols, he concluded that EGC and EGCG are the only two compounds which suffer appreciable changes in concentration during the fermentation of tea leaf. Two groups of coloured products, the theaflavins (TF) and the thearubigins (TR), are believed to result from these oxidative and condensation types of reactions.

The present paper deals with the quantitative aspects of changes undergone by different polyphenols during the processing of black tea. These changes were followed by a spectrophotometric method devised in these laboratories specifically for this purpose.[10] It has been shown that in addition to EGC and EGCG, appreciable amounts of ECG[11] and TG are also consumed during manufacture of black tea. The losses of these polyphenols during manufacture have been correlated with the amounts of TF and TR produced concomitantly. The progressive loss in the concentration of EGCG has been used as a basis for assessing the extent of fermentation and the degree of cell distortion incurred from any particular process of manufacture.

Experimental

Materials

All the plant materials used during these investigations were obtained from the experimental plots of the Station and have been previously described.[12]

Processing details

All manufactures were conducted in the miniature factory of the Station.

Conventional or Orthodox manufacture

Two lb. of leaf were allowed to wither naturally for 18 h. and then rolled in a miniature conventional roller for 1 h. Fermentation was carried out on aluminium sheets and the fermented leaf then dried by a blast of hot air at 90°. Fermentation times were calculated from the start of the rolling process.

C.T.C. manufacture

Leaf withered naturally for 18 h. was rolled for ½ h. and then passed twice through a C.T.C. machine. Fermentation and firing were done as in the conventional method. In cases where it was found necessary to modify the manufacturing procedure, the details are dealt with in appropriate places.

Legg-cut manufacture

Freshly plucked shoots were passed through a miniature Legg-cut machine and cut leaves rolled in a miniature roller for 15 min. After fermentation, leaf was dried in the usual manner.

Methods of analysis

Estimations of individual polyphenols.—Polyphenols were separated by two-way chromatography of a methanolic extract of the test material and estimations were carried out from optical extinctions of excised spots at 275 mμ using SP 500 Unicam Spectrophotometer. For further details of this method, reference may be made to a later paper in this series.

Estimations of theaflavins and thearubigins.—These were done as described by Roberts & Smith.[13]

'Tannin' content.—The A.O.A.C. (Lowenthal)[14] method for 'tannin' in tea was used. Five g. of dried leaf were refluxed with 400 ml. of water for 1 h. and the volume was made to 500 ml. after filtration through cotton wool. Ten ml. of this infusion were used for the estimation.

Total oxygen uptake.—This is the amount of oxygen in μl. consumed in 2 h. per mg. of minced tissue (calculated on dry weight basis). The measurements of oxidase activity were made as described by Roberts[15] except that 100 mg. of finely minced leaf were used instead of 200 mg.

Results

In a preliminary study, the EGCG, ECG and EGC contents of green leaf and corresponding teas manufactured by the conventional and the C.T.C. methods were determined to ascertain the relative extent of participation of these polyphenols during black tea manufacture. Results are presented in Table I.

Table I

Concentrations of individual polyphenols (% on dry wt.) in tea leaf before and after processing by Conventional and C.T.C. methods of manufacture

Test material \ Leaf source	EGCG			ECG			EGC		
	Betjan	Gauri-sankar	Lingia	Betjan	Gauri-sankar	Lingia	Betjan	Gauri-sankar	Lingia
Dried leaf	6·95	7·82	5·28	1·38	1·76	1·28	4·38	3·89	4·61
Conventional tea	0·63	0·60	0·45	0·36	0·42	0·36	—	—	—
C.T.C. tea	0·33	0·39	0·39	0·23	0·29	0·36	—	—	—

— could not be measured

It is clear that in addition to EGC and EGCG, which are known to participate in the oxidative changes during manufacture, appreciable amounts of ECG are also simultaneously consumed.

In a series of subsequent experiments, the changes in the phenolic content were measured as a function of fermentation time. Representative results are presented in Tables II and III.

Table II

Concentrations of polyphenols (% on dry wt.) after different times during the fermentation of tea leaf
(Conventional manufacture)

Fermentation time, h. \ Leaf source	EGCG				ECG			
	Clone 1/7/1	Clone 19/29/13	Clone 20/23/1	Burma Jat	Clone 1/7/1	Clone 19/29/13	Clone 20/23/1	Burma Jat
0 (dried green leaf)	6·54	4·38	5·52	6·45	1·83	2·43	1·30	1·63
1	4·80	2·98	4·62	3·51	1·52	2·37	1·31	1·29
2	2·36	1·24	2·40	1·55	1·06	1·74	0·91	0·72
3	0·85	0·68	1·31	0·67	0·73	1·06	0·67	0·44
4	0·63	0·59	0·58	0·36	0·53	0·69	0·36	0·27

Fermentation time, h. \ Leaf source	EGC				TG			
	Clone 1/7/1	Clone 19/29/13	Clone 20/23/1	Burma Jat	Clone 1/7/1	Clone 19/29/13	Clone 20/23/1	Burma Jat
0 (dried green leaf)	3·47	2·66	3·09	3·22	0·90	0·64	+	0·78
1	2·89	2·10	2·14	2·20	0·84	0·52	0·48	0·67
2	1·70	—	1·47	—	0·65	0·40	0·48	0·56
3	—	—	0·93	—	0·53	0·26	0·41	0·39
4	—	—	—	—	0·34	0·18	—	0·36

+ not estimated — could not be measured

Table III

Concentrations of polyphenols (% on dry wt.) after various times during the fermentation of tea leaf
(C.T.C. manufacture)

Fermentation time, h. \ Leaf source	EGCG		ECG		EGC		TG	
	Clone 1/7/1	Clone 20/23/1	Clone 1/7/1	Clone 20/23/1	Clone 1/7/1	Clone 20/23/1	Clone 1/7/1	Clone 20/23/1
0 (dried green leaf)	8·51	8·35	2·00	2·15	4·38	4·40	1·62	0·85
½	3·65	3·24	1·55	1·34	3·30	2·24	1·40	0·74
1	2·18	2·21	1·13	1·12	—	2·10	1·36	0·69
1½	0·89	—	0·68	—	—	—	1·11	—
2	0·66	0·51	0·52	0·41	—	—	0·78	0·37
3	0·53	0·39	0·40	0·30	—	—	0·48	—
4	0·41	0·40	0·23	0·27	—	—	0·26	

— could not be measured

Comparison of the values in Tables II and III indicates that fermentation proceeds at a faster rate in the C.T.C. than in the Orthodox method and that different polyphenols show widely varying rates of fermentation. EGC and EGCG are most rapidly oxidised, and, in comparison, ECG and TG are consumed at much slower rates. EGC was not measured at the later stages of fermentation as the boundary of this spot became indeterminate. However, it is certain that very little of this polyphenol is present in fully fermented teas. The residual EGCG content of tea after 1 h. of fermentation (in the C.T.C. method) approximates to a quarter of the total present in green leaf, whereas more than half of the ECG and about 80% of the TG are left unchanged during the same period of fermentation (Table III).

The calculated loss of polyphenols undergone by the shoots from different sources and the corresponding amounts of TF and TR produced during manufacture are recorded in Tables IV–VI.

The ratio, $\frac{\text{TF} + \text{TR}}{\text{Total decrease in (EGCG} + \text{EGC} + \text{ECG)}}$, which for convenience may be referred to as colour efficiency (C.E.), appears to depend upon the leaf source and the method of manufacture. On the whole this ratio follows the ascending order, conventional, C.T.C. and Legg-cut.

In Fig. 1 the progressive decline in the concentration of EGCG observed during different types of manufacture is plotted against the fermentation time.

In each of these curves initial high rates of decrease in the concentration of EGCG are followed by a rather flat curve representing little further decrease in its content. The steep part of the curve represents a stage in fermentation when large quantities of polyphenols made accessible to oxygen by a roller or a cutting machine are being continuously transformed into theaflavins and thearubigins. The chemical changes associated with the flat portion of the curve pertain to the transformation of theaflavins to thearubigins. As no further quantities of polyphenols are being consumed, a decrease in theaflavin content is expected and, in fact, is observed when fermentation times are prolonged.

Table IV

Relationship between TF and TR of made teas and the amounts of polyphenols consumed during manufacture by the conventional method

Sample	Date of plucking	Decrease in concn. (% on dry wt.)			Total decrease in polyphenols (%)	Concn. of transformation products (% on dry wt.)			Ratio of (TF + TR) (total decrease in polyphenols during manufacture)
		EGCG	EGC	ECG		TF	TR	TF+TR	
Betjan	16.6.60	6·31	4·37	1·03	11·71	0·60	12·42	13·02	1·11
Betjan	21.7.60	6·61	4·44	0·70	11·75	0·37	9·29	9·66	0·82
Gaurisankar	16.6.60	7·19	3·88	1·33	12·40	0·48	14·67	15·15	1·22
Gaurisankar	21.7.60	5·71	4·07	0·41	10·19	0·36	8·70	9·06	0·89
1/7/1	21.7.60	4·22	4·13	0·57	8·92	0·35	8·06	8·41	0·94
19/29/13	23.6.60	4·07	3·67	1·26	9·00	0·41	14·13	14·54	1·62
19/29/13	28.7.60	3·59	3·36	1·09	8·04	0·65	13·28	13·93	1·73
Indochina	28.7.60	6·40	4·85	1·06	12·31	0·32	12·91	13·23	1·08
Lingia	28.7.60	4·84	4·79	0·91	10·54	0·53	14·50	15·03	1·43

Table V

Relationship between TF and TR of made teas and the losses in the polyphenols during manufacture of teas by the C.T.C. process

Sample	Date of plucking	Decrease in concn. (% on dry wt.)			Total decrease in polyphenols (%)	Concn. of transformation products (% on dry wt.)			Ratio of (TF + TR) (total decrease in polyphenols during manufacture)
		EGCG	EGC	ECG		TF	TR	TF + TR	
Betjan	16.6.60	6·57	4·37	1·15	12·09	0·81	17·70	18·51	1·53
Betjan	21.7.60	6·25	4·44	1·09	11·78	0·68	18·23	18·91	1·61
Gaurisankar	16.6.60	7·42	3·88	1·45	12·75	0·85	18·86	19·71	1·55
Gaurisankar	21.7.60	5·86	4·07	0·86	10·79	0·59	15·39	15·98	1·48
1/7/1	21.7.60	5·55	4·13	1·04	10·72	0·74	14·18	14·92	1·39
19/29/13	23.6.60	4·45	3·67	1·88	10·00	0·60	20·51	21·11	2·11
19/29/13	28.7.60	3·79	3·36	1·35	8·50	0·75	19·39	20·14	2·37
Indochina	28.7.60	6·92	4·85	1·26	13·03	0·68	17·94	18·62	1·43
Lingia	28.7.60	4·90	4·79	0·92	10·61	0·73	17·84	18·57	1·75

Table VI

Relationship between TF and TR of made teas and the amounts of polyphenols consumed during manufacture by the Legg-cut process

Sample	Date of plucking	Decrease in concn. (% on dry wt.)			Total decrease in polyphenols (%)	Concn. of transformation products (% on dry wt.)			Ratio of (TF + TR) (total decrease in polyphenols during manufacture)
		EGCG	EGC	ECG		TF	TR	TF + TR	
Betjan	16.6.60	3·19	1·29	0·60	5·08	0·62	9·07	9·69	1·91
Betjan	21.7.60	5·04	2·61	0·96	8·61	0·87	15·19	16·06	1·87
Gaurisankar	16.6.61	3·72	1·16	0·85	5·73	0·65	11·94	12·59	2·20
1/7/1	21.7.60	4·87	2·72	1·14	8·73	0·97	16·60	17·57	2·01
19/29/13	23.6.60	3·86	3·67	1·90	9·43	0·66	16·60	17·26	1·83
19/29/13	28.7.60	3·10	3·36	1·07	7·53	0·87	14·48	15·35	2·04
Indochina	28.7.60	4·61	1·67	1·05	7·33	0·89	15·72	16·61	2·27
Lingia	28.7.60	3·89	2·70	0·78	7·37	0·65	12·53	13·18	1·79

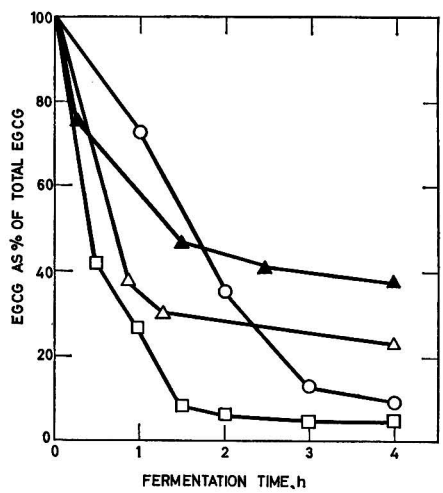


FIG. 1.—*Changes in (−)-epigallocatechin gallate content (expressed as % of total EGCG present in unprocessed tea leaf) during fermentation of tea leaf by different methods of manufacture*

○ Conventional
△ Legg-cut with rolling
□ C.T.C.
▲ Legg-cut without rolling

Compared with the conventional method of manufacture, the overall rate of oxidation in Legg-cut and C.T.C. manufacture is greater because of the relative ease with which oxygen molecules can come in contact with the polyphenols in these processes.

In unwithered turgid leaf, which has been cut by a Legg-cut machine, the polyphenols located on or near the cut surface undergo rapid oxidation and those located in the interior of the leaf are hardly effected. For all practical purposes, the cutting action does not affect the behaviour of the polyphenols remotely located from the cut surface. Hence, increasing the fermentation time does not result in further utilisation of EGCG, but considerable additional amounts of this polyphenol are consumed if leaf after passing through a Legg-cut machine is rolled.

In one experiment, the periods for which leaf was rolled and fermented were varied and estimations of residual polyphenolic contents made on dried samples. Results are recorded in Table VII.

Table VII

Effect of varying the periods of rolling and fermentation on the chemical composition of made teas

Leaf source: Betjan Jat; plucked on 10.10.61

Fermentation, h.	Rolling time, h.	EGCG %	EGC %	ECG %	TG %	Sum of polyphenols, %	TF %	TR %	TF + TR %	Tannin %	Oxygen uptake, µl/mg.
0	0 (dried leaf)	5·99	5·11	1·73	0·59	13·42	0·03	4·90	4·93	21·0	5·21
2	½	2·19	2·80	0·91	0·55	6·45	0·32	10·52	10·84	18·0	3·07
	1	1·71	2·06	1·10	0·54	5·41	0·46	12·57	13·03	19·2	2·68
	1½	1·24	—	0·78	0·50	2·52	0·48	13·06	13·54	16·9	1·57
3	½	1·47	2·29	0·74	0·47	4·97	0·39	12·92	13·31	15·9	2·33
	1	0·79	—	0·58	0·43	1·80	0·48	15·60	16·08	14·8	1·71
	1½	0·74	—	0·58	0·44	1·76	0·45	16·80	17·25	15·0	1·15
4	½	1·12	—	0·69	0·44	2·25	0·27	12·85	13·12	16·8	1·96
	1	0·47	—	0·37	0·31	1·15	0·43	17·01	17·44	13·9	1·74
	1½	0·53	—	0·37	0·35	1·25	0·43	18·64	19·07	13·4	0·88
5	½	0·74	—	0·50	0·36	1·60	0·25	15·25	15·50	15·9	1·48
	1	0·47	—	0·36	0·28	1·11	0·43	19·42	19·85	13·8	1·13
	1½	0·51	—	0·31	0·32	1·14	0·40	19·77	20·17	13·7	0·81

— could not be measured

An increase in rolling time from ½ h. to 1½ h. leads to consumption of additional amounts of EGCG indicating that cell distortion is insufficient with shorter rolling times.

Results pertaining to the effect of temperature on the consumption of polyphenols and production of colour during manufacture are recorded in Table VIII. For comparison, residual oxygen uptakes and ' tannin ' content of these teas are also included.

The relatively high residual phenolic contents of teas fermented for 1 h. only, indicate that these teas are grossly underfermented. This fact is not so obvious from the corresponding values of TF and TR. Results for polyphenols as well as TF and TR point to a greater extent of fermentation when teas are fermented for 1 h. An increase in temperature leads up to a point to an enhancement in the consumption of polyphenols; but increasing the fermentation temperature beyond 29·4° seems to be of no value in accelerating the fermentation reactions. A marked fall in TF value is observed in keeping with the poor quality of teas fermented at high temperature. There is a concomitant decrease in the TR values, presumably because of greater interaction between proteins and TR.

Discussion

Major chemical changes occurring during the manufacture of black tea involve the enzymic oxidation of polyphenols during fermentation. According to the current view, the phenolic substrates present in the vacuole and the enzyme located in the plastids are separated from each other in the undamaged cells of tea shoots by vacuolar membranes.[16] Rolling of leaf results in cell distortion (disorganisation of the vacuolar membrane) and facilitates the mixing of the polyphenols with the enzyme and penetration of oxygen to the interior of the leaf.

Table VIII

Chemical analyses of teas made from clones 1/7/1 and 19/29/13 fermented at different temperatures for varying periods

Leaf source: Clone 1/7/1; plucked on 1.11.61

Sample	Fermentation Time, h.	Fermentation Temp., °C	EGCG %	EGC %	ECG %	TG %	Sum of polyphenols, %	TF %	TR %	TF + TR %	Tannin %	Oxygen uptake, µl/mg.
Dried leaf	0		7·55	3·96	2·02	1·00	14·53	0·11	7·13	7·24	25·3	6·08
A	½	26·7	2·56	2·61	1·24	1·00	7·41	0·56	16·31	16·87	20·4	3·56
		29·4	1·57	2·18	1·18	0·78	5·71	0·26	16·33	16·59	20·1	3·11
		40·6	2·02	2·35	1·45	0·87	6·69	0·22	14·54	14·76	19·4	3·25
		51·7	2·08		1·71	1·05	4·84	0·12	14·36	14·48	13·9	3·60
B	1	26·7	1·37	1·74	0·85	0·82	4·78	0·50	18·57	19·07	17·3	2·48
		29·4	0·76		0·75	0·58	2·09	0·18	19·91	20·09	16·8	2·25
		40·6	0·84		1·07	0·70	2·61	0·13	18·36	18·49	15·6	2·65
		51·7	1·11		1·16	0·93	3·20	0·05	17·37	17·42	13·2	2·66
C	113	1·7	0·97		0·75	0·41	2·13	0·43	21·46	21·89	16·2	2·26
D	2 (Normal C.T.C.)	26·7	0·45		0·64	0·54	1·63	0·49	21·74	22·23	16·1	1·83

Leaf source: Clone 19.29.13; plucked on 1.11.61

Sample	Fermentation Time, h.	Fermentation Temp., °C	EGCG %	EGC %	ECG %	TG %	Sum of polyphenols, %	TF %	TR %	TF + TR %	Tannin %	Oxygen uptake, µl/mg.
Dried leaf	0		4·91	4·77	2·64	0·71	13·03	0·13	7·2	7·33	22·2	6·52
A	½	26·7	1·48	2·33	1·54	0·71	6·06	0·68	17·7	18·38	18·1	3·93
		29·4	0·94	1·82	1·19	0·60	4·55	0·44	18·4	18·84	17·1	3·86
		40·6	1·21	2·31	1·41	0·59	5·52	0·45	18·9	19·35	20·0	4·25
		51·7	1·25		1·63	0·62	3·50	0·30	15·3	15·60	19·8	4·85
B	1	26·7	0·33		0·38	0·48	1·19	0·56	22·3	22·86	16·0	2·48
		29·4	0·50		0·76	0·42	1·68	0·35	20·9	21·25	15·0	2·62
		40·6	0·47		0·88	0·45	1·80	0·31	20·3	20·61	15·3	2·92
		51·7	0·69		1·45	0·55	2·69	0·23	16·4	16·63	17·3	3·24
C	113	1·7	0·57		0·60	0·23	1·40	0·50	23·86	24·36	16·6	1·98
D	2 (Normal C.T.C.)	26·7	0·34		0·46	0·25	1·05	0·53	24·36	24·89	12·4	1·67

Samples A–C were prepared by passing naturally withered unrolled tea shoots twice through a C.T.C. machine followed by usual fermentation on aluminium sheets and drying. Samples A and B were fermented in a specially designed closed system wherein temperature was controlled by regulating the supply of steam.

Sample C was fermented in a refrigerator, and D was a normal C.T.C. sample made from withered and rolled leaf. All samples were dried in the miniature dryer.

From qualitative and semiquantitative work, Roberts concluded that (−)-epigallocatechin and its gallate were the only phenols which were oxidised during fermentation. Quantitative results on the oxidation of individual tea polyphenols during black tea manufacture presented here have shown that, in addition, appreciable amounts of ECG are also consumed during fermentation. The nature of the end products from this phenol and their role in liquor characters of made tea must await the results of studies on the enzymic oxidation of individual flavanols which are currently in progress. The rates of enzymic oxidations of these polyphenols, however, vary a great deal. EGC and EGCG are oxidised at faster rates than in ECG. A loss in the concentration of TG is also observed during fermentation. The relative proportions of EGCG to ECG vary a great deal in the cultivated varieties of tea grown in the Assam valley.[10] The differences in the rates of oxidation of these phenols, therefore, have obvious implications in tea manufacture. For example, clones with a high proportion of ECG will need more drastic processing if a full use is to be made of this particular phenol towards the development of colour and strength of the resulting teas.

Different manufacturing practices involve varying degrees of 'cell rupture' and these account for the accepted differences in fermentation times of C.T.C., Orthodox and Legg-cut and other processes of relatively recent origin. In the Orthodox or the Conventional method, rolling results in more or less uniform rates of oxidation at all sites within the entire bulk of the leaf. (There will, of course, be differences in the rates of oxidation from one kind of tissue to another.) In the normal undamaged tea leaf, intercellular spaces permit free exchange of gases within the leaf and with the outside through the stomata. On rolling, the intercellular path for gas exchange is probably disorganised with the result that polyphenols located in the interior are oxidised only slowly even though mixing with the enzyme may have occurred. These facts

are reflected in the relatively longer periods and the slower rates of fermentation in the conventional method of manufacture. As the oxygen molecules can freely reach the polyphenols on cut surfaces, C.T.C. and Legg-cut teas are oxidised more rapidly (Fig. 1).

Liquor characters of made teas are traditionally evaluated by tea tasters. An assessment is made on basis of the colour, strength, briskness and quality of liquors. The amount of ' cream ' and its colour are also considered in this assessment. During the fermentation of tea leaf, organoleptic characters undergo changes which can be correlated with chemical changes. An aqueous infusion of unprocessed tea leaf, which contains the polyphenols only in the unoxidised state, has a bitter taste very different from that of made tea, referred to by the tasters as ' green ', ' cabbagy ', 'raw ' and ' bitter ', etc. Underfermented teas, which contain lesser amounts of the unoxidised phenols but some quantity of TF and TR, are considered less raw, but more ' brisk ' and astringent. On further fermentation, concomitant with the decline in the concentration of phenols and production of TF and TR, the raw character disappears altogether and teas become less ' brisk ' but are considered more ' mellow ' and ' fuller '. Further fermentation may lead to a total disappearance of polyphenols and a certain loss of TF and TR is observed, the latter possibly resulting from their interaction with proteins. Such overfermented teas are completely devoid of ' briskness ' and are rated ' flat ', dull and heavy on the palate. Green or raw character of black tea is generally believed to be due solely to underfermentation (i.e., presence of large amounts of unoxidised polyphenols). Our experiments on low-temperature fermentation indicate that the situation is more complex. Black tea made by fermenting leaf for a prolonged period at an unusually low temperature (Table VIII, 113 h. at 1-7°) was reported to be ' green ', ' cabbagy ' and ' raw ' by the taster, although judged from its residual phenolic content or from the values of TF and TR, it appeared to be reasonably well fermented. It would, therefore, appear that in addition to the raw character resulting from the presence of large amounts of unoxidised polyphenols, there is another type of raw character reminiscent of the taste of an uncooked leafy vegetable. At relatively high temperatures of fermentation (23·9°–26·7°), the loss of green leafy character parallels the disappearance of polyphenols, so that during the course of normal fermentation both types of raw character disappear. However, when the leaf is fermented at a very low temperature, slow oxidation of the polyphenols continues; but it appears that, in comparison, the reactions which eliminate leafy or cabbagy character slow down disproportionally, so that these teas are not bitter but are still raw or ' cabbagy '. The association of green leafy character with underfermentation, therefore, appears to be purely fortuitous.

With the availability of quantitative data on the consumption of polyphenols and concomitant production of TF and TR, it is now possible to draw up an approximate balance sheet of the major substances involved in tea fermentation. If EGC, EGCG and ECG were the only polyphenols involved in fermentation and TF and TR were the only resulting products, one would expect the loss of these polyphenols to be equal to the amount of TF and TR produced, giving rise to a colour efficiency of 1, irrespective of the method of manufacture. That this expectation is not fulfilled is a reminder that, despite voluminous work that has been done on fermentation, the present state of knowledge of the subject is inadequate. Values of C.E. considerably higher than 1 may mean that the contribution of other polyphenols cannot be ignored. For a given leaf source, values of C.E. for Legg-cut and C.T.C. teas were found to be higher than those for conventional teas. Poorer extractibility of colour from conventional teas alone is insufficient to explain the observed orders of difference. (During the first 10 min. of treatment with boiling water, on the average, 90% and 70% of the total colour was extracted from C.T.C. and conventional teas respectively.) The conclusion must be drawn that either there is a higher proportion of colourless oxidation products (e.g., bisflavanols) in conventional teas or that the thearubigins of C.T.C. and Legg-cut teas are different from those of conventional teas and are more highly coloured. In the present method of estimation of TF and TR, no distinction is made between the quantitative contributions to colour of individual members of the thearubigin complex. In view of the facts discussed above, it is felt that this method needs to be refined to take into account the heterogeneous nature of the thearubigins.

It has been observed that C.E. of teas from clone 19/29/13 is generally of a high order. This alone, among the clones at present being cultivated, has the highest proportion of ECG

(the ratio EGCG/ECG being approximately 2). Whether the direct participation of ECG in fermentation is in itself responsible for producing superior colour, or whether hitherto unknown associated factors are responsible for the phenomenon, cannot be decided from the available results.

At present, control measures in tea manufacture are largely subjective and manufacturing practices vary a great deal from one tea estate to another. Normally, the processing conditions (e.g., numbers of rolls and fermentation times, etc.) are decided by visual examination of the withered leaf, colour and smell (called ' nose' in the trade) of the fermenting mal and the liquor characters of resulting teas. In the past, such an approach has proved satisfactory because the optimum processing conditions have been evolved from practical experience extending over a long period during which there has not been much change in tea machinery. However, with the recent development and use on a wide scale of the Rotorvane and new combinations of machines to accomplish rapid cell distortion (e.g., Rotorvane as the sole roller, Rotorvane/C.T.C. and Legg-cut/Rotorvane), need is felt for a more objective approach to the problem. By estimating the residual phenolic contents in a series of samples prepared by varying the extent of processing (e.g., number of rolls in a conventional roller, number of passes through a Rotorvane or number of cuts in a C.T.C. machine) and also the time of fermentation, correct processing conditions can be worked out for any method of manufacture. This opens up the possibility of chemical control measures in tea manufacture.

Acknowledgments

The authors are indebted to the Director, Tocklai Experimental Station, and the Tea Research Association, India, for permission to publish these results.

Tocklai Experimental Station
Cinnamara P.O.
Assam, India

Received 10 May, 1965

References

[1] Bradfield, A. E., *Chem. & Ind.*, 1946, p. 242
[2] Bradfield, A. E., Penney, M., & Wright, W. B., *J. chem. Soc.*, 1947, p. 32
[3] Bradfield, A. E., & Penney, M., *J. chem. Soc.*, 1948, p. 2249
[4] Roberts, E. A. H., & Wood, D. J., *Biochem. J.*, 1951, **49**, 414
[5] Roberts, E. A. H., & Wood, D. J., *Biochem. J.*, 1953, **53**, 332
[6] Cartwright, R. A., & Roberts, E. A. H., *Chem. & Ind.*, 1955, p. 230
[7] Roberts, E. A. H., & Meyers, M., *J. Sci. Fd Agric.*, 1958, **9**, 701
[8] Roberts, E. A. H., *J. Sci. Fd Agric.*, 1958, **9**, 212
[9] Roberts, E. A. H., *J. Sci. Fd Agric.*, 1958, **9**, 381
[10] Bhatia, I. S., & Ullah, M. R., *Nature, Lond.*, 1962, **193**, 658
[11] Bhatia, I. S., & Ullah, M. R., *Chem. & Ind.*, 1961, p. 1169
[12] Wood, D. J., Bhatia, I. S., Chakraborty, S., Choudhury, M. N. D., Deb, S. B., Roberts, E. A. H., & Ullah, M. R., *J. Sci. Fd Agric.*, 1964, **15**, 8
[13] Roberts, E. A. H., & Smith, R. F., *Analyst*, 1961, **86**, 94
[14] 'Official Methods of Analysis', 1960, 9th Edn, p. 185 (Washington, D.C.: Assoc. of Official Agric. Chemists)
[15] Roberts, E. A. H., *Biochem. J.*, 1940, **34**, 507
[16] Li, L. P., & Bonner, J., *Biochem. J.*, 1947, **41**, 105

THE DETERMINATION OF STEAM-VOLATILE FATTY ACIDS IN RUMEN LIQUOR, BLOOD PLASMA AND MILK FAT

By J. E. STORRY and D. MILLARD

Methods for the determination of steam-volatile fatty acids in rumen liquor, blood plasma and milk fat by gas–liquid chromatography are described, and the precision and advantages of the methods are given.

Introduction

The original method of James & Martin[1] for the determination of the relative proportions of volatile fatty acids in solution by gas–liquid chromatography and automatic titration of the separated free acids, has been applied to the determination of the steam-volatile fatty acids in rumen liquor[2-4] and other body fluids,[5] but not in all instances are full working details or the accuracy and precision of the methods given. In recent years studies on milk secretion in this laboratory have necessitated the measurement of these volatile acids in rumen liquor, blood plasma, and also in milk fat. During this work several modifications of the basic chromatographic method as originally described by James & Martin have been introduced, resulting in greater speed and reproducibility. Details of the procedures which are now in routine use are presented in this paper.

Experimental

Apparatus

An automatic titrator, based on that of James & Martin[1] was built in collaboration with the Engineering Dept. of this Institute. The photoelectric control circuit, introduced by Tilley *et al.*,[4] operated a magnetic clutch (type 5/1, IN, Crofts (Engineers) Ltd., Bradford) between the electric motor and the screw drive of the burette. The mechanical movements of the machine were greatly improved and greater sensitivity was obtained by using, in the burette, a rod of 0·1 in. o.d. with 26 turns of the burette screw per inch travel of the rod and 0·005N-sodium hydroxide as titrant. An improved leak-proof joint between the column tip and the titration chamber was made from a B7 cone and socket joint. The male joint was joined to the end of the column and the tip drawn out to a fine point and the female joint was incorporated into the titration chamber.

For the analysis of rumen liquor and milk fat the columns were modified to take a self-sealing plug in the end and a side-arm for the supply of nitrogen because the extracted acids (see later) in these samples were applied directly to the column by injection. To prevent the condensation of higher acids in the tip of the column at the joint between the vapour jacket and titration chamber, the joint was heated to the appropriate temperature with a thermal tape and a solution of phenol red, 0·005% w/v in 15% v/v ethyl alcohol, was used in the titration chamber to ensure complete solution of the higher fatty acids. Any loss of titration fluid from the chamber by evaporation was prevented by fitting a condenser.

Extraction of volatile acids

Rumen liquor.—Two ml. of centrifuged rumen liquor are pipetted into a 10-ml. glass-stoppered flask and made alkaline with 1 or 2 drops of 1N-sodium hydroxide, then taken almost to dryness by heating on a water-bath maintained at about 90°, air being drawn over the surface of the liquid in the flask. The flask is cooled and 1 drop of 90% sulphuric acid (90 parts of conc. acid, 10 parts of water) added and allowed to mix thoroughly with the concentrated sample. Three ml. of di-isopropyl ether (previously redistilled over sodium) are added and the mixture shaken intermittently for about 10 min. Then 0·5 g. of anhydrous sodium sulphate is added and the flask set aside for about 30 min. Depending on the concentration of volatile fatty acids in the sample, 20–40 μl. of the extract are injected on to the column with a Hamilton No. 705 microlitre syringe. Before the injection, the column is withdrawn from the vapour jacket for a distance of about 6 in. and allowed to cool and during injection of the sample the flow of nitrogen gas is stopped, and then restarted immediately and the column returned to the vapour jacket.

Blood.—Plasma is separated from the red cells by centrifugation and the total volatile fatty acids estimated by steam distillation in a Markham apparatus as described by Scarisbrick.[6] The neutralised distillates from six samples representing a total volume of 30 ml. of plasma are combined and reduced in volume to about 2 ml. by rotary evaporation in a 50-ml. glass-stoppered flask. The flask is cooled in an ice bath and 0·5 ml. of 90% sulphuric acid is added, followed by 20 ml. of diethyl ether, and the flask shaken several times during 15 min. Anhydrous sodium sulphate (15 g.) is added and the mixture kept at 4° for at least 4 h. From 0·5 to 2·5 ml. of the dried ether extract, depending on the concentration of acids, are used for the chromatographic separation. The acids are transferred to the column as described by James & Martin,[1] but also a small quantity of washed sand is added to the tube containing the acids, to prevent loss of the sample through bumping, which otherwise occasionally occurred.

Milk fat.—The fat is extracted from milk by pipetting 10 ml. of milk into 75 ml. of methanol, adding 75 ml. of chloroform and warming the mixture to 60° for 15 min. After the mixture has cooled, a further 75 ml. of chloroform are added, the mixture shaken and the precipitated protein filtered through a fast filter (Whatman No. 541). The filter is washed several times with methanol/chloroform mixture (1:2 v/v). The combined lipid extract and washing are then washed with 0·88% w/v potassium chloride[7] and the chloroform layer is separated and made up to 250 ml. with chloroform. A suitable aliquot of the extract (usually 100 ml.) is transferred to a tared flask, evaporated to dryness at 40–45° under nitrogen and finally dried to constant weight at 50°. The lipid is hydrolysed with 25 ml. of 10% w/v potassium hydroxide in methanol–water (1 : 1 v/v) under reflux for 3 h. at 100°. The resulting solution is acidified by adding 12 ml. of 5N-sulphuric acid and the lower acids (C_4 — C_{10} together with traces of C_{12} and C_{14}) are separated by steam distillation, the distillation being continued until 3 × 30 ml. fractions of distillate have been collected. The three fractions are titrated with 0·05N-sodium hydroxide and the titre of the third aliquot is usually negligible.

The neutralised steam distillate is transferred in stages to a 50-ml. round-bottomed flask with ground-glass stopper and evaporated to a final volume of about 0·5 ml. Two drops of 90% sulphuric acid are then added followed immediately by 3 ml. ' Spectrosol ' hexane (Hopkin & Williams Ltd.). The flask is stoppered and swirled for 3 or 4 min., 4 g. of anhydrous sodium sulphate are added, and 1–2 h. allowed for the hexane extract to dry. From 15 to 50 μl. of the hexane extract, depending on the concentration of acids, are injected on to the column as described for rumen liquor.

Preparation and use of chromatographic columns

Rumen liquor.—The acids are separated on 20% P.E.G.A. – Celite columns prepared as follows. To 2 g. of polyethylene glycol adipate (P.E.G.A.) dissolved in chloroform are added 8 g. of acid- and alkali-washed Celite (Celite 545, Johns Manville Co. Ltd.) of 100/200 mesh grade. After being mixed thoroughly, the chloroform is removed on a steam bath with constant stirring until visibly dry and the Celite finally dried at 100° in an oven overnight. The prepared material is then resieved, packed into the column, and conditioned at 120° for 48 h. The column is run at a temperature of 100° and the nitrogen is reduced to 4½ p.s.i. to achieve complete separation of propionic and isobutyric acids.

Blood.—The component acids are separated on columns prepared from the 120/140 mesh fraction of sieved Celite. The Celite is incinerated at 350° for 4 h., allowed to cool and washed with conc. hydrochloric acid. After repeated washing with distilled water to remove the acid, the Celite is finally rinsed in 0·5% v/v orthophosphoric acid and dried overnight at 100°. The columns are prepared by adding 10 g. of the prepared Celite to 4·5 g. of silicone DC550 and 0·5 g. of stearic acid in 100 ml. of chloroform. The chloroform is removed as previously described and the Celite finally dried by heating at 100° in an oven overnight. The stationary phase is packed by vibration into the glass column to give a nitrogen flow rate of 50 ml./min. at 8 p.s.i.

Milk fat.—The most successful column for our work was one of 20% P.E.G.A.–Celite, prepared as previously described for rumen liquor. The columns are conditioned at a temperature of 175° for 56 h. and the separation of the acids from C_4 to C_{14} is carried out at 155° and a nitrogen pressure of 17 p.s.i.

Results and Discussion

The overall accuracy of the extraction and separation of the acids was tested by using known standard solutions of mixed acids prepared from their sodium salts. Typical results of single analyses of triplicate extractions of a standard solution according to each of the three methods are given in Tables I–III. The automatic burette was also calibrated by adding standard sulphuric acid to the titration chamber and determining the number of divisions on the recording chart equivalent to a standard amount of acid. In this way the total absolute recovery of acids as well as the proportional recovery could be determined.

In all instances the agreement between replicate determinations on actual samples of rumen liquor, blood plasma and milk fat was similar to that found with standard solutions. The coefficients of variation percentages between duplicate estimations for the major component acids in rumen liquor, blood plasma and milk fat were respectively: acetic 0·80, 0·26, -; propionic 1·26, -, -; n-butyric 1·61, -, 1·93; caproic -, -, 1·32; caprylic -, -, 2·35; capric -, -, 2·35; and lauric -, -, 3·13. For the minor constituents the values were larger and varied from 6·0 to 18·0. The relative retention times of the various acids for the three columns used are given in Table IV and the separation of the isomers of butyric and valeric acids was very satisfactory.

Although blood contains formic acid it is found mainly in the red blood cells.[5] The column used in the method described for blood in this paper did separate formic acid and occasionally small amounts of this acid were detected in samples analysed. Its sporadic occurrence in low quantities in the samples investigated is due to the fact that plasma and not whole blood was used. Also, formic acid is not quantitatively distilled from the plasma by the method[6] used and the extraction technique gave low recoveries.

Table I

Analysis of a standard solution of volatile fatty acids according to the procedure for rumen liquor

Acid	Composition of standard solution, %	Composition as determined, %		
		Extraction 1	Extraction 2	Extraction 3
Acetic	64·9	64·1	64·0	64·1
Propionic	15·0	15·5	15·6	15·5
Butyric	13·0	13·0	13·3	13·0
Valeric	7·1	7·1	7·1	7·1

(The total recovery of acids was 94–96%)

Table II

Analysis of a standard solution of volatile fatty acids according to the procedure for blood plasma

Acid	Composition of standard solution, %	Composition as determined, %		
		Extraction 1	Extraction 2	Extraction 3
Acetic	88·7	88·8	89·1	89·0
Propionic	6·3	6·0	6·1	6·1
Butyric	4·9	5·2	4·8	5·0

(The total recovery of acids was 85–88%)

Table III

Analysis of a standard solution of volatile fatty acids according to the procedure for milk fat

Acid	Composition of standard solution, %	Composition as determined, %		
		Extraction 1	Extraction 2	Extraction 3
Butyric	27·9	27·7	27·5	27·0
Caproic	18·0	18·5	17·8	17·8
Caprylic	10·7	10·6	10·6	10·5
Nonylic	0·8	0·7	0·6	0·7
Capric	19·2	19·2	19·0	18·9
Undecylic	2·6	2·1	3·4	3·5
Lauric	21·0	21·3	21·2	21·7

(The total recovery of acids was 98%)

Table IV

Retention times relative to n-butyric acid of volatile fatty acids with the various columns

Acid	Stationary phase		
	20% P.E.G.A. at 155° (milk fat)	20% P.E.G.A. at 100° (rumen liquor)	Silicone–stearic acid at 100° (blood plasma)
Formic	—	—	0·10
Acetic	—	0·41	0·27
Propionic	—	0·61	0·52
Isobutyric	—	0·77	—
n-Butyric	1·00	1·00	1·00
Isovaleric	—	1·21	—
n-Valeric	—	1·81	—
Caproic	2·00	—	—
Caprylic	4·27	—	—
Nonylic	6·86	—	—
Capric	9·07	—	—
Undecylic	14·29	—	—
Lauric	18·86	—	—
Myristic	38·86	—	—

The P.E.G.A.–Celite columns for the analysis of rumen liquor has distinct advantages over that of stearic acid–silicone columns used by other workers, because they are not ruined by the presence of lactic acid in the rumen liquor. Our studies involved the use of cattle diets which caused appreciable concentrations of lactic acid in the rumen and, in preliminary investigations, it was found that with the direct extraction of these samples the presence of lactic acid in more than trace quantities quickly spoiled stearic acid–silicone columns. Also on these low roughage diets there is a significant production of caproic acid in the rumen and the elimination of any interference with the separation by lactic acid allows the separation and determination of this less volatile acid. Consequently, there is no need to purge the columns between analyses, and so far no deterioration has been observed in the efficiency of separation of the acids with continued use of the columns as has been reported by other workers.[4]

Acknowledgments

The authors would like to thank Dr. S. J. Rowland for valuable help and advice.

The National Institute for Research in Dairying,
Shinfield, Reading.

Received 7 December, 1964: amended manuscript 11 February, 1965

References

1 James, A. T., & Martin, A. J. P., *Biochem. J.*, 1952, **50**, 679
2 Bath, I. H., & Rook, J. A. F., *J. agric. Sci.*, 1963, **61**, 341
3 El-Shazly, K., *Biochem. J.*, 1952, **51**, 640
4 Tilley, J. M. A., Canaway, R. J., & Terry, R. A., *Analyst*, 1964, **89**, 363
5 Annison, E. F., *Biochem. J.*, 1954, **58**, 670
6 Scarisbrick, R., *Biochem. J.*, 1952, **50**, xxxiv
7 Folch, J., Lees, M., & Sloane-Stanley, G. H., *J. biol. Chem.*, 1957, **226**, 497

FUMIGATION OF AGRICULTURAL PRODUCTS

XIX*—Methyl bromide fumigation of narcissus bulbs infested with stem nematode, *Ditylenchus dipsaci* (Kühn)

By R. E. PURNELL and N. G. M. HAGUE

Narcissus bulbs are damaged by methyl bromide at dosages (above 500 mg.h./l.) lethal to the nematode. Warm storage before fumigation reduces phytotoxicity but decreases nematode susceptibility.

Introduction

In practice narcissus bulbs are normally lifted every 2 years and stock bulbs (those planted by the grower) are treated with hot water at 110°–111°F for 3–3½ h. to control stem eelworm, *Ditylenchus dipsaci*. Dormant infested narcissus bulbs contain mainly 4th stage pre-adult larvae or 'eelworm wool' able to withstand desiccation for long periods and very difficult to kill.

'Eelworm wool' within the bulb scales tends to survive hot-water treatment protected in air pockets.[1] Dry dormant bulbs are suitable material for fumigation and therefore the effect of methyl bromide on 4th stage larvae of *D. dipsaci* and clean and infested narcissus bulbs was investigated.

Experimental and results

Distribution of D. dipsaci *larvae in Narcissus bulbs*

Ten bulbs, variety Magnificence, were inoculated with 2000 4th-stage larvae and planted in soil in pots. In the following July they were lifted: each bulb was dissected and divided into three parts, (*a*) the outer dry bulb scales, (*b*) the middle portion of the bulb, mainly leaf scales, and (*c*) the inner portion containing the flower primordia. The nematodes were extracted from each part on the Seinhorst mistifier.[2] The results (Table I) show that nearly 3% of the total population was on the outer scales.

Table I

Recovery of nematodes from different parts of narcissus bulbs

	Nematode count (mean of 10 bulbs)	% of Total
Outer scales	1820	2·8
Middle portion	44520	69·1
Inner portion	18080	28·1
Total	64420	

Fumigation of 4th stage larvae of D. dipsaci

Fourth-stage larvae were revived from 'eelworm wool', a convenient stage in which to store *D. dipsaci* for experimental purposes, and a nematode suspension made up in water. Aliquots of 1000 larvae were filtered and the nematodes on the filter paper air-dried for 7h. and stored at 8°C in a constant-temperature room; *D. dipsaci* retains its viability longer at low temperatures.[3]

Filter papers in three-fold replication were fumigated at a range of concentration–time products in a chamber of 345 l. capacity. Chamber dosage and the determination of the effective concentration of methyl bromide were done as described previously.[4]

To estimate mortality, the nematodes on the filter papers were placed on a milk filter pad on a small brass sieve in a Petri dish of water (Oostenbrink Cotton Wool Filter[5]). Nematodes are allowed to migrate for 24 h.; those moving through the sieve were assumed to be alive and were counted.

* Part XVIII: *J. Sci. Fd. Agric.*, 1963, **14**, 577

A complete kill was not obtained (Table II), the dosage response relationship being very similar to that obtained when fumigating air-dry cysts of *Heterodera rostochiensis*.[6] The recovery from the untreated filter papers was about 50% which suggests a considerable loss of viability even when stored at 8°C.

Table II

Effect of methyl bromide on 4th stage larvae of D. dipsaci

Concentration–time products, mg.h./l.	Nematodes recovered	% Mortality
0	545	0
50	412	24
100	330	39
200	313	43
280	242	56
400	87	84
560	17	97
800	9	98

The effect of fumigating stem nematodes on filter paper after conditioning in different environments was investigated. Comparisons were made between keeping nematodes air-dry at 30°C for 24 h. and 1 week at the R.H. of the conditioning chamber and keeping nematodes at laboratory temperature for 1 week at saturated R.H. and laboratory R.H.

The nematodes were fumigated at 20, 30 and 40 mg./l. for 20 h. in a constant-temperature room. The filter papers from the saturated R.H. were maintained at approximately the same R.H. by placing dishes of water in the chamber before fumigation. The number of living nematodes was assessed as in the previous experiment.

Moist nematodes are more easily killed than air-dry nematodes (Table III), thus confirming work on other nematodes.[6] There was evidence that preconditioning nematodes at 30°C in the air-dry state decreased their susceptibility to methyl bromide.

Fumigation of Narcissus bulbs with methyl bromide

(1) *Uninfested bulbs*

To estimate the phytotoxicity of methyl bromide, 'eelworm-free' bulbs of the variety Golden Harvest were fumigated in July, August and September at a range of concentration–time products. Half the bulbs were kept at 30°C for 24h. before fumigation. The batches of 12 bulbs were weighed and immediately after fumigation were planted in drills, fully randomised. The yields, shown in Table IV, were expressed as the percentage increase in yield over the planting weight for both years of lifting. For analysis treatment yields were expressed as a percentage of the yield from untreated bulbs.

A dose of 560 mg.h./l. significantly (P=0·001) effected yields. There was no significant decrease in yields at the other three doses. There was a slight decrease in yields compared with untreated bulbs in the second year crop but it was not significant.

Table III

Effect of temperature and humidity on death of nematodes

Pre-fumigation temperature and R.H.	Concentration–time products, mg.h./l.		
	400	600	800
Laboratory temperature and R.H.	209	34	16
Laboratory temp. and saturated R.H.	4	2	1
24 h. at 30°C*	219	65	32
1 week at 30°C*	224	124	71

Number of untreated nematodes=660

* at the R.H. of the constant temperature chamber.

Table IV

Effect of methyl bromide on the yield of 'eelworm-free' narcissus bulbs: expressed as percentage increase in yield over planting weight

Month of treatment	Year of lifting	Concentration–time products, mg.h./l. 0 Preheat +	0 Preheat −	200 Preheat +	200 Preheat −	280 Preheat +	280 Preheat −	400 Preheat +	400 Preheat −	560 Preheat +	560 Preheat −
July	One	151	132	162	146	138	130	144	143	129	89
	Two	263	254	286	272	230	187	227	177	189	150
August	One	139	147	140	138	140	149	154	128	140	102
	Two	218	198	192	240	219	274	235	159	136	171
September	One	156	170	160	156	153	158	152	145	127	96
	Two	312	267	228	246	260	252	260	250	228	203

(2) *Fumigation of infested bulbs*

In a preliminary experiment 'eelworm free' bulbs were inoculated with 400 4th-stage larvae and after one year's growth the dormant bulbs were fumigated. Results were very unsatisfactory because in many treatments no bulbs survived treatment, and therefore lightly infested bulbs from a naturally infested stock of Helios were used. Treatments of batches of 500 bulbs were as follows: 30 mg./l. for 20h. (concentration–time product C.T.P. 600 mg./l.); 30 mg./l. for 20 h. (C.T.P. 600) after warm dry storage at 30°C for 1 week; and untreated bulbs.

After treatment the bulbs were planted in rows 6 ft. apart. In the following year the flowers were counted and half the rows lifted, the bulbs weighed and the number of nematodes per bulb estimated (Table V).

Table V

Effect of methyl bromide on bulbs naturally infested with stem nematodes

	Control	600 mg.h./l. +1 week at 30°C	600 mg.h./l.
Number of bulbs lifted (from 250 planted)	295	251	197
Bulbs showing severe eelworm damage and spickel lesions	112	12	42
% increase in weight over planting weight	279	223	119
Flowers produced (500 plants)	259	148	49
Nematode count per bulb	2200	1585	440

In the first year after treatment methyl bromide caused a very marked decrease in bulb growth (yield and flowers) and a high proportion of nematodes were killed. Storage at 30°C for 1 week before treatment reduces nematode kill but at the same time modified the effect on plant growth.

In the second year many of the bulbs in the untreated row did not grow as they were so heavily damaged by nematodes and counts of bulbs, yields and flower counts were impossible. Nematodes were extracted from bulbs which showed some sign of growth (Table VI).

Discussion

In vitro experiments showed that air-dry nematodes desiccated on filter papers are only effectively killed at a C.T.P. of 800 mg.h./l. At high R.H. a commercially complete kill is obtained at 400 mg.h./l.: warm storage before fumigation resulted in a slightly reduced mortality.

In field trials 'eelworm free' bulbs tolerated a methyl bromide dosage of 560 mg.h./l. although there was considerable phytotoxicity. Experiments on infested bulbs gave substantial nematode control at 600 mg.h./l. accompanied by quite severe phytotoxicity. Warm storage for 1 week at 30°C before treatment reduced phytotoxicity and decreased the susceptibility of nematodes.

Similar results have been obtained fumigating dormant tulip bulbs.[7] The present results confirm earlier work[8] that, while methyl bromide does control nematodes, it is also phytotoxic.

Table VI

Nematode counts from bulbs 2 years after fumigation with methyl bromide

	Control	600 mg.h./l. +1 week at 30°c	600 mg.h./l.
Nematodes extracted from 100 c.c. of macerated bulb tissue	32,900	1080	162

It is of interest to note that warm storage before treatment modifies the effect of the fumigation because the same pre-treatment conditioning reduces the phytotoxicity caused by subsequent hot-water treatment,[9] a result for which no satisfactory explanation has been given.

Although fumigation of dormant bulbs with methyl bromide under polythene sheets or in air-tight storage chambers is a practical proposition, experimental results suggest that the limits of nematode control and phytotoxicity overlap.

Acknowledgment

This investigation has been carried out with the aid of a grant from the Agricultural Research Council whose interest and assistance is hereby acknowledged. The work also forms part of a thesis for the Ph.D. degree, University of London.

Imperial College Field Station,
Ashurst Lodge,
Sunninghill, Ascot,
Berkshire.

Received 2 February, 1965

References

[1] Staniland, L. N., *J. Helminth.*, 1950, **24**, 91.
[2] Webster, J. M., *Nematologica*, 1964, **8**, 245.
[3] Palo, A. V., *Nematologica*, 1962, **7**, 122.
[4] Lubatti, O. F., & Blackith, R. E., *J. Sci. Fd Agric.*, 1956, **7**, 149.
[5] Oostenbrink, M., *Meded. LandbHoogesch. Gent*, 1954, **19**, 377.
[6] Hague, N. G. M., *Plant. Path.*, 1959, **8**, 68.
[7] Griffin, D. E., private communication
[8] Thomas, P. R., M.Sc. Thesis, Univ. Wales, 1960
[9] Purnell, R. E., & Hague, N. G. M., *Hort. Res.*, in the press.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1965

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Soil genesis in temperate humid climates. III. Some other soil groups. J. van Schuylenborgh (*Neth. J. Agric. Sci.*, 1964, **12**, 190–203). Characteristics of the formation of Grey-Brown/Red-Yellow Podzolic Intergrade, Rendzina, Grumusol and Grumusolic, Braunerde and Brown Forest soils are discussed. A. H. CORNFIELD.

Soil/water relations during rain infiltration. III. Water uptake at incipient ponding. J. Rubin and R. Steinhardt (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 614—619).—Theoretical expressions for the bounds of water uptakes at rain-induced incipient ponding were derived. These expressions represented well the measured relation between rain intensities and cumulative rain uptakes by a soil on the surface of which puddles just started to form. A. H. CORNFIELD.

Circulation of water in soil under a temperature gradient. R. D. Jackson, D. A. Rose and H. L. Penman (*Nature, Lond.*, 1965, **205**, 314—316).—When a temp. gradient is applied to a uniform closed soil column a circulating system is set up with a vapour flow moving from hot to cold and a return liquid flow from cold to hot.
S. A. BROOKS.

Oxygen diffusion and aerobic respiration in columns of fine soil crumbs. D. J. Greenwood and D. Goodman (*J. Sci. Fd. Agric.*, 1965, **16**, 152—160).—The aeration status of a soil, with regard to microbial respiration, can be defined approx. in terms of the proportion of soil having an O_2 concn. $>3\mu M$ and is therefore an aerobic zone. The validity of a given equation was tested by two methods, one was based on the equation, the other was not. The mean lengths of the aerobic zones were approx. the same, 4·3 and 3·7 cm., respectively. What is required is a method of assessing the probability of a field soil having aerobic zones over a period of, e.g., a year's duration. Methods based on the proposed equation appear to offer a better but imperfect means of assessing the aeration of field soils than would any direct method. E. M. J.

Measuring root responses to soil properties with a single plant. M. L. Giskin and H. Kohnke (*Agron. J.*, 1965, **57**, 96—97).—The seed is planted in a small central chamber filled with sand. Surrounding this chamber are 2—4 compartments (containing the soils under test) separated from it by wax membranes, which are impervious to air and water, but permeable to plant roots. The relative amount of root growth in the outer compartments is a reflection of soil properties. The method showed clearly the deleterious effects of weed root residues on the development of maize roots. A. H. CORNFIELD.

Relationship between oxygen diffusion rate and maize growth. J. Letey, L. H. Stolzy and N. Valoras (*Agron. J.*, 1965, **57**, 91—92).—The O_2 diffusion rate (ODR) in the surface layer of a silt loam growing maize and subjected to air containing <1 to 21% O_2 increased with level of O_2 in the air. At lower soil depths ODR also increased with level of O_2 in the air, but at a lower rate. Plant growth was reduced only when the air contained $<2\%$ O_2. N, P and K % in the tissue were reduced when the air contained $<2\%$ O_2. Na% in the tissue was high when the air contained $<2\%$ O_2, but was negligible when the air contained 2% or more of O_2. A. H. CORNFIELD.

Sterile culture of excised tomato roots in sands of different grain size. L. K. Wiersum (*J. exp. Bot.*, 1964, **15**, No. 45, 568—573).—Root development in sand was related to the degree of aeration rather than to mechanical impedance due to particle size.
A. G. POLLARD.

Performance of tillage implements in a stubble mulch system. I. Residue conservation. II. Effects of soil cloddiness. III. Effects of tillage sequences on residues, soil cloddiness, weed control and wheat yield. N. P. Woodruff, C. R. Fenster, W. S. Chepil and F. H. Siddoway. (*Agron. J.*, 1965, **57**, 45—49, 49—51, 52—55).—I. The amount of crop residue retained on the soil surface after initial tillage of winter wheat stubble varied with the height of stubble, amount of pretillage residue and spacing between stubble rows. During subsequent tillage residue retention was most strongly influenced by the previous method of tillage.

II. Soil cloddiness varied with type of initial and subsequent tillage and soil moisture at time of tillage.

III. Tillage sequences produced no noticeable effect on soil cloddiness at the end of the season. Better weed control was obtained with tillage sequences which included one-way disking. Tillage sequences influenced wheat yields more in dry than in wet years. A. H. CORNFIELD.

Cation status of soil moisture. V. Effect of soil moisture tension on growth and cation uptake by plants. P. Moss (*Amer. Potato J.*, 1964, **20**, 271—286).—Increasing soil moisture tension (pF 0—3) resulted in an increase in the cation concn. of both soil solution and plant material (carrot). The value of $\log K - 0{\cdot}51\log(Ca + Mg)$ in the plant was constant over the soil moisture tension range studied and was a reflection of the characteristic K intensity status of the soil solution. The concn. ration K/(Ca + Mg) in the plant varied with moisture tension in the same way as the ratio varied in the soil solution and was therefore a reflection of both the soil solution composition and soil moisture tension. A. H. CORNFIELD.

Ion adsorption on charged surfaces. W. R. Heald, M. H. Frere and C. T. DeWit (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 622—627).—Equations describing ion adsorption on a charged surface are based on a model of equilibrium between ion pairs at the surface and a diffuse Gouy layer. Selectivity for adsorbed ions is related to the charge density, the constants for ion-pair formation, and the total concn. of ions in the bulk solution. Material with high charge density is a special case which results in simplified equations. These simplified equations were confirmed with Dowex-50 resin.
A. H. CORNFIELD.

Iron and aluminium oxide coatings in relation to sulphate adsorption characteristics of soils. T. T. Chao, M. E. Harward, and S. C. Fang (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 632—635).—The extent of adsorption of SO_4^{2-} from aq. solution increased with the amount of Al or Fe oxides coated on to the soil particles. The extent of adsorption decreased with increasing pH (3—7) with Fe oxide-coated soil. With the Al oxide-coated soil adsorption increased with pH up to 4 and then decreased with further increase in pH.
A. H. CORNFIELD.

Longevity of soil reaction effects following lime and alum additions. T. B. Hutcheson, jun., and J. F. Freeman (*Agron. J.*, 1965, **57**, 89—90).—Incorporation of $Ca(OH)_2$ or $Al_2(SO_4)_3$ into the upper 4 in. of a silty clay loam resulted in the greatest changes in pH 6 weeks after application, but thereafter pH tended to approach that of the controls, although small differences due to treatment were still evident after 3 years. The treatments had little effect on exchangeable Al, Ca or Mg below the 8-in. depth. A. H. CORNFIELD.

Relationship of exchangeable sodium percentage at different soil pH levels to hydraulic conductivity. J. P. Martin, S. J. Richards and P. F. Pratt (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 620—622).—For any level of Na (as % of exchangeable Ca + Mg + K + Na + Al) in the exchange complex hydraulic conductivity was less in acid than in neutral or alkaline soils. For six soils there were highly significant negative correlations between hydraulic conductivity and exchangeable Na expressed as a % of exchangeable Ca + Mg + K + Na.
A. H. CORNFIELD.

Ion diffusion. I. Quick-freeze method for the measurement of ion diffusion in soil and clay systems. D. A. Brown, B. E. Fulton and R. E. Phillips (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 628—632).—The method combines a radio-isotope tracer technique for measuring ion distribution by quick-freezing the exchange medium and sectioning it with a refrigerated microtome. After radiation assay of the sections the distribution resulting from ion diffusion in montmorillonite and kaolinite clays and two soils was obtained.
A. H. CORNFIELD.

Competition of ammonia and water for adsorption sites on clay minerals. D. W. James and M. E. Harward (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 636—640).—Ammoniation of air-dry Ca-, Mg-, and Al-montmorillonite and bentonite resulted in dehydration of the exchangeable cations and a change in C axis spacing from 15—16Å to 12·6—13Å. Ammoniation of a vac.-dried system resulted in a

change in c-axis spacing from 10·1Å to 12·3Å. NH_3 retained by the minerals upon ammoniation was replaced by water under controlled conditions of R.H. A. H. CORNFIELD.

ABS [alkylbenzenesulphonate] adsorption on soils. M. J. Suess (*J. Wat. Pollut. Control Fed.*, 1964, **36**, 1393—1400).—The factors affecting adsorption are considered. The rate and intensity of adsorption was determined for a variety of siliceous, calcareous and silty clay soils. Adsorption obeys the Freundlich equation and the intensity of adsorption increases with grain size and concn. Adsorption is affected by mineralogical composition but no soil was completely covered by an ABS monomol. layer. (10 references.)
E. C. DOLTON.

Effect of soil moisture tension on carbon dioxide evolution, nitrification and nitrogen mineralisation. R. D. Miller and D. D. Johnson (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 644—647).—Incubation (30°) of four soils at varying moisture tensions resulting in increasing CO_2 production with decreasing moisture tension from the air-dry state (>50 bars) to a max. at tensions ranging from 0·50 to 0·15 bars and then decreasing CO_2 production with further decrease in moisture tension to zero bar. After 14 days' incubation there was little difference in CO_2 release in the tension range 0—0·15 bar. Max. nitrification and N mineralisation occurred in the tension range, 0·50—0·15 bar, but N mineralisation usually increased again at zero tension, due mainly to NH_3 accumulation. A little nitrification occurred sometimes even at moisture tensions >15 bars.
A. H. CORNFIELD.

Relationships between soil cation-exchange capacity and the toxicity of ammonia to the nitrification process. J. H. Smith (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 640—644).—The rate of nitrification of NH_3 from lucerne particles decomposing in soil decreased with diminution in exchange capacity of the soil, obtained by dilution with sand. NO_2^- which accumulated in the early period of incubation disappeared most rapidly in the soil of highest exchange capacity. Nitrification decreased and NO_2^- accumulation increased with decreasing particle size of lucerne. A. H. CORNFIELD.

Soil nitrogen loss as a result of alternate submergence and drying W. H. Patrick, jun., and R. Wyatt (*Proc. Soil Sci. Soc. Amer.*, 1964, **28**, 647—653).—Up to 20% of the total N of a silt loam was lost by alternate submergence and drying, with most of the loss occurring during the first 2—3 cycles. No loss of N occurred from a sample kept moist but not waterlogged, and a little loss occurred from a sample kept permanently waterlogged. Soil redox potential decreased rapidly after the initial emergence, but decreased more slowly with successive submergences after drying. The ratio of C/N lost was similar to that in the soil. A. H. CORNFIELD.

Phosphorus mobilisation in a calcareous soil in relation to surface properties of roots and cation uptake. R. L. Fox and B. Kacar (*Plant & Soil*, 1964, **20**, 319—330).—Several legume and grass species were grown in pots in a calcareous silty clay loam (11% $CaCO_3$), following which a barley or lettuce indicator crop was grown to evaluate change in the P status of the soils. Yields and P% of the indicator crop were higher following legumes than following grasses. Although there were indications of greater P mobilisation concurrent with growth of legumes (which have high root cation-exchange capacity) than with grasses (low root cation-exchange capacity), there was little evidence that differences among legumes or grasses in P mobilised was related to the cation-exchange capacity of the roots. The legumes and grasses themselves extracted about the same amounts of P. Since grasses absorbed more soil-K than did legumes, whilst the reverse was true for soil-Ca, the changes in cation status of the soil may be as important as the effects of root cation exchange capacity in affecting P availability to the following crop.
A. H. CORNFIELD.

Changes in phosphate potential on re-wetting air-dry soil. R. C. Salmon (*Nature, Lond.*, 1965, **205**, 316).—When dry soil was moistened to field capacity the $Ca(H_2PO_4)_2$ potential and the pH gradually increased to approach a constant value after 4—5 days.
S. A. BROOKS.

Effect of organic manures on natural enemies of nematodes in soil. B. A. Oteifa, D. M. Elgindi and H. Z. Abduleid (*Plant Dis. Reptr*, 1964, **48**, 894).—The effects of different types of org. manures on the occurrence of predaceous nematodes and nematode-trapping fungi were studied. Many of the materials encouraged the development of groups or specific varieties of these nematode-controlling organisms.
A. H. CORNFIELD.

Effect of nitrogen and phosphorus on the decomposition of straw and the accumulation of individual B-vitamins in soil. J. Szegi and F. Gulyás (*Z. Bakt.*, 1964, II, **118**, 491—499).—Addition of NH_4NO_3 to a limed chernozem or to a Brown Forest soil, to each of which had been applied 2% of powdered straw, greatly increased CO_2 production in the soil. $CaHPO_4$ slightly increased straw decomposition only in the forest soil. In absence of added straw neither N nor P affected CO_2 production in the soil. Addition of straw greatly increased the formation of thiamine, pantothenic and nicotinic acids, pyridoxine, and biotin. Production of the vitamins was further increased by applications of K and P. A. G. POLLARD.

Aspergilli. XIV. Effect of foliar sprays of urea on the Aspergilli of the rhizosphere of *Triticum vulgare* L. V. P. Agnihotri (*Plant & Soil*, 1964, **20**, 364—380).—Treatment of *Triticum vulgare* with urea sprays increased the no. of Aspergilli present in the rhizosphere and increased the concn. of amino-acids (particularly γ-aminobutyric acid and glutamine) but reduced that of org. acids in the plant.
A. H. CORNFIELD.

Anti-microbial effects of sorbic acid. V. Destruction and fermentation of sorbic acid by various micro-organisms. H.-J. Rehm, C. Nummermann-Vaupel and P. Wallnöfer (*Zbl. Bakt.* 1964, **II**, **118**, 472—482).—*Aspergillus niger* and *Pseudomonas fluorescens* decomposed sorbic acid in sub-lethal concn., particularly in respiratory processes. *Escherichia coli* and *Bacillus. mycoides* were strongly inhibited by **I** and were unable to utilise or decompose it. *Lactobacillus buchneri* and *L. arabinosus* developed in presence of high concn. of **I**. A. G. POLLARD.

Determination of urease activity in soils. P. E. le R. van Niekerk (*S. Afr. J. agric. Sci.*, 1964, **7**, 131—134).—The soil sample is sterilised by γ-radiation and transferred with a standard sterile urea solution and a PO_4^{3-} buffer (pH6·7) to a graduated flask and incubated at 37°. The suspension is decolorised (activated animal C) and filtered. Residual urea in the clear solution is determined by the *p*-dimethylaminobenzaldehyde method (Watt and Chrisp, *Analyt. Chem.*, 1954, **26**, 452). A. G. POLLARD.

Irrigation and land disposal of pulp mill effluents. R. O. Blosser and E. L. Owens (*Wat. Sewage, Wks.*, 1964, **111**, 424—432).—Problems associated with this method of disposal are briefly surveyed. In four representative soils (sand, silt and two clay loams) BOD, colour, pH, lignin, conductivity and ion measurements were carried out. The significance and value of cover vegetation is discussed and it is considered doubtful if land can be used economically for irrigation disposal unless the programme can be tailored around this factor. Effluents having a sodium adsorption ratio of >8 can be used satisfactorily as irrigants on most soils except on some clays in which lower values are indicated if soil deflocculation is to be avoided. C. V.

Soil analysis as guide to magnesium fertilisation of oats. C. M. J. Sluijsmans (*Verslv landbouwk. Onderz.*, 1964, 643, 37 pp.).—Requirements of soil-MgO for satisfactory yields on sandy soils vary from 32 p.p.m. at soil-pH 5·5 to 66 p.p.m. at pH 3·5; at lower pH and soil-MgO, Mg fertilising will be necessary; if the soil-pH is increased to <4·5 by liming, 50 kg. of MgO per hectare (applied as $MgSO_4$) will generally suffice even at low soil-MgO values. (14 references.)
P. S. ARUP.

Suggested foliar sampling and handling techniques for determining the nutrient status of some field, horticultural and plantation crops. H. D. Chapman (*Fruits, Paris*, 1964, **19**, 367—377).—Results published on the nutrient status of plants deduced from leaf analysis are not comparable with each other due to variations in sampling technique. Suggested standard sampling techniques for many plants, to overcome this difficulty, are tabulated. (105 references.)
W. ELSTOW.

Adsorption of ammonium ion by clay from different fertiliser grades of ammonium compounds. S. Mukherjee, H. Roy and B. K. Banerjee (*Technology* [*Quart. Bull. Fertil. Corp. India*], 1964, **1**, No. 3, 8—11).—Max. adsorption of NH_4+ ions occurs from $(NH_4)_2SO_4$, NH_4NO_3 + $(NH_4)_2SO_4$ double salt 2 : 3 by wt. and $CaCO_3$ + NH_4NO_3 (mixture 2 : 3 by wt.) and min. adsorption from NH_4NO_3. Addition of small amounts of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ to NH_4NO_3 increases NH_4+ adsorption. E. C. DOLTON.

Direct estimation of calcium nitrate in calcium ammonium nitrate fertiliser. A. K. Roy and R. M. Bhatnagar (*Technology* [*Quart. Bull. Fertil. Corp. India*], 1964, **1**, No. 3, 31—32).—$Ca(NO_3)_2$ is extracted with ammoniacal MeOH, the extract is evaporated to dryness, and the residue is dissolved in water. Ca is determined by the $KMnO_4$ method. Results agree well with those obtained using Devarda's alloy. E. C. DOLTON.

Vacuum oven method for [determining] free water in fertilisers. J. H. Caro and N. A. Heinly (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1040—1047).—If the sample is dried for 2 h. ± 10 min. at 50 ± 1·5° under an absolute pressure of 9—11 in. the results obtainable are as precise as those afforded by the official (A.O.A.C.) vac. desiccation method. A. A. ELDRIDGE.

Separation of various forms of nitrogen in fertilisers. J. M. O'Neal and K. G. Clark (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1054—1056).—By the use of cation (Dowex 50W—XS, Na+ form) and anion (Dowex-21 K, Cl^- form) resin-exchange columns in series, PO_4^{3-} is removed from the solution containing urea, and the N present as NH_3, NO_3 and amide can be determined without double pptn. A. A. ELDRIDGE.

Improved ammonium molybdophosphate method for [determining] phosphorus in fertilisers. J. A. Brabson, F. J. Johnson, J. W. Williard and W. G. Burch (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1028—1034).—A study of the official (A.O.A.C.) method for the volumetric determination of P in fertilisers has led to modifications that improve its precision and accuracy. NH_3 is complexed by addition of formaldehyde, glass filter pads are used and if the sample contains SO_4^{2-} extra HNO_3 is added before addition of molybdate. Pptn. must be limited to the temp. range 20—30°. A. A. ELDRIDGE.

Improved alkaline citrate method for evaluation of phosphorus in fertilisers. J. A. Brabson and W. G. Burch (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1048—1054).—In the alkaline NH_4 citrate extraction method for determining available P in fertilisers the water-insol. portion of the sample is extracted for 2 h., with shaking, at 65°. Ca_2HPO_4, but not apatite, dissolves. Results correlate well with those of field tests. A. A. ELDRIDGE.

Composts. Eclipse Peat Co. Ltd. (Inventor: T. M. W. Alexander) (B.P. 948,735, 31.12.59).—There is claimed a loam-free potting compost comprising assimilable N (10—6000), assimilable K (50—3700), assimilable P (30—1700) p.p.m.), peat (of specified grain size and 1—25 wt.-% colloidal content), and inert material, e.g., crushed glass, crushed brick, vermiculite or perlite, of grain size >0·375 in. (up to 80 vol.-% on compost). Such a compost has good water retention and excellent aeration; it has very large plant retention capacity so that fertilisers can be applied with greater safety; and it is much lighter than John Innes type of composts requiring addition only of water before use. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Effects of day length and light intensity on growth of barley. III. Vegetative development. D. Aspinall and L. G. Paleg (*Aust. J. biol. Sci.*, 1964, **17**, 807—822).—The effects of variations in light intensity, photoperiod and light quality on shoot dry wt., tillering and leaf growth of barley were examined in controlled environments. The rates of tillering and dry matter production were primarily dependent on the total radiant energy on the plants. The rate of leaf emergence was relatively insensitive to changes in light intensity but mature leaf size and shape were indirectly affected by the spectral composition of the light source. (17 references.) S. A. BROOKS.

Carbohydrate utilisation as a factor in plant growth. R. L. Burt (*Aust. J. biol. Sci.*, 1964, **17**, 867—877).—The growth of potato plants (*Solanum tuberosum* L.) was studied over a period of 13 days. The dry wt. of the principal organs, leaf areas and sol. sugar contents of selected leaves were determined on four occasions. Removal of tubers 21 days after the commencement of tuber initiation reduced the absolute growth rates and net assimilation rates of the plants. It is suggested that the assimilisation of plants under high light intensities may be restricted by their ability to utilise or store products of photosynthesis. (19 references.) S. A. BROOKS.

Influence of alien genome combinations on protein synthesis in cereals. F. C. Yong and A. M. Unrau (*Canad. J. Biochem.*, 1964, **42**, 1647—1657).—Starch-gel electrophoresis of the water-sol., salt-sol. and alcohol-sol. preoteins of *Triticale*, *Triticum durum*, *Secale cereale*, *Triticum vulgare* and *Tritipyron* revealed both qual. and quant. differences. Experimental evidence obtained indicated that the biosynthetic potential of the alien genomes in the synthetic species (*Triticale*) was not fully maintained. A variable influence of the tetraploid wheat (*T. durum*) genomes on protein synthesis in the three hexaploid cereals (*Triticale*, *T. vulgare* and *Tritipyron* was observed. (18 references.) S. A. BROOKS.

[A]. **Potassium uptake and transport in roots of *Ricinus communis*.** D. J. F. Bowling and P. E. Weatherley. [B] **Active transport of ions across the root of *Ricinus communis*.** D. J. F. Bowling and R. M. Spanswick (*J. exp. Bot.*, 1964, **15**, No. 45, 413—421, 422—427).—[A] Seedlings of *R. communis* were grown in nutrient solutions and de-topped after 3—4 weeks. Exudates from the cut stumps were collected. The transport of K from the nutrient to the plant vessels was closely related to the [K] of the nutrient; the amount translocated was only a small portion (~1%) of that taken up by the roots. K retained by the roots tended to become unavailable for transport. A definite relationship was apparent between the [K+] in the nutrient (as KNO_3) and that in the exuded sap. This relationship was altered by the presence of other ions in the nutrient.

[B] Measurements of the difference in electrical potential between the exudate from de-topped plants and that of the nutrient medium in which they had been growing are compared with the corresponding [K+]. The latter indicate that the movement of K into the sap is a passive process whereas that of Cl^- is an active process against a potential gradient. Probably K+ and Cl^- pass through the diffusion barrier at which active transport occurs before exchanging with other ions which appear in the sap. A. G. POLLARD.

Influence of anions on the uptake of calcium and magnesium by plants and on calcium and magnesium movement in soils. J. T. Gillingham and N. R. Page (*Agron. J.*, 1965, **57**, 83—88).—The uptake of Ca by sunflowers was increased by soil application of Cl^- and NO_3^-, but not by SO_4^{2-} or PO_4^{3-}. Mg uptake was increased by all anions except Cl^-. In a lysimeter experiment the downward movement of Ca and Mg added with various anions decreased in the order NO_3^-, Cl^-, SO_4^{2-}, PO_4^{3-}. A. H. CORNFIELD.

Manganese uptake by excised oat roots. E. R. Page and J. Dainty (*J. exp. Bot.*, 1964, **15**, No. 45, 428—443).—The uptake of Mn from aq. $MnCl_2$, labelled with ^{54}Mn, by excised roots of oat plants is not a metabolic process. The initial 'fast' phase of intake is an ion exchange completed in about 30 min. The subsequent 'slow' phase represents the movement of Mn initially absorbed on cell walls etc., the process occupying >3 h. The two phases probably take place simultaneously and independently. A. G. POLLARD.

Foliar uptake and translocation of caesium. J. Moorby (*J. exp. Bot.*, 1964, **15**, No. 45, 457—469).—The uptake of ^{137}Cs by pea leaves and its subsequent translocation was examined in plants grown for a period in light or in darkness. Both processes were restricted in darkness and the amount of Cs transported downwards in the plants was lessened. This limitation of movement was associated with changes in carbohydrate metabolism in the treated leaves. A. G. POLLARD.

Absorption of phosphorus-32 by leaves of *Glycine max* of different ages. G. E. Ahlgren and T. W. Sudia (*Bot. Gaz.*, 1964, **125**, 204—207).—A buffered solution (pH 3·4) of ^{32}P-labelled NaH_2PO_4 was applied to individual leaves of soya-bean plants of different ages. Absorption of P was greatest in immature leaves and decreased with advancing age to a fairly steady rate. Cotyledons translocated ^{32}P but the second and third tri-foliate leaves did not do so. A. G. POLLARD.

Foliar, floral and root absorption of strontium-90 by crops. E. J. Evans and A. J. Dekker (*Agron. J.*, 1965, **57**, 82—83).—The ^{90}Sr% in lucerne, timothy, oats and wheat grown in the open was considerably higher than that of comparable species grown under clear plastic tents. A. H. CORNFIELD.

Effect of divalent cations on the uptake of salt by beetroot tissue. M. G. Pitman (*J. exp. Bot.*, 1964, **15**, No. 45, 444—456).—The uptake of Cl^- from aq. $CaCl_2$ and KCl by beetroot slices may become greater than from aq. KCl of the same total [Cl^-]. Probably the uptake of Cl^- limits the uptake of K+ and Ca tends to increase the Cl^- uptake. A possible mechanism of this process is discussed. A. G. POLLARD.

Rôle of boron in plant growth. III. Effects of differentiation and deficiency on radicle metabolism. C. R. Slack and W. J. Whittington (*J. exp. Bot.*, 1964, **15**, No. 45, 475—513).—In field beans grown in culture media the influence of B on some cell constituents was traced by observing the incorporation of ^{14}C in various components of root cells in different zones of the roots in presence and in absence of B. B deficiency increased the incorporation of ^{14}C into pectic matter and also increased acid resistance in the early stages of the deficiency. B is probably concerned with the bonding of the cell wall. (34 references.) A. G. POLLARD.

Comparison of the boron requirements of intact tomato plants and excised tomato roots grown in sterile culture. T. F. Neales (*J. exp. Bot.*, 1964, **15**, No. 45, 647—653).—In excised tomato roots grown in a culture solution B-deficiency inhibited growth after 3 days. Concn. of B which supported 50% of normal growth were similar in intact plants and in excised roots. The bearing of these observations on the function of B in plants is discussed. A. G. POLLARD.

2, 6-Dichlorobenzonitrile and boron deficiency [in plants]. B. V. Milborrow (*J. exp. Bot.*, 1964, **15**, No. 45, 515—524).—Symptoms of 2, 6-dichlorobenzonitrile (**I**) poisoning in a no. of plant species are compared with those produced by phenylboronic acid and B-deficiency. Macro- and micro-scopic effects and the influence on ability to translocate growth regulators are sufficiently similar to suggest that **I** and B-deficiency affect the same basic process(es) in the plants. (31 references.) A. G. POLLARD.

Translocation in sugar-beet. I. Assimilation of $^{14}CO_2$ and distribution of materials from leaves. K. W. Joy (*J. exp. Bot.*, 1964, **15**, No. 45, 484—494).—Leaves were exposed to labelled CO_2 and the subsequent movement of ^{14}C within the plant was examined. Young leaves assimilated ^{14}C for their own growth and very little passed into other organs of the plant. Fully grown leaves exported most of the photosynthate to younger leaves and to the root. No movement of ^{14}C occurred from any particular leaf to any other which had emerged before it. Transported $^{14}CO_2$ was stored in leaves as insol. material or in roots as insol. matter plus sucrose.
A. G. POLLARD.

Lateral movement of inorganic solutes in plants. D. A. Baker and J. A. Milburn (*Nature, Lond.*, 1965, **205**, 306—307).—Rooted stumps of *Coleus frederici* cuttings were examined by the root technique. In this way it was shown that lateral tensions are normally small but can develop and induce lateral movement of both water and solutes.
S. A. BROOKS.

Effects of various growth regulators on *Datura meteloides*. J. H. Bennett and L. A. Sciuchetti (*J. pharm. Sci.*, 1964, **53**, 1254—1256).—Growth of *Datura* plants was not significantly affected by treatment for 4 weeks with 4-hydroxy-5-isopropyl-2-methylphenyltrimethylammonium chloride 1-piperidinecarboxylate (**I**), (2-chloroethyl) trimethylammonium chloride, allyltrimethylammonium bromide, *N*-dimethylaminomaleamic acid (**II**) or *N*-dimethylaminosuccinamic acid (**III**) (applied as spray) or 2,4-dichlorobenzyltributylphosphonium chloride (**IV**) (applied as soil drench). The concn. of alkaloids in the roots was increased by **I** (26%) and **IV** (17%) and the total alkaloid content of the roots increased by **I** (32%) and **III** (19%) and of the leaf tops by **II** (25%). Chlorophyll content of the leaf tops was increased (18%) by **IV**, **III** and **II**. Selective solvent extraction of dry leaf tops indicated further differences, and more pronounced effects on growth would probably be caused by the compounds under different conditions. (20 references.) J.A.C. Abstr.

Effects of gibberellin and growth-retarding chemicals on respiration and catalase activity in various organs of cucumber seedlings. A. H. Halevy (*J. exp. Bot.*, 1964, **15**, No. 45, 546—555).—The seedlings were grown in darkness on filter paper treated with aq. gibberellin (**I**) or the growth retardant Amo-1618 (**II**). **I** increased the respiration and catalase activity of the hypocotyl and cotyledons, but not that of roots, and diminished root respiration. **II** decreased the respiration of hypocotyl and cotyledons and increased root respiration and also increased catalase activity in all three plant organs. The opposite effects of the two reagents were mutually antagonised when both were present. (35 references). A. G. POLLARD.

Increase in fruit set of *Vitis vinifera* by treatment with growth retardants. B. G. Coombe (*Nature, Lond.*, 1965, **205**, 305—306).—Single inflorescences of various grape vines have been treated with 2-chloroethyltrimethylammonium chloride, tributyl-2,4-dichlorobenzylphosphonium chloride and gibberellic acid at 2—3 weeks pre-anthesis, anthesis and 2—3 weeks post-anthesis. All three substances increased fruit set in three out of four cultivars with different types of berry development, when applied before anthesis.
S. A. BROOKS.

Crops and Cropping

Relative water requirement and its relationship to tiller number and heading dates in eight oat varieties. N. W. Widstrom, D. D. Harpstead and D. B. Shank (*Agron. J.*, 1965, **57**, 68—70).—There were significant differences among eight varieties of oats in water requirement even allowing for variations in tiller no. and heading date. Neither character was closely associated with water use. Although environment affected water requirement considerably the varieties retained their ranks with respect to water requirement.
A. H. CORNFIELD.

Yields of barley following leys based on different grass species. J. L. Hammerton and R. S. Edwards (*J. agric. Sci.*, 1965, **64**, 3—9).—Both the grass species of the previous ley and the fertiliser applied to it affect the succeeding barley field. In most years yield following a timothy ley was the lowest, whilst the highest followed perennial rye-grass and occasionally cocksfoot. A significant linear relationship exists between yield and fertiliser treatment in the range of 0, 1, 3, 5, 7 and 9 cwt./acre. M. LONG.

Competition of quackgrass, *Agropyron repens*, with oats and lucerne. J. H. Ohman and T. Kommedahl (*Weeds*, 1964, **12**, 222—231).—Poor growth of oats and lucerne when grown with quackgrass in solution cultures was due to the ability of quackgrass to withdraw P, K and N (especially). No evidence was obtained of any phytotoxic substance from living quackgrass roots or rhizomes that could inhibit germination or growth of lucerne and oats. When quackgrass residues were added to soil growing oats or lucerne chlorosis and stunting occurred, but this was probably due to the wide C/N ratio of the residues causing N immobilisation. Extracts of soil containing decomposing quackgrass residues were phytotoxic only when decomposition was occurring at high moisture levels under anaerobic conditions. A. H. CORNFIELD.

Influence of oxygen and carbon dioxide on germination and seedling development of maize. P. W. Unger and R. E. Danielson (*Agron. J.*, 1965, **57**, 56—58).—The germination rate of maize seeds in closed containers was similar with pure O_2 pressure ranging from 0 to 150 cm. Hg. Radicle length of 65-h.-old seedlings increased with O_2 pressure up to 20 cm. Hg and then decreased with further increasing pressure. When seedlings were transferred from atm. which inhibited radicle elongation to an atm. of 20 cm. O_2 pressure, growth rates increased rapidly to values which were considered normal. Addition of CO_2 to the O_2 atm. increased radicle growth even when more CO_2 than O_2 was present. Reduced O_2 supply rather than build-up of CO_2 may be responsible for reduced growth of young maize plants under poorly drained conditions. A. H. CORNFIELD.

Soil moisture as a factor influencing the degree of response of an upland rice plant to increasing supply of nitrogen and phosphorous. B. A. C. Enyi (*J. agric. Sci.*, 1965, **64**, 15—18).—' Dry ' management results in greater leaf and shoot no., leaf area, dry wt. of grain, root and root length per plant, leaf area of main shoot and lamina area of main shoot leaves, root dry wt. per shoot and shoot to root wt. ratio than do flooded conditions, almost regardless of N and P levels. Flooded soil conditions hasten maturity by causing earlier ear emergence. The beneficial effects of N are less noticeable in flooded conditions, due to smaller absorption of N and loss of NO_3^- as N_2, caused by the reducing action of flooded soil. M. LONG.

Influence of mulching on soil moisture and temperature and yield of potatoes. A. B. Awan (*Amer. Potato J.*, 1964, **41**, 337—339).—In the tropics (Honduras), where soil temp. is above optimum for potato cultivation, a surface mulch of hay (2—4 tons per acre) reduced soil temp., increased available moisture and increased tuber yields by about 30%. A. H. CORNFIELD.

Effect of nitrogen, phosphorus and potassium on the specific gravity, ascorbic acid content and chipping quality of potato tubers. A. H. Teich and J. A. Menzies (*Amer. Potato J.*, 1964, **41**, 169—173).—At three locations over 2 years tuber sp.gr. was usually reduced by application of N (30—60 lb.) or K (30 lb. of K_2O per acre), but was unaffected by P (30—90 lb. of P_2O_5 per acre). Tuber ascorbic acid was sometimes slightly reduced by N or K applications, but was usually unaffected by application of P. The treatments had no consistent effects on chip colour, which was acceptable with all treatments. There was a high correlation between tuber sp.gr. and ascorbic acid content. Differences in sp.gr. and ascorbic acid content were much greater between years than were those due to treatment. A. H. CORNFIELD.

Potassium manuring of potatoes grown for starch on peaty clay soils. K. Boskma and D. van der Hey (*Versl. landbouwk. Onderz.*, 644, 33 pp.).—A useful correlation exists between economic optimum applications of K and the soil-K; the amounts are higher on soils with high than with low clay content, irrespective of soil -pH or org. matter content. For the calculation of profitable applications a table of factors is given for the conversion of the soil-K into ' K-values ' in relation to the clay content (10—70%) of the soils. A second table gives the optimum applications of K_2O/hectare for different K-values. (11 references.) P. S. ARUP.

Cumulative effects of supplemental irrigation on fertiliser requirement, yield and dry matter content of early potatoes. J. M. Fulton and W. I. Findlay (*Amer. Potato J.*, 1964, **41**, 315—318).—The effects of a 5-10-13 (N–P_2O_5–K_2O, 400—1600 lb. per acre) fertiliser and irrigation on potato yields on a sandy loam over 5 years was studied. In the first year, irrigation increased yields considerably whilst fertiliser had no effect. In the third and fifth years irrigation increased yields on fertilised, but not on unfertilised, plots. Irrigated plots required 1600 lb. and non-irrigated plots 400 lb. of fertiliser for max. yields. At the end of the experiment soil K and P were lower on irrigated than on non-irrigated plots. Irrigation did not affect the dry matter content of the tubers and values were lower on plots fertilised at high rates than on unfertilised plots.
A. H. CORNFIELD.

Influence of variety and time upon the resistance of potatoes to mechanical damage. E. E. Finney, C. W. Hall and N. R. Thompson (*Amer. Potato J.*, 1964, **41**, 178—186).—Kennebec was significantly lower in resistance to injury (stress required to rupture the tuber skin and the tissue immediately below the skin) than were Russet Burbank, Katahdin, Sebago and Onaway. The resistance to injury decreased with increasing time of sampling before harvest, and increased with time after harvest. Susceptibility to injury was not related to sp.gr. of tubers. A. H. CORNFIELD.

Field plot technique for the estimation of specific gravity of potatoes. D. A. Young and H. B. Cannon (*Amer. Potato J.*, 1964, **41**, 349—356).—A statistical study of the effect of no. of plots and no. of hills per plot on the errors obtained during the determination of the sp.gr. of potatoes. The standard error of mean sp.gr. decreased particularly with increasing no. of plots (1—20), but also decreased somewhat with increasing no. of hills per plot (1—100).
A. H. CORNFIELD.

Effect of potassium chloride on the infestation of sugar beet by the beet eelworm, *Heterodera schachtii*, Schmidt. G. T. Curtis (*Ann. appl. Biol.*, 1964, **54**, 269—280).—Application of K increased sugar beet size and reduced the no. of cysts of *Heterodera schachtii* on the roots. Infestation decreased with increasing level of application of KCl (0·001—0·100M). Larvae stimulated to emerge from cysts by leachates from K-treated plants were more infective than those stimulated by leachates from control plants. Leachates from K-deficient plants induced quicker and greater hatching of cysts than did leachates from plants receiving K. In the field large doses of KCl applied as top dressings reduced the reproduction of the eelworm to below maintenance level without affecting sugar content and yield of marketable roots. A. H. CORNFIELD.

Storage of fodder beets. IV. Influence of diseases and other forms of damage on keeping quality. V. Relationships between mineral composition and keeping quality. W. A. P. Bakermans (*Versl. landbouwk. Onderz.*, 1964, 648, 32 pp., 650, 20 pp.).—IV. A review with 45 references.

V. Regression equations expressing the effects of the contents of K, Mg, Ca and total cations are of limited significance. Keeping quality is partly favoured by a high content of K and Mg in correct proportions and a moderate Na and Ca content. P. S. ARUP.

Carotenogenesis in carrot roots. O. Banga and J. W. de Bruyn (*Neth. J. Agric. Sci.*, 1964, **12**, 204—220).—The carotenoid content of carrot roots in relation to age, plant density, soil moisture content, temp., and supply of plant food was studied. The results are discussed in relation to the evaluation of field trials and the breeding of carrots of a good colour. A. H. CORNFIELD.

Effects of N, P and K fertilisers on yield and N, P and K contents of grass. F. V. Widdowson, A. Penny and R. J. B. Williams (*J. agric. Sci.*, 1965, **64**, 93—100).—Application of 0·3, 0·6 and 0·9 cwt. of N/acre as Nitrochalk increased yields by 150, 223 and 241% respectively. Neither P nor K greatly affected yields on soil already well supplied. The % of K in the grass was decreased by N in proportion to the amount applied. Recovery of applied N was high, that of P very small and of K intermediate. M. LONG.

Field experiments comparing winter and spring applications of ammonium sulphate, ammonium nitrate, calcium nitrate and urea to grassland. J. R. Devine and M. R. J. Holmes (*J. agric. Sci.*, 1965, **64**, 101—107).—Early winter applications give rise to lower yields than do late winter or spring applications, especially when winter rainfall is high and when $Ca(NO_3)_2$ was used. Late winter applications sometimes give rise to higher yields than early spring. Urea generally leads to lower yields than do the other three fertilisers.
M. LONG.

Effect of seeding rate, fertility and weed control on the spring establishment of lawn grasses. H. R. Kemmerer and J. D. Butler (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 599—604).—When sown in May the coverage by Aug. of Kentucky bluegrass increased with rate of seeding (4—120 lb. per acre). Application of Na_2 methyl arsonate (1 lb. per 25 gal.), a post-emergence weed control chemical, in early Aug. gave better coverage than did the use of a vertical cut mower. None of the four varieties tested was significantly better than the others in preventing the development of weedy summer grasses. Extra application of NPK at sowing or in July did not improve the coverage of bluegrass or reduce the prevalence of weedy grasses.
A. H. CORNFIELD.

Effect of nitrogen and irrigation on yield and botanical composition of rangeland. M. G. Klages and D. E. Ryerson (*Agron. J.*, 1965, **57**, 78—81).—When both N and irrigation water were applied over 3 years, yields were increased but botanical composition was not affected. During a further 2 years without N or irrigation undesirable species increased where N had been applied and yields were lower where water had been applied for the 3 previous years.
A. H. CORNFIELD.

Specific and varietal differences in sodium and potassium in grasses. G. ap Griffith, D. I. H. Jones and R. J. K. Walters (*J. Sci. Fd Agric.*, 1965, **16**, 94—98).—The Na content varies widely between grass species and also between varieties of the same species. The species studied (in descending order of Na content) were *Lolium perenne, Dactylis glomerata, L. multiflorum, Festuca arundiracea, F. pratensis, Phleum pratense.* A low herbage-Na (mequiv. % of dry matter) is not always accompanied by a high K level. N fertilisers applied as Nitro-chalk, $(NH_4)_2SO_4$ or $NaNO_3$ increased the Na content of *L. perenne* and *D. glomerata* but had little effect on *P. pratense.* E. M. J.

Nitrogen fractions and soluble carbohydrates in Italian ryegrass. I. Effects of soil temperature, form and level of nitrogen. T. Z. Nowakowski, R. K. Cunningham and K. F. Nielsen (*J. Sci. Fd Agric.*, 1965, **16**, 124—134).—The grass was grown for 9 weeks (Oct.—Dec.) in clay loam at 11, 19·5 or 28° and treated with N (NH_4^+- or NO_3^--) at six levels (0—500 p.p.m.). Much N (>300 p.p.m.) given to grass grown at high soil-temp. and low light-intensity as NH_4^+-N gives better growth than does NO_3^--N. Most of both forms of N was taken up at 19·5°. Yields of grass at 11 and 19·5° were smaller when NO_3^- content was >1·1% of the dry matter and at 28° were still smaller at 1·9% of the dry matter. Smaller growth was probably caused by inadequate light intensity; with all treatments plants contained very little carbohydrate. Grass fertilised with NH_4^+-N had a larger % of total N as protein N than had that given NO_3^--N. Grass grown at 28° synthesised protein less efficiently than at 11°. (33 references.) E. M. J.

Effects of an evaporation retardant, a surfactant, and an osmotic agent on development of Kentucky bluegrass. E. C. Roberts and D. P. Lage (*Agron. J.*, 1965, **57**, 71—74).—In solution culture tests increasing the osmotic pressure of the solution (by addition of polyethylene glycol) to 2·6 atm. increased top growth, but higher osmotic pressure levels reduced it. Reducing surface tension (by addition of a non-ionic org. wetting agent) depressed top growth. Addition of an evaporation retardant (mixed C_{16} and C_{18} alcohols) increased foliage yields and had little effect on osmotic pressure or surface tension. A. H. CORNFIELD.

Effects of phosphorus on development of red fescue, Merion and common Kentucky bluegrass. F. V. Juska, A. A. Hanson, and C. J. Erickson (*Agron. J.*, 1965, **57**, 75—78).—Top growth and P% in Merion bluegrass increased more rapidly with increasing P level than did those in common bluegrass. Red fescue outyielded bluegrass and both grasses grew better at pH 6·5 than at pH 4·5. Yields of tops increased with level of applied P, but yields of underground parts were not consistently related to P level. Grasses tolerated very high levels of applied P (1527 lb. per acre) without ill effects.
A. H. CORNFIELD.

Lucerne root activity as measured by uptake of strontium and phosphorus-32. R. L. Fox and R. C. Lipps (*Plant & Soil*, 1964, **20**, 337—350).—Estimates of root activity of lucerne obtained by uptake of Sr placed at various depths agreed in general with those obtained from uptake of ^{32}P placed similarly. Root activity was high in surface horizons which received intermittent moisture from rainfall and moderate in the zone of capillary moisture above the water table (8—12 ft.). Activity was low between these two zones. Activity was low from the cold soil of the capillary zone early in the season, but increased as the season advanced. Abnormal amounts of Sr in the first growth of lucerne from plots which received deep applications of Sr indicated some winter root activity. Effects of excessive and early clipping on deep root activity were seen in decreased Sr uptake from deep-placed Sr following a year of heavy sample taking.
A. H. CORNFIELD.

Yield and quality of apples grown in uncultivated sod in relation to rate of application of nitrogen. J. L. Mason (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 42—47).—Apple trees growing on uncultivated grass sod mown monthly were treated with 0·5—4·0 lb. of N (NH_4NO_3) each autumn for 6 years. The only effects of the heavy compared with the lighter rates of N were to decrease red fruit colour, delay maturity and increase leaf N slightly. Yields, flavour and size of fruit and titratable acidity, conductivity and sol. solids content were not significantly different between the different N rates.
A. H. CORNFIELD.

Non-destructive detection of water core in Delicious apples. G. S. Birth and K. L. Olsen (*Proc. Amer. Soc. Hort. Sci.*, 1964, **85**, 74—84).—A non-destructive measure of the extent of water-core in apples was obtained by measuring the optical density of the whole apple at 785 mμ. An even better indication was obtained by measuring the difference in optical density at 760 and 810 mμ. Measurement of the difference in optical density at 740 and 690 mμ., usually used for measuring chlorophyll content, was only moderately successful in measuring the extent of water-core. A. H. CORNFIELD.

Biochemical patterns in leaf tissue from virus-infected and disease-free apple. I. Nucleic acid constituents. D. F. Millikan and E. E. Pickett (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 48—52).—Leaves of virus-infected apple trees were considerable higher in both RNA and DNA (dry leaf basis) contents than were leaves from comparable healthy trees. Virus infection also reduced the carbohydrate% and increased the N% in the leaves. A. H. CORNFIELD.

Effects of blackcurrant yellows virus and a strain of reversion virus on yield. R. Cropley, A. F. Posnette and J. M. Thresh (*Ann. appl. Biol.*, 1964, **54**, 177—182).—Blackcurrant yellows virus restricted the growth of bushes, decreased the size and no. of fruits, retarded ripening in one season and reduced fruit yields by 70% over 6 seasons. A strain of reversion virus did not restrict vegetative growth but reduced fruit yields by 33—50%. A. H. CORNFIELD.

Control of eggplant yellows. D. A. Wolfenberger (*Plant Dis. Reptr*, 1964, **48**, 811—814).—Of a no. of materials tested for control of flea beetle damage and eggplant yellows the most effective was Monsanto CP 40294 (*O*-p-nitrophenyl *O*-phenyl methylphosphonothioate, 1 lb. per acre) applied as a spray. A. H. CORNFIELD.

Ascorbic acid content of citrus during growth and development. I. L. Eaks (*Bot. Gaz.*, 1964, **125**, 186—191).—Data showing the ascorbic acid (**I**) concn. and total content in whole fruits of orange and lemon at various stages of growth, of the peel, pulp and juice of lemons (flavedo and albedo separately) and of lemon leaves are presented. During the development of lemon fruits the **I** concn. increases rapidly from that in small fruits to a max. followed by diminution with increasing wt. and maturity. Changes in leaves followed a similar pattern. A. G. POLLARD.

Epidemiology of tomato mosaic. VII. Effect of tomato mosaic virus on tomato fruit yield and quality under glass. L. Broadbent (*Ann. appl. Biol.*, 1964, **54**, 209—224).—With a winter-sown crop, plants inoculated with tomato strains of tobacco mosaic virus when first trusses began to flower suffered 15% loss in wt. of fruit; inoculation at the fifth truss produced 18% loss and inoculation when most fruit had set, a 14% loss. The latest infection had the least effect on fruit set and mean fruit size decreased with later infection date. Extent of decline of fruit quality increased with later infection date. With spring-sown plants fruit yields usually decreased with later infection dates. A tobacco strain of tomato mosaic virus that caused no symptoms in tomato failed to protect plants from later infection with a tomato strain. Fruit quality was poor in the late-infected plants. A. H. CORNFIELD.

Seasonal changes of nutrient elements in the leaves and bulbs of the Easter lily, *Lilium longiflorum*. A. N. Roberts, L. T. Blaney and O. C. Compton (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 611—630).—The greatest growth increases of the Easter lily were obtained with N and P applications. Seasonal changes in leaf- and bulb-N and -P were of equal value in diagnosing nutrient status. In July bulb-P, but not leaf-P, was related to available soil-P. P status was most effectively determined when yearling bulbs had 50—60 fully expanded leaves. The greatest differences in N status due to treatment were found after flowering. Leaf and bulblet wt. and flower no. were more responsive to N and P applications than was the parent bulb with its daughter. Liming increased bulblet production only. A. H. CORNFIELD.

Keeping quality, flower size and flowering response of the Easter lily, *Lilium longiflorum*, to gibberellic acid. J. D. Kelley (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 631—634).—Application of gibberellic acid (100—2000 p.p.m.) to 1—6 in. long buds of the Easter lily had no significant effect on time of flowering or flower size, but prolonged the keeping quality of uncut flowers up to 35%. A. H. CORNFIELD.

Changes in metabolic activity and composition of Wedgewood iris bulbs during maturation of bulbs. A. H. Halevy and J. Shoub (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 605—610).—The dry wt.% of iris bulbs increased from mid-Feb. (blossom time) until mid-May (ultimate desiccation of leaves). Total sol. sugars decreased initially and then increased, whilst the reverse occurred with ascorbic acid. Respiration of the scales and peroxidase activity of the bulbs and scales decreased, whilst catalase activity of the buds increased, with time. O_2 consumption of the buds and catalase activity of the scales decreased sharply at the time of bulb maturation. A. H. CORNFIELD.

Abnormal cyathia production in poinsettias. R. A. Larson and M. L. McIntyre (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 635—638).—Drenching the soil with the growth retardant Cycocel (2-chloroethyltrimethylammonium chloride, 1 quart per 10 gal.) increased the no. of cyathia produced in axillary positions and reduced bract length. There were significant differences due to variety in response to the treatment. Varying light treatment following Cycocel treatment had no significant effect on cyathia formation. A. H. CORNFIELD.

Effect of N^6-benzyladenine on chilling injury, respiration and keeping quality of *Anthurium andraeanum*. T. Shirakawa, R. R. Dedolph and D. P. Watson (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 642—646).—Treatment of cut flowers of *Anthurium andraeanum* by a momentary dip in N^6-benzyladenine (10 p.p.m.) increased tolerance to the subsequent short-term chilling period and extended the keeping quality of the flower at 22·2°. The chemical treatment reduced the respiration rate of immature more than that of mature flowers. A. H. CORNFIELD.

Physiology of cocoa, *Theobroma cacao*, L. I. Suppression of swollen-shoot virus symptoms by light. E. J. A. Asomaning and R. G. Lockard (*Ann. appl. Biol.*, 1964, **54**, 193—198).—Shading retarded the growth of cocoa seedlings infected with cocoa swollen-shoot virus proportionately more than the growth of uninfected seedlings. Infected seedlings grown in bright light did not produce the swellings on stems and roots which were consistent symptoms of infection on plants grown under shade. The type of leaf symptoms also differed in shaded and unshaded seedlings. A. H. CORNFIELD.

Genetic control of the caffeine content of coffee. A. Carvalho, J. S. Tango and L. C. Monaco (*Nature, Lond.*, 1965, **205**, 314).—Genetic analysis of various cultivated coffee species has indicated that the presence of the Laurina (*lr*) alleles is responsible for a drastic reduction in the caffeine content. The *xanthocarpa* (*xc*) and *bourbon* (*t*) factors also have a slight tendency to diminish the caffeine content, while the *maragogipe*(*Mg*) *mokka* (*mo*) alleles seem to increase it. S. A. BROOKS.

Methods of applying boron for cotton. J. L. Keogh, R. Maples and G. W. Hardy (*Ark. Farm Res.*, 1964, **13**, No. 2, 13).—Comparison is made of applications of B to a B-deficient soil by broadcasting (*a*) before or (*b*) after planting, (*c*) with pre-emergence chemicals or as a side dressing in a 12 in. band, (*d*) as a foliar spray. Yields of cotton were increased in all cases, there being no consistent differences between the effects of the different methods of application. A. G. POLLARD.

Growth, phosphorus uptake, and fibre cell dimensions of the jute plant as affected by silicate treatment. D. H. Khan and A. C. Roy (*Plant & Soil*), 1964, **20**, 331—336).—Application of Na_2SiO_4 (104 lb. per acre) to limed and unlimed clay soils of low P status increased plant height and stem thickness and elongation and fineness of the fibre cell. P uptake was also increased, particularly on the unlimed soil, and the effects obtained were considered to be due to increased availability of soil P resulting from the silicate treatment. A. H. CORNFIELD.

Sulphur deficiency in wattle, *Acacia mearnsii*. J. M. Gosnell (*E. Afr. agric. For. J.*, 1964, **30**, 1—7).—Bronzed wattle leaves were lower in S% than were normal leaves. Pot and field tests showed growth increases and reduction in extent of bronzing as a result of S application. There were significant responses to application of $CaSO_4$ in 7 of 13 field tests. In the field S deficiency in wattle may be induced by competition for the nutrient by grasses. A. H. CORNFIELD.

Pest Control

Physico-chemical studies on agricultural sprays. VI. Survey of methods for measuring the wetting ability of spray formulations. C. G. L. Furmidge. **VII. Visual assessment of spray coverage.** D. I. Conibear and C. G. L. Furmidge (*J. Sci. Fd Agric.*, 1965, **16**, 134—144, 144—149).—VI. Wetting tests were surveyed to find if they will predict the wetting ability of a range of wetting agents, on several types of leaf surfaces. A satisfactory wetting test must involve the appropriate spray target surface and take into account the effects of spray droplet impaction; these requirements are satisfied by a test based on the visual assessment of wetting under practical spraying conditions. None of the other wetting tests considered can be accepted as fully satisfactory for the measurement of the wetting efficiency of high-vol. sprays except in certain limited cases, e.g., when the receding contact angle is zero. The tape-sinking test can be used as a guide to the wetting of certain leaf types by anionic and non-ionic systems and it provides a rough classification of the overall efficiency of wetters on leaf surfaces. (22 references.)

VII. Difficulties in assessing the wetting ability of spray formulations are discussed. Wetted from non-wetted areas can be distinguished more readily if fluorescent materials are included in the wetting liquid and assessment is made under u.v. light or by photographing the leaves under u.v. light and making the assessments from the negatives. Addition of fluorescent tracers to the spray modifies its wetting properties, but by this method and by direct leaf assessments, together with experience, visual assessments of all degrees of leaf wetting can be reasonably accurate. E. M. J.

Direct photomicrography of air-borne droplets and its application for particle size determination of insecticidal aerosols in the field. C. B. Rathbone, jun. (*Dissert. Abstr.*, 1964, **25**, 1801).—Details of the construction and operation of the aerosol camera are described. Dropets as small as 2μ in dia. were measured directly from the negative and by projection on a screen. F. C. SUTTON.

Water-base insecticides. A. S. Glessner (*Soap, N.Y.*, 1964, **40**, No. 8, 83—86, 112).—The use of water as a base for insecticides in relation to corrosion of the container is discussed with special

reference to the effect of lacquering or interior-lined cans. Pyrethrin, piperonyl butoxide, *N*-octylbicycloheptenedicarboximide, methoxychlor, allethrin, DDT and Thanite are examined and various formulations are presented. Lacquered cans showed a slight underfilm corrosion and rusting along the seams but with a thicker film, a shelf-life of 18—24 months could be expected. Purging, vac. crimping and N_2-sparging are recommended. A low-tin solder gives better results than pure Sn; a single coat of diphenolic lacquer gives better protection than a double coat (epoxy + vinyl). C. V.

Ecology of plant pathogens in soil. I. Influence of chitin and lignin amendments on the development of bean root rot. C. L. Maurer and R. Baker (*Phytopathology*, 1964, **54**, 1425—1426).—Addition of lignin or chitin to soil infested with *Fusarium solani*, f. sp. *phaseoli*, prior to planting with beans had no consistent effect on the severity of disease symptoms. Addition of both substances together diminished the severity of the disease which was further reduced by addition of KNO_3 (1225 p.p.m.). A. G. POLLARD.

Relation between the re-colonisation of partially sterilised soil and the attack of seedlings by *Pythium debaryanum*. D. Seidel (*Zbl. Bakt.*, 1964, **II, 118**, 500—509).—Attack of *Brassica napus* by *P. debaryanum* was more severe in partially sterilised soil than in unsterilised soil. This was not related to change in soil pH produced by partial sterilisation or to differences in the nutrient status of the soils. The no. of healthy plants and of micro-organisms in the sterilised and re-contaminated soil were significantly correlated. A. G. POLLARD.

Effect of seed protectant and planting depth on *Pythium* and *Rhizoctonia* damping-off of *Tephrosia vogelii* in Puerto Rico. E. G. Ruppel, D. K. Barnes, R. H. Freyer and A. Santiago (*Plant Dis. Reptr*, 1964, **48**, 714—717).—*Rhizoctonia solani* and *Pythium aphanidermatum* are reported for the first time as incitants of damping-off of *Tephrosia vogelii* seedlings (source of rotenone). Seed treatment with thiram (water slurry or dry treatment) gave good control of damping-off. Incidence of damping-off increased with depth of planting of the seed. A. H. CORNFIELD.

Effect of repeated applications of nematocides on vegetable yields and nematode populations in a muck soil. J. D. Wilson and O. Hedden (*Plant Dis. Reptr*, 1964, **48**, 698—702).—Definite differences in crop yields and nematode populations of a muck soil treated over 7 years with different nematocides appeared in the last year. EDB (12 gal. per acre) gave the best control of root-knot, followed by DBCP (10 gal.). Larval no. were higher in plots treated with chloropicrin (35 gal.) than in control plots. SMDC (50 gal.) did not control root-knot but controlled the lesion nematode. *Pratylenchus* was best controlled by D-D (45 gal.), and the pin nematode by D-D and EDB. EDB and DBCP delayed maturity of onions. EDB reduced potato yields and increased the incidence of scab, but gave the highest yields of carrot and celery with the lowest incidence of damage by root-knot. SMDC gave the highest yields of onion, beet, spinach and potato. A. H. CORNFIELD.

Rapid centrifugal-flotation technique for separation of nematodes from soil. W. R. Jenkins (*Plant Dis. Reptr*, 1964, **48**, 692).—A modification of the centrifugal-flotation technique (*Proc. helminthol. Soc.*, 1955, **22**, 87) permits the separation of nematodes from soil so as to be available for examination within 10 min. A. H. CORNFIELD.

Organophosphorus and carbamate insecticides as soil treatments for control of wireworms. D. C. Griffiths and R. Bardner (*Ann. appl. Biol.*, 1964, **54**, 241—254).—Of a no. of materials tested in the laboratory for control of wireworms in soil the most promising were Thionazin (diethyl *O*-2-pyrazinyl phosphorothionate), fenthion (dimethyl 3-methyl-4-methylthiophenyl phosphorothionate) and Bayer 38156 (*O*-ethyl *S*-*p*-tolyl ethylphosphonodithioate). When the materials were applied as sprays at 2·7 lb. active ingredient per acre to soil growing cereals fenthion was ineffective, but Thionazin and Bayer 38156 increased cereal yields significantly. Yield increases were associated with reduced wireworm populations. A. H. CORNFIELD.

Chemical control of wheat rusts in the United Arab Republic. H. A. Mohamed (*Plant Dis. Reptr*, 1964, **48**, 681—685).—Good control of rust in wheat was obtained by spraying with 1% Sabethane Z (zineb 2·85 parts; $NiCl_2$ 1 part) before, but not after, inoculation with rust. In the field grain yields were increased 30—40% by application of Sabethane Z (1·5kg. per feddan) in large-plot tests, but were not significantly affected in small-plot tests. A. H. CORNFIELD.

Urea–formaldehyde for the control of common scab of potato. A. R. Weinhold, T. Bowman and J. Bishop (*Amer. Potato J.*, 1964, **41**, 319—321).—Application of urea–formaldehyde (Uracide, 60% HCHO–25% urea, 10—20 gal. per acre) in the furrow at planting time considerably reduced the incidence of potato scab without affecting yields. A. H. CORNFIELD.

Control of root-knot nematode, *Meloidogyne hapla*, in potatoes with dimethoate in the greenhouse. A. W. Helton (*Plant Dis. Reptr*, 1964, **48**, 881—885).—Immersion of infected seed pieces in dimethoate (800 p.p.m.) for 24 h. before planting eliminated the nematodes from tubers and roots. Spraying plants from infected seed-pieces with dimethoate, 7—28 days after emergence, was only partially effective in reducing nematode damage. A. H. CORNFIELD

Carrot motley dwarf and parsnip mottle viruses. M. Watson, E. P. Serjeant and E. A. Lennon (*Ann. appl. Biol.*, 1964, **54**, 153—166).—Characteristics of the two diseases are presented. A. H. CORNFIELD.

Influence of pesticides on fruit set, return bloom, yield and fruit size of the apple. C. W. Donoho, jun. (*Proc. Amer. Soc. Hort. Sci.*, 1964, **85**, 53—59).—The effects of seven pesticides in various combinations were studied over 3 consecutive years. The largest fruit set and yields were obtained with captan + DDT, glyodin + DDT, and dodine + DDT and the lowest usually with Phygon XL + DDT, Guthion + captan and, especially, Phix + DDT. Sevin + captan reduced fruit set and yields only when applications began at petal fall, but not when applications began at a later date. The treatments had no consistent effect on return bloom or fruit size. A. H. CORNFIELD.

Strawberry latent ringspot: a new nemotode-borne virus. R. M. Lister (*Ann. appl. Biol.*, 1964, **54**, 167—176).—The properties and mode of transmission of strawberry latent ringspot virus are described. A. H. CORNFIELD.

Control of a nematode of the genus *Hypsoperine* on Zoysia and Bermuda grasses in Maryland. A. A. Bell and L. R. Krusberg (*Plant Dis. Reptr*, 1964, **48**, 721—722).—The pest was fairly well controlled by application of Bayer 25141 (*OO*-diethyl *O*-*p*-(methylsulphinyl)-phenyl phosphorothioate (12—20 oz. active material in 10 gal. water per 1000 sq.ft.). A. H. CORNFIELD.

Control of root-knot nematodes on celery transplants. J. A. Winchester (*Plant Dis. Reptr*, 1964, **48**, 782—783).—Excellent control of root-knot nematodes on celery plants was obtained by immersing the roots in 1500—2500 p.p.m. zinophos (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) for 10—60 min. before planting out. A. H. CORNFIELD.

Effect of chemicals on infection of shortleaf pine stumps by *Fomes annosus*. F. H. Berry and T. W. Bretz (*Plant Dis. Reptr*, 1964, **48**, 886—887).—Of a no. of chemicals tested by application to cut stumps of shortleaf pine 20% urea, 40% Ammate, 5% NH_4F and 10% borax were the most effective in reducing infection by *Fomes annosus*. A. H. CORNFIELD.

Weed science—revolution in agricultural technology. W. C. Shaw (*Weeds*, 1964, **12**, 153—162).—An address. A. H. CORNFIELD.

Kinetics of *Chlorella* inhibition by herbicides. J. V. Gramlich and R. E. Frans (*Weeds*, 1964, **12**, 184—189).—Treatment of *Chlorella* with naphth-1-ylacetic acid and 2,4-dichlorophenoxyacetic acid resulted in an inhibitory response that was characterised satisfactorily by kinetic analysis. This is consistent with the fact that both of these compounds function as auxins at low concn. The response of *Chlorella* to atrazine was more satisfactorily interpreted by probit analysis, and growth inhibition was due to its effect on photosynthesis. A. H. CORNFIELD.

Chlorophyll formation in *Euglena gracilis* as a test for herbicides. C. A. Price and M. G. Estrada (*Weeds*, 1964, **12**, 234—235).—A method is described using the extent of chlorophyll formation by the alga *Euglena gracilis* for screening potential herbicides which act as inhibitors of chlorophyll synthesis. A. H. CORNFIELD.

Use of grass-killing herbicides in establishing grass-legume mixture. J. Vengris (*Agron. J.*, 1965, **57**, 59—61).—Pre-emergence and post-emergence applications of Na trichloroacetate (6—10 lb.) and post-emergence applications of dichloroacetic acid (2—3 lb. per acre) controlled common annual weedy grasses. Although the treatments injured orchardgrass, timothy and lucerne initially, the plants regained normal growth later in the season and produced satisfactory stands. Post-emergence treatments with 4-(2,4-dichlorophenoxy)-butyric acid controlled broadleaved weeds. A. H. CORNFIELD.

Translocation and metabolism of radioactive 2,4-D in Jimsonweed, *Datura stramonium*. R. C. Fites, F. W. Slife, and J. B. Hanson (*Weeds*, 1964, **12**, 180—183).—The leaf of Jimsonweed was treated with COOH-labelled ^{14}C2,4-D. Activity rapidly decreased in the leaf and accumulated in the stem, but there was little accumulation of activity in the roots. With the exception of the stem tissue, where minor alteration of the herbicide occurred, 2,4-D was the only radioactive material recovered from the plant. A loss of activity from root tissue indicated a possible detoxification mechanism. Up to 25% of the applied 2,4-D was expelled from the roots in 6 weeks. A. H. CORNFIELD.

Photolysis of 3,5-di-iodo-4-hydroxybenzonitrile (ioxynil) as a factor in its herbicidal action. E. N. Ugochukwu and R. L. Wain (*Chem. & Ind.*, 1965, 35).—When ioxynil in benzene was irradiated in a special apparatus in which the cooled test solution surrounds the lamp it was converted into 3,5-diphenyl-4-hydroxybenzonitrile (71%). The corresponding mono-iodo deriv. gave 3-phenyl-4-hydroxybenzonitrile (25·7%). The reaction occurs by a free radical mechanism which may also occur in the plant and affect the photosynthetic transport system. J. B. WOOF.

Control of swainsonpea, *Swainsona salsula*. W. C. Robocker, H. D. Kerr and V. F. Bruns (*Weeds*, 1964, **12**, 189—191).—Swainsonpea, an unpalatable legume which is a problem in poorly drained or saline areas, was controlled by spray application of 2,4-5-trichloro- or 2,4-dichloro-phenoxyactic acid, or 2-(2,4,5-trichlorophenoxy)-propionic acid (2 lb. per acre) for 2 or more years. A. H. CORNFIELD.

Control of timber milkvetch, *Astragalus miser*, and effects on associated vegetation. E. H. Cronin and M. C. Williams (*Weeds*, 1964, **12**, 177—179).—Of a no. of materials tested for control of timber milkvetch spray application of 2,4,5-trichlorophenoxyacetic acid and -propionic acid (2—4 lb. per acre) were the most effective. Control of the weed increased both cover and frequency of associated grass species. A. H. CORNFIELD.

Bioassay methods for 4-amino-3,5,6-trichloropicolinic acid. J. K. Leasure (*Weeds*, 1964, **12**, 232—233).—Three procedures using bean seedlings for the bioassay of 4-amino-3,5,6-trichloropicolinic acid in solutions and in soil are described. A. H. CORNFIELD.

Application of 4-(*p*-nitrobenzyl)pyridine as a rapid quantitative reagent for organophosphate pesticides. M. E. Getz and R. R. Watts (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1094—1096).—A method based on the reaction between *p*-nitrobenzylpyridine and the phosphate pesticide in presence of cyclohexylamine at 175—180°, followed by dilution with ethyl acetate and reading the extinction at 520 mμ, is described. For strawberries, potatoes, apples and cabbage, fortified at 1 p.p.m., recoveries were 60·0—114·0%. A. A. ELDRIDGE.

Identification and analyses of five organophosphate pesticides: recoveries from crops fortified at different levels. R. W. Storherr, M. E. Getz, R. R. Watts, S. J. Friedman, F. Erwin, L. Giuffrida and F. Ives (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1087—1093).—By the 'gross column cleanup' procedure described, good average recoveries for crops fortified at 5·0, 1·0 and 0·1 p.p.m. with diazinon, methyl parathion, parathion, malathion and Trithion were obtained. Paper chromatography gave good recoveries, but results obtained with *p*-nitrobenzylpyridine reagent were variable. A. A. ELDRIDGE.

Colorimetric determination of residues of the dithiolane insecticides in cottonseed and on cotton foliage. R. C. Blinn and J. E. Boyd (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1106—1111).—Acid hydrolysis converts 2-ethoxyphosphinothioyl- and 2-ethoxyphosphinyl-imino-1,3-dithiolane into 2-imino-1,3-dithiolane, which on treatment with alkali affords thiocyanate. This is converted into CNBr which on reaction with benzidine in pyridine gives an intensely red solution, permitting spectrophotometric determination at 530 mμ. Recoveries of 63—93% at 0·1—1·0 p.p.m. are reported. A. A. ELDRIDGE.

Gas chromatographic determination of small vapour pressures. Determination of the vapour pressures of some triazine herbicides. K. Friedrich and K. Stammbach (*J. Chromatogr.*, 1964, **16**, 22—28).—A chromatographic method for the determination of small v.p. is described in detail. The method does not require an absolutely pure sample, and the v.p. of several substances can be determined simultaneously. V.p. and v.p. constants are presented for 10 substituted symmetrical triazines, important as herbicides, over the temp. range 50—130°, and extrapolated values at 20° are also given. S. M. MARSH.

Amino esters of dithiocarbamic acids. VEB Farbenfabrik Wolfen (Inventors: T. Waag and W. Löttage) (B.P. 949,641, 19.10.61).—There are claimed fungicidal compositions (for use against, e.g., *Septoria apii*, *Phytophthora infestans*, *Peronospora viticola*) in which the active ingredient is a compound of the general formula $(NR'R''\cdot CS_2\cdot CH_2)_2NR$, wherein R—R'' are alkyl of 1—4 C, e.g., *NN*-bis-(*NN*-dimethyldithiocarbamoylmethyl)methylamine. F. R. BASFORD.

Nuclear chlorinated benzonitrile derivatives. N. V. Philips' Gloeilampenfabrieken (B.P. 949,619, 27.4.60. Neth., 28.4.59).—Preparation details are given for 2-*chloro*-6-*nitrobenzonitrile*, m.p. 117—119°, useful as a fungicide, and 2-*amino*-6-*chlorobenzonitrile*, m.p. 132—134°, useful for influencing the growth of plants, and from the latter 2,6-*dichlorobenzonitrile*, m.p. 142—144°, a selective herbicide. H. S. R.

α-Substituted benzaldoximes. Shell Research Ltd. (Inventors: J. T. Hackman and P. A. Harthoorn) (B.P. 949,371, 1.1.60).—Compounds of formula $C_6H_{4-n}XW_n\cdot CY{:}NOZ$ are claimed as herbicides and as fungicides for protecting seeds against fungal attack (X is halogen in *o*-position to CY:NOZ; W is halogen or alkyl; *n* is 1—X; Y is a substituent such as halogen, OH, ester group substituted amino; Z is H, carbalkoxy or aliphatic acyl radical). In one example (out of 77), the prep. is described of α-*chloro*-2,4-*dichlorobenzaldoxime*, m.p. 95—97°. F. R. BASFORD.

Nitrile oxides. Shell Research Ltd. (Inventors: J. T. Hackman, P. A. Harthoorn and J. Kidd) (B.P. 949,372, 1.1.62).—Stable nitrile oxides of the aromatic series with halogen in each *o*-position to the C:NO group are claimed as insecticides and molluscicides and also as fungicides against *Alternaria brassicola*. A representative compound is 2,6-*dichlorobenzonitrile* N-*oxide*, m.p. 74—77°. H. S. R.

Complex manganese salts of ethylenebisdithiocarbamic acid and fungicidal compositions containing same. Badische Anilin- u. Soda-Fabrik A.-G. (Inventors: H. Windel and E.-H. Pommer) (B.P. 949,286, 21.8.62. Ger., 26.8.61).—There is claimed any complex compound of the Mn salt of ethylenebisdithiocarbamic acid with NH_3 and/or an amine (4—9%). It has fungicidal properties superior to those of the simple salt. F. R. BASFORD.

Chloroethylsulphenyl chloride. Hooker Chemical Corp. (B.P. 949,375, 18.3.60. U.S., 23.3.59).—Chlorine is passed at −20° into bis-trichloroethyl disulphide, then the mixture is allowed to attain temp., and is fractionally distilled, to give *tetrachloroethylsulphenyl chloride* (1,2,2,2-*tetrachloro*-1-*chlorothio*-*ethane*), b.p. 53—57°/0·5 mm., d_4^{23} 1·76. It is a malodorous, yellow oil with nematocidal properties. F. R. BASFORD.

Carbamoyl and thiocarbamoyl derivatives and their applications. État Française (Inventors: R. Levy, Y. Menoret and C. Magnoux) (B.P. 949,170, 15.3.60).—Compounds claimed (fungicides and herbicides) comprise 3-amino-1,2,4-triazoles substituted in the 2- or 4-position by $CX\cdot NRR^I$ and optionally in the 5-position by alkyl of 1—4 C (X is O or S; R and R^I are H, alkyl or alkenyl of 1—18 C, Ph, or halogenophenyl). They are prepared by reacting (where R^{II} is H) an aminotriazole with $R^{III}\cdot NCX$, or by condensing aminoguanidine bicarbonate with a fatty acid and then with KCNO in presence of mineral acid (to give products in which R^{II} and R^{III} are H). A representative compound is 3-*amino*-2(or 4)-*carbamoyl*-1,2,4-*triazole*, m.p. 173°. F. R. BASFORD.

Pyridazone derivatives and herbicidal agents containing them. Badische Anilin- u. Soda-Fabrik A.-G. (Inventors: F. Reicheneder, K. Dury, H. Stummeyer and A. Fischer) (B.P. 949,132, 21.3.62. Ger., 25·3 and 17.11.61).—Selective herbicides claimed comprise 5-halogeno-1-R^I-4-$NR^{II}R^{III}$-6-oxo-1, 6-dihydropyridazine·(-6-pyridazones), wherein R^I is alkyl, Ph or cycloalkyl, optionally substituted; R^{II} is H; R^{III} is CO·NHR (R is substituted or unsubstituted alkyl, substituted or unsubstituted pyridazonyl-ureidoalkylene, Ph or C_6H_4Cl group) or NH_2 optionally substituted; or R^{II} and R^{III} together comprise :N:N or $:CR^{IV}\cdot NR^VR^{VI}$ [R^{IV} is H or alkyl; R^V and R^{VI} are (substituted) alkyl; or NR^VR^{VI} is heterocyclyl]. One example (prep. described) is 1-*phenyl*-5-*chloro*-4- (1-*methylpyrrolid*-2-*ylideneimino*)-6-*oxo*-1,6-*dihydropyrazole*, m.p. 121—122° (*hydrochloride*, m.p. 230°). F. R. BASFORD.

Disulphides of the thiophosphoric, thiophosphonic and thiophosphinic acid series. Farbenfabriken Bayer A.-G. (B.P. 949,083, 10.2.61. Ger., 12.2.60).—Insecticidal compounds of the general formula $R'R''\cdot PS_2\cdot SR$ are claimed, wherein R' and R'' are alkyl or alkoxyl and R is alkyl substituted by alkylthio, Ph, chlorophenyl, nitrophenyl, alkylcarbamoyl, dialkylcarbamyl, carbalkoxy or $S\cdot S\cdot P(S)R'R''$. They are prepared by interaction of $R'R''\cdot PS_2H$ with $M\cdot SO_3\cdot SR'''$ at 20—60° in aq. alcoholic or ketone medium (M is metal). One compound prepared is *OO-Me*$_2$ S-*ethylthioethylthiophosphorothiolothionate*. F. R. BASFORD.

Chlorothiolformates. Stauffer Chemical Co. (B.P. 948,831, 29.8.61. U.S., 6.9.60).—Compounds SR-COCl [R is alkyl of 5—11 C or of 14 C, cycloalkyl, alkenyl of 2—5 C, alkaryl, aralkyl, halogenoalkyl, halogenoaralkyl, carbalkoxyalkyl, aryl other than Ph, or $R'(S\text{-}COCl)_2$; R' is polymethylene of 3—5 C] are obtained in high yield by reacting RSH with phosgene in presence of finely divided active C. Directions are given for the prep. of Me chlorothiolformate which is effective against fungi and nematodes. F. R. BASFORD.

Thiophosphoric acid ester derivatives having insecticidal properties. Stauffer Chemical Co. (B.P. 948,783 14.7.60. U.S., 24.7.59).—The esters, which are useful as insecticides and acaricides, have the general formula $(OR)_2PY'\cdot Y''\cdot[CH_2]_2\cdot O\text{-}CO\text{-}NR'R''$, wherein R is alkyl of $\geqslant$4 C; Y' and Y'' are O or S but at least one of them is S; R' and R'' are H, alkyl of $\geqslant$ 5 C, allyl, CH_2Ph, Ph optionally substituted; or NR'R'' is heterocyclyl. One example is *OO-Me*$_2$ S-

(2-*carbamoyloxyethyl*) *phosphorothiolothionate*. A further 48 compounds are described and activities against American cockroach, large milkweed bug, pea aphid, confused flour beetle, and housefly are tabulated. F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (Inventor: G. Schrader) (B.P. 948,530, 6.11.51. Ger., 5.11.60).—Insecticidal compounds of the general formula SR′(SR″)·PS·XR‴ are claimed, wherein R′ and R″ are alkyl of 1—4 C or R″ is Ph optionally substituted; R‴ is (substituted) Ph or naphthyl, chloroalkyl, alkylthioalkyl, aralkylthioalkyl or arylthioalkyl; X is O or S. In an example, SS-*di-isopropyl phosphorochloridodithiolate*, b.p. 86°/1 mm., is converted into Pr^{i}_{2} 2-*ethylthioethyl phosphorotetrathioate*, which is 100% lethal to flies and spiders at 0·1% concn. F. R. BASFORD.

Carbonyldithio compounds and compositions containing them. Shell Internationale Research Mij. N.V. (B.P. 948,489, 11.7.60. U.S., 13.7.59).—Compounds claimed have the general formula R·S·S·COX, in which R is hydrocarbon radical optionally halogenated; X is OR, SR, $S{\cdot}CH_2{\cdot}CO_2R$, $S{\cdot}CH_2{\cdot}SR$ or NR_2. They are active against soil fungi and nematodes, and may be prepared by interaction of RSY with X·CS·OR′ (Y is halogen; R′ is hydrocarbon radical) in a solvent). The prep. is detailed of *Me(methyldithio)thioformate*, MeS·S·CO·SMe, b.p. 84—85°/3·5 mm., in 71% yield. F. R. BASFORD.

Thiuram monosulphides. Farbenfabriken Bayer A.-G. (B.P. 948,649, 11.4.61. Ger., 14.4.60).—Compounds with fungicidal properties are obtained by oxidation of the primary diamine salts of bisdithiocarbamic acids (e.g., Na ethylenebis-dithiocarbamate) with K ferricyanide, Cl_2, persulphate etc. and treatment of the products with water on an org. solvent (acetone, $CHCl_3$, ligroin) at 40—100° (50°) H. S. R.

Dithiophosphoric acid ester. Shell Research Ltd. (Inventor: J. L. Melles) (B.P. 948,158, 29.7.60.)—A 3-halogenodihydrothiophen-1, 1-dioxide (obtained by dehydrohalogenation of a 3,4-dihalogenotetrahydrothiophen-1.1-dioxide) is reacted with a salt of $(OMe)_2PS_2H$ at 35—100° in an inert solvent to give OO-Me_2 S-1, 1-*dioxodihydrothien-3-yl phosphorothiolothionate*, m.p. 66—67° (34·6% yield). The product has high toxicity towards aphids and *Tetranychidae*, and insecticidal compositions containing it are also claimed. F. R. BASFORD.

OO-**Dimethyl dithiophosphoryl-acetic acid monomethylamide.** Boehringer Ingelheim Ltd. (B.P. 948,117, 28.6.62. Ger., 3.7.61).—The above pesticide is obtained in improved (83—95%) yield by interaction of the ethylene glycol diester of *OO*-Me_2 *S*-carboxymethyl phosphorothiolothionate with NH_2Me in water and/or alcohol at < 30°. The product is the *M*-Me_2 ester of *S*-methylaminocarbonylmethyl phosphorothiolothionate. F. R. BASFORD.

Pyrazinylphosphoroamidothionates. American Cyanamid Co. (B.P. 948,522, 6.2.61. U.S., 17.5.60.)—Pesticidal compositions of the general formula $OR^{II}{\cdot}PS{\cdot}RR^{I}$ are claimed (R and R^{I} are $NR^{III}R^{IV}$ or ethylenimino, or R is low-mol. alkyl; R^{III} and R^{IV} are H or low-mol. alkyl; R^{III} is pyrazin-3-yl) are obtained by interaction of $OR^{II}{\cdot}PSCl_2$ with at least 1 mol. of $R^{I}H$ and ≯ 1 mol. of an alkanol at 15—100°. *Pyrazin-3-yl phosphorodichloridothionate* (prep. described) is treated with $NHMe_2$ to give *pyrazin-3-yl* NN^{1}-*dimethylphosphorodiamidothionate*, m.p. 54—55°. F. R. BASFORD.

6-Trichloromethyl-2,4-diamino-*s*-triazines. Deutsche Gold- u. Silber-Scheide-anstalt (B.P. 948,175, 25.7.61. Ger., 10.8.60).—Fungicidal compounds are produced by interaction of 2,4,6-tris-trichloromethyl-*s*-triazine with NH_3 or an amine in presence of an alkali metal alkoxide. One example is 6-trichloromethyl-2,4-bismethylamino-*s*-triazine. E. ENOS JONES.

Thiocyanomethyl-substituted heterocyclic compounds and fungicidal compositions containing them. Rohm & Haas Co. (B.P. 948,060, 30.3.60. U.S., 15.4.59).—There are claimed 1-(*thiocyanatomethyl*)-1H-*benzotriazole* (**I**) and 3-(*thiocyanatomethyl*)-4-*oxo*-3,4-*dihydro*-1,2,3-*benzotriazine*, also fungicidal compositions containing them (preferably in admixture). They can be obtained by interaction of a corresponding halogenomethyl analogue with a thiocyanate in an inert solvent. **I**, m.p. 128—131° is active at a concn. of 5 p.p.m. against *Alternaria solani* and *Monolinia fructicola*, and at a concn. of 5—10 p.p.m. against *Stemphylium sarcinaeforme*. F. R. BASFORD.

Esters of *OO*-**dialkyl-dithiophosphorylacetic acids.** K. Thomae G.m.b.H. (B.P. 948,039, 9.3.62. Ger., 13.3.61).—Compounds $(OR^{I})_2$ PS·S·$CH_2{\cdot}CO_2R$ (R and R^{I} are alkyl of 1—3 C) are conveniently obtained by interaction of the corresponding acid or salt with ROH or RX respectively (X is halogen) in presence of a catalyst. In an example, the Na salt of *OO*-dimethyl *S*-carboxymethyl phosphorothiolothionate is boiled with EtOH and EtI to give the *trimethyl* ester in 72% yield. The products are insecticides or intermediates therefor. F. R. BASFORD.

Esters of phosphorus-containing acids. Farbenfabriken Bayer A.-G. (B.P. 948,221 27.3.62. Ger., 27.3.61).—The esters, which are useful as pesticides, have the general formula $R^{I}R^{II}{\cdot}PX{\cdot}S{\cdot}CH_2{\cdot}NR^{III}{\cdot}CY{\cdot}R^{IV}$, wherein R^{I} and R^{II} are alkyl or alkoxy of 1—4 C; R^{III} is alkyl of 1—4 C; R^{IV} is H (substituted) aliphatic or aromatic hydrocarbon radical, or OR^{V} (R^{V} is aliphatic or aromatic hydrocarbon radical optionally substituted); X and Y are O or S. They are obtained by reacting $R^{I}R^{II}{\cdot}PX{\cdot}SH$(**I**) with CH_2O and $NHR^{III}{\cdot}CYR^{IV}$, or where Y is O, reacting **I** with $R^{IV}{\cdot}CY{\cdot}NR^{III}{\cdot}CH_2OH$. One compound prepared is OO-Me_2 S-(*N-ethoxycarbonyl-N-isopropylaminomethyl*) *phosphorothiolothionate*, which is active against spider mites at a concn. of 0·1%. F. R. BASFORD.

New nitrogen-containing organo-phosphorus compounds. Farbenfabriken Bayer A.-G. (Inventors: H. Malz, G. Oertel, H. Holtschmidt and K. Wagner) (B.P. 948,581, 31.1.62. Ger., 5.4.61).—Compounds claimed have the general formula $R^{I}R^{II}{\cdot}PZ{\cdot}S_n{\cdot}CO{\cdot}N{\cdot}(CR^{III}R^{IV}R^{V})_2$, wherein R^{I} and R^{II} are org, radicals optionally substituted and are linked to P directly or via O, NH or NR^{I}, Z is O or S; *n* is O or 1; R^{III}-R^{IV} are H or halogen, or R^{III} is $S_n{\cdot}P(Z)R^{I}R^{II}$ but at least one of R^{III}-R^{V} is other than H. They are useful as pesticides (active against flies mites aphids) and in veterinary medicine. One example (prep. described) is OO-Et_2 S-*di(chloromethyl)carbamoyl phosophorothiolothionate*. A 0·1% solution of this is 100% lethal to caterpillars, grain weevils and spider mites. F. R. BASFORD.

Borates. U.S. Borax & Chemical Corp. (B.P. 948,209, 11.1.62. U.S., 1.2.61).—A solid, flaked product consisting of $Na_2B_2O_4$ containing < 4 mol. of water of hydration is produced by forming a dry mixture of $Na_2B_4O_7{,}10H_2O$ and NaOH in a mol. ratio of 1 : 2 and, when the reaction commences, transferring the mixture to a drum dryer the drying surfaces of which are between 80° and the m.p. of the product and below the decomposition temp. of any additional components, and heating the mixture in the dryer until the reaction is completed. The mixture may also contain $NaClO_3$ 13—60 and/or an org. herbicide, pesticide or insecticide 1—18 wt.-% based on the total wt. of $Na_2B_4O_7{\cdot}10H_2O$ and NaOH. J. M. JACOBS.

Animal Husbandry

Determination of digestibility and soluble carbohydrate content of fodder crop varieties in trial. J. W. Dent (*J. nat. Inst. agric. Bot.*, 1963, **9**, 282—284).—The *in vitro* method described is based on a first-stage digestion by saliva + strained sheep-rumen liquor and a second stage using pepsin–HCl. Results obtained agree well with those given by *in vivo* methods. A. G. POLLARD.

Simplified technique for *in vitro* comparison of cellulose and dry matter digestibilities of forages. J. D. Pettyjohn, J. M. Leatherwood and R. D. Mochrie (*J. Dairy Sci.*, 1964, **47**, 1102—1104).—A procedure is described for comparing the cellulose digestibilities of forages in which the samples, mixed with rumen fluid, are suspended in bags made from dialysis tubing in the rumen of a fistulated steer for 24 h. and the residual cellulose determined by a modification of the Crampton–Maynard procedure. M. O'LEARY.

Pasture quality and ruminant nutrition. I. Carbohydrate composition of ryegrass varieties grown as sheep pastures. R. W. Bailey (*N.Z.J. agric. Res.*, 1964, **7**, 496—507).—Short-rotation ryegrass had consistently lower cellulose and possibly higher sol. sugars than perennial ryegrass. Italian ryegrass had lower cellulose and possibly lower hemicellulose but higher sol. sugars than had perennial ryegrass. There were no significant differences in lignin levels, which were always low, and only Italian differed substantially from perennial ryegrass in total N. Clover had no effect on the carbohydrate composition of the grasses but contained a higher proportion of readily fermentable carbohydrate. (19 references.) E. G. BRICKELL.

Nutritive value of N.Z. dairy pastures. III. Comparative value of various feed–fæces relationships in herbage intake studies with dairy cattle. J. B. Hutton and K. E. Jury (*N.Z.J. agric. Res.*, 1964, **7**, 583—595).—Results of digestibility trials with fresh pasture herbage fed at two levels to non-lactating twin cattle, are reported. Feed/fæces ratios were calculated from feed org. matter, feed energy, and fæcal org. matter. Deviations from intake factor regressions were association with level of fæcal output. (15 references.) E. G. BRICKELL.

Digestibility and voluntary intake of S22 and H.1 ryegrass, S170 tall fescue, S48 timothy, S215 meadow fescue and Germinal cocksfoot. D. J. Minson, C. E. Harris, W. F. Raymond, and R. Milford (*J. Brit. Grasslandt Soc.*, 1964, **19**, 298—305).—All species showed a fall in digestibility with increasing maturity when fed to sheep under conditions of voluntary intake. The digestibility of the two ryegrasses, tall fescue and timothy, began to fall well before ear emergence, whilst that of Germinal cocksfoot fell only slowly up to time of emergence. The monthly regrowths within each herbage were of

similar digestibility, but the mean digestibility of regrowths differed between herbages, being highest with meadow fescue and lowest with Germinal cocksfoot and tall fescue. The grasses with the highest digestibility in the early spring, in particular S22 ryegrass, were not always the most digestible in subsequent regrowths. The no. of sheep fed on each herbage were insufficient to show whether there were significant differences in voluntary intake between different herbage species of the same digestibility. A. H. CORNFIELD.

Animal preference in relation to the chemical composition and digestibility of varieties of cocksfoot. B. F. Bland and J. W. Dent (*J. Brit. Grassland Soc.*, 1964, **19**, 306—315).—The relationship between animal preference, measured as mean % herbage removed, and chemical composition of 11—14 varieties of cocksfoot was studied at 3 locations. In Yorkshire there was a significant positive relationship between sol. carbohydrates and animal (cows) preference and a negative correlation between fibre and animal preference. In Scotland the relationship between sol. carbohydrates and animal (sheep) preference was less pronounced. Animal preference in the spring was for the early varieties which have thick and succulent stems up to the stage of ear emergence. In the aftermath animal preferences were less marked. At Cambridge the early varieties were higher than the late varieties both in soluble carbohydrates and in digestibility. This tendency was also present in Scotland but was less pronounced. A. H. CORNFIELD.

Browse plants in Ghana. III. Determination of the free-choice Griffonia/grass ingestion ratio for West African shorthorn cattle. R. Rose Innes and G. L. Mabey (*Emp. J. exp. Agric.*, 1964, **32**, 180—190).—When given free choice in the field the cattle ingested 40% and 60% respectively of Griffonia and grass. Total dry-matter intake was 40% higher than by stall-fed cattle. Digestible protein intake exceeded estimated requirements for rapid growth by 30%. A. H. CORNFIELD.

Feeding value of rations containing sewage sludge and oak sawdust. C. B. Ammerman and S. S. Block (*J. agric. Fd. Chem.*, 1964, **12**, 539—540).—The substitution of 20% of this waste mixture for 20% of Bermuda-grass hay in a ration of maize and cottonseed meals for lambs resulted in somewhat lower nutrient digestibility, gains in wt., and feed intake. Palatability was appreciably improved after the waste mixture had been composted during 17 days. The mixture should prove useful where roughage and sources of N are scarce. (14 references.) P. S. ARUP.

Plant juices in relation to silage fermentation. III. Effect of water activity of juice. W. L. Greenhill (*J. Brit. Grassland Soc.*, 1964, **19**, 336—339).—The water activity (v.p.) of juice available for fermentation, after breakdown of the cell walls during the ensiling of plant material, depended largely on the moisture content of the sample. Water activity increased with moisture content, but probably never became so high as seriously to limit lactic-acid fermentation, although high moisture contents have other detrimental effects. At low moisture contents, the limited availability of the juice rather than its lowered water activity was most probably the factor primarily responsible for poor lactic-acid production. A. H. CORNFIELD.

Relationship between growth and carcass quality in cattle and sheep. J. C. Tayler (*Emp. J. exp. Agric.*, 1964, **32**, 191—204).—A review. A. H. CORNFIELD.

Development of indirect methods for determining the chemical composition and energy value of living cattle. J. W. Stroud (*Dissert. Abstr.*, 1964, **15**, 2133—2134).—Feeding and slaughter methods were used to establish the relation of the gross composition of the animal body to energy intake and digestibility. Indirect estimates of body-water showed the antipyrine dilution technique to over-estimate the empty body water (digesta free) by an average of 12·6%. *N*-Acetyl-4-aminoantipyrine gave values similar to those obtained by direct drying. Analyses of tissues served to evaluate the calorific value of proteins and fat as the whole body. Protein and fats from different parts of the body differed in calorific value. Reliable values for the protein and fat of the whole body are 5·447 and 9·499 kcal./g. A. G. POLLARD.

Effect of feeding high-grain restricted-roughage rations with and without bicarbonates on the fat content of milk produced and proportions of volatile fatty acids in the rumen. C. L. Davis, R. E. Brown and D. C. Beitz (*J. Dairy Sci.*, 1964, **47**, 1217—1219).—Feeding trials with lactating Holstein cows showed that the depression of the milk fat caused by feeding a high-grain–restricted-roughage diet could be prevented by the incorporation of 3% $NaHCO_3$ and $KHCO_3$ in the diet. Bicarbonate feeding increased the acetate : propionate ratio of the rumen fatty acids. M. O'LEARY

Pectic enzymes of rumen fluid. W. W. C. Smart, jun., T. A. Bell, R. D. Mochrie and N. W. Stanley (*J. Dairy Sci.*, 1964, **48**, 1220—1223).—γ-Pectinglycosidase was detected in rumen fluid from Holstein steers fed a diet of hay and concentrate. Pectinesterase was not detected. The addition of citrus pulp to the diet resulted in a significant increase in γ-pectinglycosidase activity of the rumen fluid. (14 references.) M. O'LEARY.

Relationship of certain milk-fat-depressing diets to changes in the proportions of the volatile fatty acids produced in the rumen. D. C. Beitz and C. L. Davis (*J. Dairy Sci.*, 1964, **47**, 1213—1216).—Experiments with Holstein cows indicated that feeding of cod-liver oil lowered milk fat content by affecting milk production somewhere beyond the rumen, whereas a high-grain–restricted roughage ration exerted a similar effect by changing the proportions of volatile fatty acids produced during rumen fermentation. (12 references.) M. O'LEARY.

Physiological effects of high-level concentrate feeding. E. M. Kesler and S. L. Spahr (*J. Dairy Sci.*, 1964, **47**, 1122—1128).—The literature dealing with the effects of high-level concentrate feeding on milk production is reviewed. (63 references.) M. O'LEARY.

Feeding value of limestone-treated maize silage for lactating dairy cows. J. H. Byers, C. L. Davis, and C. E. Baylor (*J. Dairy Sci.*, 1964, **47**, 1062—1064).—Feeding trials with 24 high-producing Holstein, Brown Swiss and Jersey cows revealed no significant difference between the feeding value of maize-silage containing 1% limestone and that of untreated maize-silage. M. O'LEARY.

Effect of excessive iron intake upon the health and production of dairy cows. M. R. Coup and A. G. Campbell (*N.Z. J. agric. Res.*, 1964, **7**, 624—638).—High intake of iron [as $Fe(OH)_3$] caused scouring, loss of body wt., and lowered production of milk and butterfat. Intakes below 30 g./day affected milk yield and digestibility of ingested herbage, and between 30 and 60 g./day lowered butterfat yield and caused losses of body wt. (18 references.) E. G. BRICKELL.

Nitrate toxicity in dairy heifers. I. Effect on reproduction, growth, lactation and vitamin-A nutrition. K. L. Davison, W. Hansel, L. Krook, K. McEntee and M. J. Wright (*J. Dairy Sci.*, 1964, **47**, 1065—1073).—Trials with 45 dairy heifers fed with NO_3^- at levels of 0, 440 and 660 mg./kg. body wt. showed that conception rate was lowest in the heifers fed 660 mg. NO_3^-. The no. of abortions was higher the greater the NO_3^- level but the results were not statistically significant. The level of NO_3^- fed had no effect on growth, length of oestrous cycles, length of gestation, birth wt. and performance of calves, vitamin A and carotene nutrition, or on milk production. (40 references.) M. O'LEARY.

Vitamin deficiencies in farm animals in the tropics. P. J. Larkin and R. J. Yates (*E. Afr. agric. For. J.*, 1964, **30**, 11—20).—A review. A. H. CORNFIELD.

Nutritive value losses of hay by ventilation by suction in comparison with blowing. N. K. Dijkstra (*Versl. landbouwk. Onderz.*, 1964, 647, 24 pp.).—Average losses of digestible crude fibre and starch equiv. found in experiments over two years were slightly (but not significantly) higher in suction-ventilated stacks than in stacks ventilated by blowing. P. S. ARUP.

Effects of grazing management on plasma-calcium and -magnesium concentrations of ewes in early lactation. R. G. Hemingway, N. S. Ritchie, N. A. Brown and J. N. Peart (*J. agric. Sci.*, 1965, **64**, 109—113).—Plasma-Mg and, unexpectedly, plasma-Ca declined on transfer of the animals to the experimental plots. The Ca concn. increased within 24 h. of each successive grazing change. Cases of clinical grass tetany were associated with a marked hypocalcaemia (< 6·0mg. of Ca/100ml.) and a marked hypomagnesaemia (< 0·5mg. of Mg/100ml.). Where only low Mg without low Ca concn. occurred, clinical tetany was not found. Old ewes with twins developed lower plasma-Ca concn. than did those with single lambs. M. LONG.

Theoretical aspects of nitrogen economy in grazing experiments. R. L. Davidson (*J. Brit. Grassland Soc.*, 1964, **19**, 273—280).—Results of small-plot tests and grazing trials on natural grassland in South Africa are used to develop an hypothesis relating to the circulation of N between soil, plants and animals. Where the value of land is low in comparison with the cost of fertiliser, it is essential that the max. response be obtained from fertiliser as well as from excreted N. Live-wt. data indicated that 80% of herbage N is returned as excreta, of which 25% is recovered by the grasses in the first season and 25% in the second season. In trials over 5 years the average apparent recovery of fertiliser N was 22·4% where 30 lb. of N per acre per annum (with an initial heavy dressing) was applied and 11·6% where 60 lb. of N per acre per annum was applied. A distinction is drawn between the initial build-up of N in circulation and the long-term maintenance of a N level providing max. recovery of fertiliser N. A. H. CORNFIELD.

Nitrogen dioxide production from silage. II. Field survey. J. V. Scaletti, J. J. Jezeski, C. E. Gates, and L. M. Schuman (*Agron. J.*, 1965, **57**, 65—67).—Over 5 years the presence of NO_2 gas produced from maize silage, in concn. considered hazardous, occurred on 42%

of 554 farms. The highest incidence occurred during a drought year and the following year. There were indications that NO_3 production was related to temp. and rainfall and was also associated with soil texture, org. matter, available P and K. A. H. CORNFIELD.

Losses in the conservation of grassland herbage in lined trench silos. I. Comparison of long and lacerated silages made by the warm fermentation process. II. Comparison of lacerated silages of low and high dry-matter content made by the cold fermentation process. W. O. Brown and J. A. M. Kerr (*J. Agric. Sci.*, 1965, **64**, 135—141, 143—149).—I. Laceration does not reduce losses in dry matter or in starch equiv. and is the preferred method with regard to rapid filling of the silo and to fermentation quality.

II. Losses in dry matter are lower where wilted lacerated silages are made than where the fodder is unwilted. Wilting also produces silages having lower volatile acid and lactic acid contents. Digestible N-free extractives losses are lower with wilting. M. LONG.

Effects of different processing temperatures on the utilisation of solvent-extracted cottonseed protein by sheep. L. B. Sherrod and A. D. Tillman (*J. Anim. Sci.*, 1964, **23**, 510—516; cf., *idem.*, *ibid.*, 1962, **21**, 901).—Portions of cold hexane-extracted cottonseed meal were autoclaved at 15 p.s.i. (250° F) for varying periods (20—240 min.). When the products were fed to sheep the faecal loss of N increased and urinary-N diminished in substantially linear proportions with increase in time of autoclaving. N retention as % of intake was max. when the meal was heated for 60 min. Autoclaving raw cottonseed meal for 60 min. increased the rate of gain in wt. and the feed efficiency more than did heating for 120 or 240 min. or the unheated control. The possible bearing of heat treatments on the detoxication of gossypol in the rumen is discussed in the light of analyses of the ruminal volatile fatty acids obtained in the course of the metabolism trials. (29 references.)
A. G. POLLARD.

Rate of passage of chromic oxide and composition of digesta along the alimentary tract of wethers. D. E. Johnson, W. E. Dinusson and D. W. Bolin (*J. Anim. Sci.*, 1964, **23**, 499—505).—The efficiency of Cr_2O_3 impregnated in paper is compared with that of the powdered oxide as an indicator in digestibility studies. In experiments with sheep, 1500 g. of Cr_2O_3 were given daily in the ration. Powdered oxide was excreted appreciably quicker than that in the 'paper' form. Digestion coeff. determined by the conventional 'powder' method agreed with those resulting from the ratio procedure using Cr_2O_3-paper. Recoveries of Cr_2O_3 powder were low in all cases. Cr_2O_3 passed out of the rumen faster than did lignin or the bulk of the dry matter; its rate of passage was similar to that of Ca^{2+}. A. G. POLLARD.

Relationship between the nitrogen content and the heat of combustion value of sheep urine. O. L. Paladines, J. T. Reid, B. D. H. Van Niekerk and A. Bensadoun (*J. Anim., Sci.*, 1964, **23**, 528—532).—Sheep were fed rations containing different amounts of chopped hay, the same hay ground and pelleted or a pelleted mixture of the ground hay, 55%, with maize meal 45%. Urine samples from all groups of animals were collected periodically and calorific values (*C.V.*) and N contents of each were determined. From the data obtained, relationships were established leading to formulae for the prediction of *C.V.* from the N concn. or content of the urine.
A. G. POLLARD.

Microbial digestion in ruminants; nitrogen metabolism in the rumen. G. A. McLaren (*J. Anim. Sci.*, 1964, **23**, 577—590).—A symposium. (147 references.) A. G. POLLARD.

Effect of a synthetic diet containing non-protein nitrogen on acetate metabolism in the ruminant. J. R. Sabine, E. D. Mayfield and B. C. Johnson (*J. Anim. Sci.*, 1964, **23**, 555—557).—Sheep were fed a semi-purified diet in which the sole N source was 4% of urea with 0·5% of methionine. The acetate metabolism in the rumen was examined during continuous infusion of ^{14}C-labelled acetate *via* the jugular vein (cf. Sabine and Johnson, *J. biol. Chem.*, 1964, **239**, 89). There was no significant difference in acetate utilisation whether the ration contained protein or non-protein-N. A. G. POLLARD.

Effects of fluorine on dairy cattle. III. Digestion and metabolism-trials. L. E. Harris, R. J. Raleigh, G. E. Stoddard, D. A. Greenwood, J. LeG. Shupe and H. M. Nielsen (*J. Anim. Sci.*, 1964, **23**, 537—546; cf. Shupe *et al.*, *Amer. J. vet. Res.*, 1963, **24**, 924).—Over a 7-year period commencing with calves aged 3—4 months, NaF was added to the feed in proportions to make the total F in the ration 10, 28, 55 or 109 p.p.m. of the dry matter. Feed intake digestion coeff. and nutrient intake were not affected by the F supplement over the first two years prior to lactation. In the following years the upper level of F lowered feed consumption and nutrient absorption; similar effects were produced in some animals receiving F at 55 p.p.m. Increase in the proportion of concentrate (grain pellets) in the ration tended to increase the energy-intake but did not affect the retention of F; the latter was also unaffected by increasing the level of Ca-P minerals in the ration. Over the 7-year period the average tolerance of F (as NaF) was 27—49 p.p.m. of the total dietary dry matter. A. G. POLLARD.

Comparative effects of fluorine from soft phosphate, calcium fluoride and sodium fluoride on steers. C. B. Ammerman, L. R. Arrington, R. L. Shirley and G. K. Davis (*J. Anim. Sci.*, 1964, **23**, 409—413).—Steer calves and 2-year steers for 91 days were fed soft phosphate (**I**), CaF_2 (**II**), each providing F at 134 p.p.m. or NaF (**III**), providing 67 p.p.m. in the ration. The F content of metacarpals (% of boneash) was higher when **I** than when half its equivalent of F was fed as **II**; more F was deposited from 67 p.p.m. dietary F as **III** than from 134 p.p.m. dietary F as **II**. Feed consumption and conversion, and average daily gain in wt. were not affected by any of the F sources tested. A. G. POLLARD.

Variations in the intraruminal fatty acid ratios of sheep fed ryegrass harvested at different stages of maturity. P. F. Parks, M. E. Rieme, C. M. Lyman and H. O. Kunkel (*J. Anim. Soc.*, 1964, **23**, 344—349).—The mol. proportions of acetic (**I**), propionic (**II**) and butyric (**III**) acids in the rumen fatty acids in sheep fed Gulf ryegrass harvested at different stages of maturity are recorded. As the grass matured the mol. % of **I** increased and those of **II** and **III** diminished. Changes in mol.-% were paralleled by changes in nutrient value and digestibility of the grass. Constituents of the grass most closely related to the ratio of intraruminal acids were sol. sugars, proteins and lignin, the two first-named diminishing and the third increasing with the advancing age of the grass. A. G. POLLARD.

Evaluation of cattle foods and diets in terms of the ruminal concentration of volatile fatty acids. II. Roughages and succulents. L. H. Bath and J. A. F. Rook (*J. Agric. Sci.*, 1965, **64**, 67—75).—Hays, silages and dried grass give rise to high values for the molar proportion of acetic acid (**I**) of about 70% in the ruminal volatile fatty acids (*VFA*). Silage tends to give a lower proportion of propionic acid (**II**) and higher concn. of butyric acid (**III**) than do the hays. Replacement of half the hay by concentrate decreases **I** slightly and increases **III**. Mangolds, dried sugar-beet pulp, sugar-beet tops and brewers grains give rise to lower proportion of **I** and a variable increase in **II** and **III**. Marrow stemmed kale, stall fed, leads to higher **III** and valeric acid levels, but if grazed leads to a slightly lower proportion of **I** and much more **II**. Leafy herbage in springtime acts similarly to other succulent foods, whilst mature growth and autumn regrowth resemble hays in their effects. Relations are shown between structural carbohydrate, water-sol. carbohydrate and protein contents of the diet and the relative proportions of *VFA* in the rumen liquor. M. LONG.

Influence of Aureomycin on rumen metabolism. T. J. Klopfenstein, D. B. Purser and W. J. Tyznik (*J. Anim. Sci.*, 1964, **23**, 490—495).—In trials with rumen-fistulated wethers (5 months old) Aureomycin (**I**) was administered by infusion of 20 mg./day via fistula, 90 min. before feeding or by addition to the feed at 20 or 60 mg./day. Rumen fluid samples were taken immediately before and at intervals (1—12 h.) after feeding. Bacterial concn. in the rumen changed very little during the treatment but protozoal concn. increased considerably. Addition of **I** significantly increased the apparent digestibility of the dry matter and of N, both N balance and urinary N tending to increase. In *in vitro* tests of rumen organisms from treated animals, inhibition by **I** was greater in samples from control animals than from those fed 60 mg. of **I** daily. Gas production *in vitro* by rumen contents 1 h. after feeding animals receiving 20 mg. of **I** daily exceeded that form control animals. (27 references.) A. G. POLLARD.

Rate of feed consumption and body weight of beef cattle. P. A. Putnam, R. Lehmann and R. E. Davis (*J. Anim. Sci.*, 1964, **23**, 425—429).—Steers of different wt. (500—1150 lb.) were fed individually, *ad lib.*, on rations differing in physical form (pelleted, ground or in bulk). The times and periods of feeding were recorded automatically as also were food consumption and wt. of the animals. The rate of increase in live-wt. was directly related to the speed with which the feed was consumed (lb./h.), the relationship being influenced by the physical form of the ration used. A. G. POLLARD.

Effects of varying protein levels in finishing rations for beef cattle. M. L. Ray and R. D. Child (*Ark. Farm Res.*, 1964, **13**, No. 2, 15).—Diminution in the proportion of protein in the ration as the end of the finishing period is approached increased fat disposition only in the older animals. Increasing the protein level at this stage lowered the rate of gain in wt. to less than that resulting from the reverse order of change or that produced by an intermediate protein level maintained throughout. A. G. POLLARD.

Growth and nitrogen balance with steers fed 'Hi-N-molasses'. C. A. Putnam and R. E. Davis (*J. Anim. Sci.*, 1964, **23**, 339—343).—The material is a by-product of a recent sugar-refining process based on ion-exchange principles; it contains 1·7—2·2% of N as NH_4+ salts of S, P and C. In trials with steers 'Hi-N-molasses' (**I**),

proved palatable when fed with hay and had no apparent deleterious effects on the nervous or digestive system. The performance of the steers was similar whether the ration contained (*a*) 30% of **I** or (*b*) 30% of molasses with equivalent N as urea. Digestibility data were also similar except that the value for crude protein was lower in (*a*). In a further trial, in which 40% of the ration was (*c*) **I** + urea or (*d*) molasses + cottonseed meal, the digestibility of dry matter, fibre and calories was lower and that of crude protein higher in (*c*) than in (*d*). No differences were apparent between the rations in respect of N retention expressed either as g./day or as % of the N consumed. A. G. POLLARD.

Grazing and drylot cattle fed grain and a cellulolytic enzyme. W. W. Heinemann (*J. Anim. Sci.*, 1964, **23**, 450—453).—Yearling steers received implants of progesterone, 200 + oestradiol benzoate 20 mg. and were fed an all-roughage ration for 49 days followed by roughage + ground maize for 117 days. A group of the animals (*A*) was turned out to graze lucerne and a comparison group was fed a similar proportion of lucerne as hay, in dry lot. The cellulytic prep. was incorporated in lucerne pellets. The average daily gain in wt. was not affected by the enzyme prep. Feed efficiency was higher in dry lot but was not associated with the enzyme. At slaughter the dressing % was higher in the *A* animals. Marbling was the only carcass characteristic significantly correlated with final carcass grade in both groups of animals. A. G. POLLARD.

Effect of feeding coconut oil meal on milk production and composition. K. Mohammed, W. H. Brown, P. W. Riley and J. W. Stull (*J. Dairy Sci.*, 1964, **47**, 1208—1212).—Feeding trials with Holstein cows indicated that addition of coconut oil or coconut meal to a ration consisting of 60% good-quality lucerne hay and 40% concentrate resulted in an increase in the amount of C_{12} fatty acids in the milk and in a depression of the total butyrate and valerate content of the rumen fluid but had no effect on gross milk composition. (23 references.) M. O'LEARY.

Bacteria of the ovine rumen. IV. Effect of change of diet on the predominant type of cellulose-digesting bacteria. L. Gouws and A. Kistner (*J. agric. Sci.*, 1965, **64**, 51—57).—*Ruminoccus albus* (**I**) strains producing rhizoid colonies and well-defined zones of cellulolysis are the predominant cellulose digesters when sheep are fed with lucerne hay (14% crude protein). After a period, varying from 2—5 or more weeks on a diet of teff hay (3% crude protein) *Butyrivibrio* species take over. Resumption of a diet of lucerne hay brings **I** into prominence again after a 2 week period. Immediately after a change in diet, cocci, giving rise to atypical colonies and zones of cellulolysis occur transiently, as the most abundant cellulose digesters. M. LONG.

Effect of water deprivation upon the rumination behaviour of housed sheep. John G. Gordon (*J. agric. Sci.*, 1965, **64**, 31—35).—After 1 or 2 days without water, except in the air-dry food offered, no important changes occur. However, by the fourth day without water rumination falls by 30% in terms of time spent in chewing and by 34% in terms of the no. of boli regurgitated; food intake falls by 46%. M. LONG.

Low-level diethylstilboestrol-implantation for lambs grazing lucerne. W. N. Garrett (*J. Anim. Sci.*, 1964, **23**, 430—435).—Implants of stilboestrol (3—12 mg.) were given to lambs and wethers of white-face (*W*) and black-face (*B*) phenotypes. The response of lambs in increased average daily gain in wt. was more consistent in *W* than in *B* types at all levels of implantation, wethers gaining more than ewes. Energy used per lb. of gain in wt. was lower for implanted than for untreated animals. Improved feed efficiency, frequently recorded as due to the implantations, may not be real if calculated on an energy basis. A. G. POLLARD.

Comparative effects of diethylstilboestrol and diallylhexoestrol on digestion and nitrogen metabolism in sheep. P. D. Whanger, J. A. Welch, G. C. Anderson, G. A. McLaren and K. M. Barth (*J. Anim. Sci.*, 1964, **23**, 506—509).—Lambs were fed a semi-purified diet based on molasses, concentrates (66% of the N being as urea) ,and roughage (maize cobs), supplemented with diethylstilboestrol (**I**), 1·6 mg. daily or with diallylhexoestrol (**II**) (4·2 or 12·5 mg. daily). In a further trial all dietary N was supplied as urea and wheat straw replaced maize cobs as roughage. Neither **I** nor **II** had any appreciable effect on nutrient digestibility or on blood levels of amino-acid-N, urea-N, NH_3-N or glucose. **I** increased the % of absorbed N retained. A. G. POLLARD.

Protein and energy nutrition of the bacon pig. IV. Digestible energy values of cereals in pig diets. D. W. Robinson, J. H. D. Prescott, and D. Lewis (*J. agric. Sci.*, 1965, **64**, 59—65).—The procedure and cage used in the trials are described. Total digestible nutrients (TDN) and digestible energy (*DE*) values agree well, an average factor being 44·2 for converting TDN into *DE* (kcal/kg), although this varies from food to food. The *DE* for barley, wheat, maize and milo are 2880, 3300, 3430 and 3300 kcal./kg., respectively, when the values are corrected for absorbed N. *DE*, as determined by bomb calorimetry of feed and faeces and corrected for N absorbed provides a rapid and accurate means of assessing the potential energy value of the feed to the animal. M. LONG.

Relation between ingested fats, body lipids and fatty acids of milk in the sow. E. Salmon-Legagneur (*Ann. Biol. anim. Biochim. Biophys.*, 1964, **4**, 141—155).—Sows, fed iso-caloric diets of three types (details given) during pregnancy and lactation, were studied for relative contribution of dietary fatty acids and depot fats to milk lipogenesis. Samples of adipose fats and milk fats were analysed by gas–liquid chromatography. During pregnancy, diet affected the type of fats deposited in the body but during lactation there were no further differences attributable to diet. The composition of fatty acids of milk was affected by and originated from fatty acid of the diet, from fatty acid synthesis and a decreasing proportion from body fats, during both pregnancy and lactation. (21 references.) J. V. RUSSO.

Comparison of three practical rations for growing pigs. R. Braude, M. R. Lyon and J. G. Rowell (*J. agric. Sci.*, 1965, **64**, 87—91).—Comparisons of the 'Shinfield ration', simpler and cheaper, the 'old' National Pig Progeny Testing Station ration and the 'new' ration carried out at 26 centres indicate that the Shinfield ration does not give significantly worse growth rates than the new ration which is significantly better than the old. M. LONG.

Barley rations for swine. III. Lysine and methionine supplementation; effects on rate and efficiency of gain and on carcass characteristics. D. Reimer, R. J. Meade and R. S. Grant (*J. Anim. Sci.*, 1964, **13**, 404—408).—Addition of lysine and/or methionine to a 14%-protein pelleted ration, based on barley and soya-bean meal, did not improve the rate or efficiency of gain in wt. of growing pigs nor did it affect carcass leanness. A. G. POLLARD.

Effects of dietary protein level and environmental temperature on the performance and carcass quality of growing-finishing swine. E. W. Seymour, V. C. Speer, V. W. Hays, D. W. Mangold and T. E. Hazen (*J. Anim. Sci.*, 1964, **23**, 375—379).—In feeding trials carried out in summer and in winter, significant interaction was shown between the effects of dietary protein and of temp. on the amount of feed required per lb. of increase in wt. from age 3 weeks to 110 lb. live-wt. in summer and up to 200 lb. in winter; a further interaction was shown with the % of lean cuts in the winter feeding experiment. Pigs maintained at 60°F grew faster than did those kept at 90°F in summer; those maintained at 36°F required more feed per unit increase in wt. than did those kept at 60°F. The latter, in time, required slightly more feed per unit gain than did others raised at 90°. A. G. POLLARD.

Effects of the inclusion of dried skim milk, whole milk or maize oil in maize–soya-bean meal diets from early weaning to market weight on performance and carcass characteristics of pigs. J. R. Jones and W. G. Pond (*J. Anim. Sci.*, 1964, **23**, 481—484).—Weanling (3-week) pigs were fed rations based on maize–soya-bean meal (21 or 18%-protein) or others in which part of the concentrate was replaced by (i) maize oil, (ii) dried skim milk or (iii) dried whole milk. During the first 7 weeks (iii) produced the most rapid growth, followed by (ii), rates of gain being unrelated to levels of dietary fat. Subsequently the high-fat diets [(i) and (iii)] produced the greater gains in wt. The digestible energy consumption was substantially the same for all rations. The I val. of the back fat varied with the ration viz., (i) 97·7, (ii) 51·6, (iii) 50·2 and for the original unsupplemented ration 57·8. A. G. POLLARD.

Amino-acid supplements to milo–groundnut meal rations for growing pigs. S. K. Ranjhan, A. H. Jensen, J. L. Cox, B. G. Harmon and D. E. Becker (*J. Anim. Sci.*, 1964, **23**, 461—464).—In feeding trials with pigs of 18—50 kg. live-wt. on pasture or in drylot, a 16%-protein ration based on milo–groundnut meal (**I**) was nutritionally inadequate. Supplements of lysine, 0·13% or lysine 0·13% + methionine 0·13% or fish meal 3%, or fish meal + methionine 0·1%, improved the growth rate, which, however, was not in any case as good as that obtained by a comparable maize–soya-bean meal ration. A 20%-protein **I** ration was supplemented with various proportions of fish meal (4—10%), best results were obtained with 8% of fish meal but this was still inferior to the maize–soya-bean ration. A. G. POLLARD.

Effect of amino-acid supplementation of rations containing meat and bone scraps on rate of gain, feed conversion and digestibility of certain ration components for growing-finishing swine. W. G. Luce, E. R. Peo jun. and D. B. Hudman (*J. Anim. Sci.*, 1964, **23**, 521—527).—In trials with 234 growing-finishing pigs the effects of lysine (**I**), methionine (**II**) and tryptophan (**III**), supplements to rations containing 5 or 10% of meat and bone scraps (*MB*) are examined. Neither **I** nor **II** added to the 5% *MB* ration significantly

increased the rate of gain in wt.; some increase was produced by **III** supplements in most cases. Addition of **I** and **II** to the 10% *MB* ration diminished growth rates and lowered feed consumption but did not affect the feed required per unit gain in wt. The digestibility of dry matter, energy and N tended to diminish as the no. of supplementary amino-acids was increased and usually was greater in the morning than in later feeds. In general, *MB* with or without supplementary amino-acids, did not improve a maize–soya-bean meal ration but could effectively replace part of the soya-bean meal if an increase in total protein content of the ration is desired.

A. G. POLLARD.

Fluorometric determination of chlortetracycline in low-level mixed feeds. S. E. Katz and J. Spock (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1157—1161).—The chlorotetracycline is extracted with aq. acetone acidified with HCl, retained on a Permutit Decalso Na-form resin, and eluted with hot aq. Na_2CO_3. The isomer so produced is determined fluorometrically using an activation wave-length of 350 mμ and an emission wave-length of 420 mμ. Recoveries of 75·8—100·0% are reported. A. A. ELDRIDGE.

Low-level feeding of ronnel for controlling horn flies or cattle grubs. J. Simco and J. L. Lancaster jun. (*Ark. Farm Res.*, 1964, **13**, No. 2, 16).—Ronnel (**I**) was added (5%) to a mineral mixture for cattle and the rate of consumption was adjusted so that each animal received approx. 2 lb. of **I** per month. Counts of horn flies and cattle grubs were markedly lowered (to 1—2% and to 30% respectively).

A. G. POLLARD.

Poultry feed and supplements therefor. Celanese Corp. of America (B.P. 948,623, 24.2.60. U.S., 27.2.59).—There is claimed a poultry feed containing as growth stimulant 5—10 wt.-% of β-nitropropionic acid or an alkali metal or alkaline-earth metal salt thereof.

F. R. BASFORD.

Penicillins. Smith, Kline and French Laboratories (B.P. 948,952, 9.6.61. U.S., 31.3.61).—Acylated 6-aminopenicillanic acid deriv. are prepared for use against penicillin-resistant organisms and for administration to livestock and poultry, e.g., in animal feeding stuffs. A representative compound is *K* 6-(*o-phenylbenzamido*)*penicillanate*, m.p. 212—215°. H. S. R.

[A] **Stabilisation of fermentation harvest mash solids containing tetracyclines.** [B] **Stabilised animal feeds containing antibiotics.** American Cyanamid Co. (B.P. 949,631—2, 30.5.61. U.S., 22.6.60. [B] out of [A]).—[A] A fermentation harvest mash containing a tetracycline antibiotic is adjusted to pH 0·1—3 (with, e.g., aq. HCl or H_2SO_4), then the mixture is further adjusted to pH 8·4—13, and the solids are filtered off and dried, to give a stabilised product. [B] A stabilised animal feed composition is produced by adding to such a feed composition containing or comprising the stabilised product of [A], enough of a base, e.g., $Ca(OH)_2$, to maintain pH 6—12 (as measured when the feed is diluted with water to ~ 10% of solids).

F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Curing of freshly harvested rice by heat treatment. K. R. Bhattacharya, H. S. R. Desikachar and V. Subrahmanyan (*Indian J. Technol.*, 1964, **2**, 378—380).—Fresh rice may be cured by heating in a humid atm.; the rate of cure increasing with increasing temp. and humidity. Heating fresh rice in a closed rotating drum for 40 min. at 90—95° and keeping overnight in incubated storage gives satisfactory curing. E. C. DOLTON.

Functional characteristics of edible soya flours. T. M. Paulsen, F. E. Horan and T. L. Daniels (*Cereal Sci. Today*, 1965, **10**, 14—17).—Criteria used for correlation of data for measurement of functional or physical characteristics of edible soya flours with the quality of white bread containing soya flours included water-dispersibility of protein, wettability, foaming characteristics and visco/amylo/graph curves. Data obtained demonstrated the effect of heat, salts, and acids on soya-protein dispersibility for flours at several pH. Soya flours treated with H_2O_2 alone or H_2O_2 and $CaCl_2$ appear to have more desirable functional characteristics for bread-making than heat-treated flours. (12 references.) G. W. FLINN.

Characteristics of humidity elements used in flour moisture measurements. F. J. Hughes, B. M. Tessem and R. B. Koch (*Cereal Sci. Today*, 1965, **10**, No. 1, 6, 8, 12).—A Honeywell relative humidity instrument, consisting of a W611A balancing bridge and Q229A gold grid sensing elements, which could be inserted directly into the flour sample, was used to determine R.H. rapidly and accurately. Equilibrium was reached in 8 to 15 min. Temp. correction was simple, and variations in protein content had little effect on moisture measurements. A specially designed cycling apparatus was used for life tests on the sensing elements which were little affected by 2000 measurements or storage in flour for 16 days. G. W. FLINN.

Determination of particle size distribution in flour. H. Bolling and F. Springer (*Getreide u. Mehl*, 1964, **14**, 128—132).—The Coulter counter is described and experiences in its use for flour examination are reported. The results obtained are compared with those obtained by use of the Sartorius sedimentation balance.

E. C. APLING.

Flour protein solubility and baking quality. XI. Protein solubility in doughs. XII. Influence of γ-radiation. E. Maes (*Getreide u. Mehl*, 1964, **14**, 133—136, 136—138).—**XI.** Results are reported for total sol. protein and protein sol. in water, propanol, lactic acid and KOH in doughs prepared with or without additions of yeast and/or salt from a series of flours of protein contents (N × 5·7) varying from 6 to 14·2% and mixed and/or rested for varying times. Protein solubilities were modified in the presence of salt and/or yeast, but in general, water soluble protein in dough was higher and protein sol. in propanol was lower than in the parent flour, and protein solubility in all solvents but KOH increased with mixing time. On resting the dough, total sol. protein and protein sol. in propanol decreased during the first 30 min. and subsequently increased again. (12 references.) (Paper duplicated in *Brot u. Gebäck*, 1964, **18**, 216—219.)

XII. Treatment of European soft wheat (6·9% protein) with a high radiation dose (5000 krad) markedly reduced Zeleny sedimentation value, test no., dextrin figure (Lemmerzahl), falling no. and gassing power, increased grade figure (Kent-Jones) and produced an off-flavour. Protein solubility in water and in lactic acid was increased and solubility in propanol and in KOH was decreased, but the changes were much less than would be predicted from the changes in baking quality. With smaller doses (from 50 to 500 krad), bread vol. from European and mixed flours increased up to a dose of 100 krad, and then fell to below that from untreated control flour at about 500 krad; in this treatment range only very minor changes in protein solubility were found. E. C. APLING.

Maize gluten, a new ingredient for bakery products. C. Feldberg (*Cereal Sci. Today*, 1965, **10**, No. 1, 18—19, 28).—Maize gluten was analysed. Addition of 3% maize gluten to bakery products improved colour, crumb moistness, moisture retention and tenderness, and storage stability. Formulae for egg, cheese, raisin and maize breads, pound cake and devil's food cake are given with vol. and compressibility data. G. W. FLINN.

Significance of wedge protein of rye flour for dough properties. F. Voneš, V. Podrazký, J. Šimová and Z. Veselý (*Cereal Chem.*, 1964, **41**, 456—464).—The influence of wedge protein on the mechanical properties of rye flour doughs was studied by Farinograph, Amylograph and baking test evaluations of mixtures of wedge protein and the flour residue from wedge protein separation. Wedge protein was indispensable for the formation of normal rye dough and a significant determinant of water adsorption. The probable formation of complexes between rye proteins and gums was shown from determinations of intrinsic η of solutions of model mixtures of rye gluten (washed out from separated wedge protein) and rye gums (precipitated with ethanol from deproteinised and dialysed flour extracts). (15 references.) E. C. APLING.

Polarographic behaviour of gluten and thiosulphogluten. H. Matsumoto and T. Kuninori (*Cereal Chem.*, 1964, **41**, 491—502).—Studies of the polarographic catalytic reduction waves of gluten and thiosulphogluten (gluten in which part of the disulphide bonds were cleaved to SSO_3^- groups by the action of sulphite) are reported. For both materials the first wave occurred at −1·4 v and the second at −1·65 v, measured against an Hg pool electrode; the waves increased in height with increase in protein concn. (Electrolyte: $1·2 \times 10^{-3}$ M $CoCl_2$, 0·2-N NH_4Cl and 0·05-N NH_4OH in 0·2 M urea at pH 9·4). (21 references.) E. C. APLING.

Properties of wheat flour proteinases. C. E. McDonald and L. L. Chen (*Cereal Chem.*, 1964, **41**, 443—455).—Wheat flour (wholemeal or patent) shows the presence of proteinases of max. activity at pH 3·8 (haemoglobin substrate) which are readily extractable by water at pH 8 or by pH 3·8 acetate buffer, but not by distilled water (at pH 5·8), and also of unextractable proteinases of max. activity at pH 4·4. On self-digestion flour shows max. proteolytic activity at pH 4·0 (much less than that found in haemoglobin substrate). Only part of the activity is accounted for by papainase-type enzymes; the action of *p*-chloromercuribenzoate and *p*-chloromercuriphenylsulphonic acid indicated that a papainase enzyme is present in both the extractable and unextractable fractions, but *N*-ethylmaleimide and iodoacetamide produced inhibition of the unextractable fraction

only. Trypsin and chymotrypsin inhibitors were ineffective against flour proteinases. The proteolytic activity of flour is substantially reduced both by dough mixing and by additions of NaCl. (19 references.) E. C. APLING.

Evaluation of durum wheat and durum products. II. Separation and identification of the sitosterol esters of semolina. K. A. Gilles and V. L. Youngs (*Cereal Chem.*, 1964, **41**, 502—513).—Sitosteryl palmitate was isolated from the lipids of wheat, barley and rye by thin-layer chromatography and identified by gas chromatography, colour change, m.p. and mixed m.p., and R_F comparisons, and sitosteryl oleate, linoleate and linolenate were similarly separated and identified. Only a trace of sitosteryl palmitate was found in durum varieties and the light petroleum extracts from mixtures of farina and durum semolina gave spot-densities of the palmitate (on thin-layer chromatoplates developed with CCl_4) which were proportional to the known farina content of the mixtures. The technique may provide a basis for the convenient detection of other cereal grains in durum semolina. (11 references.) E. C. APLING.

Results of ball-milling Buhler experimentally milled hard winter wheat flour. J. Schlesinger (*Cereal Chem.*, 1964, **41**, 465—473).—Ball milling of experimentally-milled flour resulted in smaller average particle size, higher maltose value, increased Farinograph absorption, longer Farinograph curve, lower loaf vol. and poorer baking score. Starch damage and the consequent increase in water absorption, rather than gluten damage, was shown to be the main cause of deterioration in baking results. (16 references.) E. C. APLING.

Simplified gel electrophoresis apparatus used in wheat gel protein research. J. E. Cluskey (*Cereal Chem.*, 1964, **41**, 551—553).—The construction of a simplified and inexpensive apparatus and it uses in the separation of cereal proteins is described. E. C. APLING.

Modification of cereal flours with hydrochloric acid. E. B. Lancaster, K. J. Moulton, D. Uhl and V. E. Sohns (*Cereal Chem.*, 1964, 41, 484—491).—The rate of modification of a soft white winter wheat flour, predried to < 3% moisture, and treated with from 1·3 to 1·6% of HCl applied as 4N solution doubled for each 10°F increase in temp. in the range from 90 to 110°F, but was little affected by the amount of acid. Comparative tests with other materials showed that starch reacts much more rapidly than flour and that for various flours the rate varies both with flour type and protein content. The cost of producing acid modified flour is estimated at 1·9 cents per lb. above the price of the raw material E. C. APLING.

High-protein rice flours. D. F. Houston, Ali Mohammad, T. Wasserman, and E. B. Kester (*Cereal Chem.*, 1964, **41**, 514—523).—Investigations of the separation of high-protein flours from milled rice by (i) fine-grinding (turbomilling) and air classification and (ii) by scouring off successive layers from whole kernels in an abrasive cone mill, are reported. Turbomilling and air classification yielded about 8—10% of fine-particle flour with protein content increased by about 75%, and a residual flour with a slightly lower protein content than the milled rice. The abrasion process gave a 10—15% yield of flour from the outermost layers with about twice the protein content of the whole kernels, and a residue of whole kernels of slightly reduced size and protein content, together with some broken kernels. The high protein flours from both processes had higher ash, fat, thiamine and riboflavin contents and amylase activities than the original milled rice. (23 references.) E. C. APLING.

Electronic method for the measurement of heat-damage in artificially dried maize. C. E. Holaday (*Cereal Chem.*, 1964, **41**, 533—542).—The method is based on consideration of the relation between total and surface moisture contents of the kernels, determined by measurements of electrical capacitance and d.c. resistance respectively. For undamaged samples the relation between capacitance and log d.c. resistance is linear in the moisture range from 10 to 20%; values from heat damaged samples show displacements from this line (conveniently measured in capacitance units) which increase with increase in heat damage. Results presented for two sets of artificially dried samples of maize for which the yields of prime starch were determined show that the electrical measurements yielded an accurate index of drying damage and a highly significant correlation with the glutamic acid decarboxylase activity of the samples. (12 references.) E. C. APLING.

Carotenoids of maize and sorghum. VI. Determination of xanthophylls and carotenes in maize gluten fractions. C. W. Blessin, J. D. Brecher and R. J. Dimler (*Cereal Chem.*, 1964, 41, 543—548).—Carotenoid contents of two samples of hybrid yellow dent maize and the commercial maize gluten fractions derived from them are reported. Variations in total carotenoid content were: whole maize, 19—30 p.p.m., maize gluten (60—70% protein), 247—379 p.p.m., gluten feed (21% protein), 14—34 p.p.m., and gluten meal (41% protein), 65—253 p.p.m. The gluten fractions contained a much larger proportion of non-carotenoid pigments than the whole maize. In several samples, saponification of extracts before chromatography reduced the total carotenoid content found, presumably due to conversion of hexane-sol. alkylated flavonoid compounds to water-sol. types during the saponification. E. C. APLING.

Separation and composition of 'polar' wheat-flour lipids. J. J. Wren and A. D. Szczepanowska (*J. Sci. Fd. Agric.*, 1965, **16**, 161—169).—The lipids of untreated white flour (< 2% by wt., of which ~¼ are 'polar' lipids) were examined. They were extracted at −23° with $CHCl_3$–methanol and freed from contaminating non-lipids by aq. K citrate. The lipid was fractionated on silicic acid columns by elution with continuous, concave gradients of methanol in $CHCl_3$ and pure methanol. The fractions were examined by i.r. spectrophotometry, chromatography on aminoethylated paper and deacylation. Eleven known classes of polar lipids were identified, others classes were detected but not identified. (69 references.) E. M. J.

Action of chlorine on semi-dry starch. T. R. Ingle and R. L. Whistler (*Cereal Chem.*, 1964, **41**, 474—483).—Wheat starch (13·2% moisture) was oxidised with Cl_2 at starch/Cl_2 molar ratios of 1 : 1 and 1 : 3, in the dark and under illumination of 500 ft. candles. The oxidation rate was photosensitive, and aldehyde, carboxyl and total carbonyl groups in the products increased with the extent of oxidation. Oxidation increased solubility and decreased η due to depolymerisation of the starch by oxidative and hydrolytic cleavage of D-glucosidic bonds. The major sugar deriv. found in the oxidised material was D-gluconic acid, together with small amounts of D-glucuronic acid and D-glucosone; the only other oxidation products observed were oxalic acid and CO_2. In contrast to the findings on the oxidation of starch slurries, no cleavage between the C2 and C3 carbon atoms was observed. (16 references.) E. C. APLING.

Hydration of starch. Á. Dávid and E. Orbán (*Stärke*, 1964, **16**, 391—393).—Varying conditions of moisture content and temp. may affect the stability of starch used for pharmaceutical tablets. A sensitive calorimeter with an automatic recorder was used to study the thermal dependence of the hydration of potato starch; the results were analysed on a derivatogram. Heat of hydration of starch containing 14·3% water decreases with increasing temp. between 10 and 40° and an exponential correlation was found with the reciprocal of abs. temp.; at higher temp. the heat of hydration changes only slightly. Moisture is almost completely removed at about 150°. The processes involved in the mechanism of hydration are complicated and the possibility of discrete thermal movement of the starch mol. within the granules must be considered as well as certain physico-chemical processes. (13 references.) A. T. CARPENTER.

Preparation and use of starch dialdehyde.—II. K. Babor, V. Kaláč, and K. Tihlárik (*Listy cukrovar.*, 1965, **81**, 30—33).—The laboratory prep. of periodic acid and of starch dialdehyde is described. The optimum conditions for the oxidation of HIO_3 to HIO_4 at the anode consisting of a Pb dioxide layer previously deposited on the lead electrode, are specified. In oxidation of starches to starch dialdehyde with a slight excess of the oxidising agent the cereal starches are to be preferred to potato starch. J. S. B.

Microscopical characteristics of high-amylose maize starches. M. J. Wolf, H. L. Seckinger and R. J. Dimler (*Stärke*, 1964, **16**, 375—380).—Starch grain characteristics of commercial varieties of amylomaize were compared with ordinary maize starch. With increasing amylose content the endosperm of many cells shows two distinct types of starch body; the amylomaize granules occur near the centre, stain reddish with I, show little or no birefringence, are irregularly shaped and include filamentous starch bodies, and are smaller than the usual maize starch granules. Isotropism of many morphologically normal starch granules as well as of the filaments of the other bodies suggests a higher than normal amylose to amylopectin ratio. Variations in depth of staining suggest an uneven distribution of amylose between granules as well as between different parts of the same starch body. A. T. CARPENTER.

Properties of agglutinated bakery yeasts. A. Ginterová, L. Mitterhauszerová, and O. Janatková (*Kvasný průmysl* 1965, **11**, 41—43.)—There exist two basically different types of agglutination taking place in bakery yeasts: one in the form of flakes, and the other in grains. The two types differ in several aspects, e.g., in the appearance of the yeast agglomerations, resistance against acids, and behaviour in extended cultivation. Some inherent reasons of the differences are presented and discussed. In yeast production plants, the flake agglutination prevails. J. S. B.

Flour brew studies. VII. The use of malt syrup and enzymes. E. G. Bayfield and W. E. Young (*Baker's Dig.*, 1964, **38**, No. 6, 52—56).—Enzymic supplementation in the form of malt syrup or fungal prep. produced advantageous results when added to the flour brew in baking. Malt syrup improved the bread flavour at normal syrup levels. (12 references.) S. A. BROOKS.

Hydrophilic colloids of rye. I. Influence of the colloids on amylographic values of starches and flours. V. Podrazky (*Z. Lebensmitt-Untersuch.*, 1964, **126**, 110—118).—Increases in the (max. η) Brabender Amylograph values of suspensions of starch, wheat flour, and rye flour were obtained by the addition of 0·5% or 1% of the rye flour colloids (calculated on the wt. of starch or flour). The increases were proportional to the concn. of the colloids and on their η values, and much greater than could be attributed to the additive effect of the η of the added colloids. The colloids had no effect on the activity of the amylose of the flours; their activity was impaired by the addition of cellulase. (14 references.) P. S. ARUP.

Water as a constituent of flour, dough and bread. W. Bushuk and I. Hlynka (*Baker's Dig.*, 1964, **38**, No. 6, 43—92).—The various functions of water in flour, dough and bread have been reviewed with particular reference to the type of bonds by which the water mol. are attached in these substances. (19 references.) S. A. BROOKS.

Investigations with the Maturograph and oven spring apparatus. G. N. Irvine and C. J. Marek (*Brot u. Gebäck*, 1964, **18**, 193—197).—Relationships between Maturograph max. and baking test loaf vol., fermentation time, yeast level and bromate dosage, between test loaf vol. and loaf vol. indicated by the oven spring apparatus, and between the shape of the experimental curves and baking value found in testing Canadian strong wheat flour are reported and discussed. Some examples of the use of the equipment for the investigation of bakery problems are briefly reported. E. C. APLING.

Buffering and dough flora. G. Spicher and A. Angermann (*Brot u. Gebäck*, 1964, **18**, 201, 204—208).—Studies of the effects of water hardness and whey solids on the course of souring over 24 h. (pH changes, acidity development, and bacterial multiplication) are reported for doughs inoculated with cultures of *Lactobacillus plantarum, L. brevis, L. fermentii*, and mixed cultures. Buffering agents in general retarded bacterial multiplication during the first h. of the souring process; the effect was most marked in the case of addition of whey solids to doughs soured with *L. fermentii*. (12 references.) E. C. APLING.

Dough-improving effect of some aliphatic hydrocarbons. II. Dough lipids. J. G. Ponte, jun., S. T. Titcomb, J. Cerning and R. H. Cotton (*Cereal Chem.*, 1964, **41**, 431—442).—Studies of possible relationships between dough lipids and the improving effects of small amounts of hexane or heptane are reported and discussed. The solvents showed improving effect only in doughs containing added fat; in other cases bread quality deteriorated, and in doughs prepared from lipid-extracted flour, addition of solvent led to inability of the dough to prove to standard height. Binding of lipid (including preferential binding of linoleic acid), lipid P and water-sol. protein in unyeasted doughs was increased by additions of hexane. (19 references.) E. C. APLING.

Frozen bread dough. K. Lorenz and W. G. Bechtel (*Baker's Dig.*, 1964, **38**, No. 6, 59—63).—Experimental batches of dough were made by the straight dough method, sponge and dough procedure and by the continuous mixing processes; dough was moulded in Al foil pans and frozen at −10° or −40°F and stored at −10°F in polythene bags. The best results were obtained with continuously mixed dough; frozen straight doughs were satisfactory for 12 weeks but frozen sponge doughs only for 2 weeks. S. A. BROOKS.

Flour and continuous dough mixing. J. A. Gillis and D. W. Pitts (*Baker's Dig.*, 1964, **38**, No. 6, 64—69).—The effects of different mixing rates, oxidation levels, malt supplementation and starch damage in flours from spring and winter wheats are described. It is emphasised that flour, formulation and processing must be adjusted to each other. S. A. BROOKS.

Cleaning of continuous dough mixing systems. L. A. Timm (*Baker's Dig.*, 1964, **38**, No. 6, 70—73).—Full details are given of a mechanical clean-in-place method for cleaning continuous dough mixing systems. S. A. BROOKS.

Volatile components of bread. M. P. DeFigueiredo (*Baker's Dig.*, 1964, **38**, No. 6, 48—51).—Investigations on the volatile components of bread were reviewed. The compounds detected were mainly alcohols and carbonyl compounds and their total contribution to bread odour seems to be very small. (25 references.) S. A. BROOKS.

Loss of essential amino-acids during baking. G. Gorbach and E. Regula (*Fette Seif. Anstrichm.*, 1964, **66**, 920—925).—The extraction and hydrolysis of proteins from bread and flour are discussed. Four series of R_F values on 18 amino-acids were obtained by paper chromatographic tests carried out with four different solvent mixtures. Paper chromatography was used to separate the amino-acids into groups and determine them quant. No loss of N occurs when bread is baked. Crumbs and crusts from loaves were hydrolysed and a Sorensen titration on the hydrolysed material showed that amino-acids were lost during the baking process. The % loss of eight different amino-acids from crumbs and crusts during baking is reported. (26 references.) W. E. ALLSEBROOK.

Action of shortenings in pastry goods preparation and its explanation. H. J. Hommers (*Brot u. Gebäck*, 1964, **18**, 208—209).—Comparative evaluations of dough characteristics, and the texture, shape, colour, taste and storage properties of pastry goods prepared from recipes differing only in the type of fat used are reported. Reasons for the improvements in dough properties and quality of the finished goods resulting from the use of special shortenings are briefly discussed. E. C. APLING.

[A, B] **Oxidatively treating** [A] **flour,** [B] **maize flour.** [C] **Oxidatively active compositions and treatment of flour therewith.** J. R. Short Milling Co. (B.P. 948,741 and 948,743—4, 23.6.60. U.S., 23.6.59).—[A] Simultaneous bleaching and maturing of (wheat) flour is accomplished by incorporation of an oxidatively active composition having a substantial titratable peroxide content constituted by at least 1 acyclic acetone peroxide, especially bis-(2-hydroperoxyprop-2-yl) peroxide (**I**). [B] The physical properties of yellow maize flour, especially the imbibing properties, are improved (and the flour possibly simultaneously bleached) by heating (below temp. of caramelisation) in presence of **I**. If desired, the active composition may be formed *in situ* from acetone and aq. H_2O_2. [C] Product formed by interaction of acetone and aq. H_2O_2 (e.g., during 1 h. at the boil) is extracted with a low-boiling hydrocarbon, e.g., hexane, to effect removal of **I**, leaving an aq. residue containing water-sol-peroxides. This residue is more active for the bleaching of flour than the original reaction mixture or isolated **I**. F. R. BASFORD.

Sugars and confectionery

Periodical method of softening thin juice by cation-exchange resins. J. Buriánek and D. Marešová (*Listy cukrovar.* 1965, **81**, 53—58).—Softened thin juice, even at full operation scale production, is able to dissolve incrustation in the evaporator. From an evaporator wherein non-softened juice was evaporated for 72 h., ~ 12 kg. of Ca contained in the incrustation was dissolved by the softened juice during 15—20 h. The conditions of optimum regeneration of the cation-exchanger serving for softening were examined. However, after introducing periodical softening methods the advantages thereof became irrelevant. There is a probable connexion between the Ca content and the content of elemental O_2 in the juice. (15 references.) J. S. B.

Composition of molasses from the campaign 1963—64. J. Bureš (*Listy cukrovar.* 1965, **81**, 49—53).—The composition of molasses produced during the campaign 1963—64 corresponded with that of vegetation of a year with normal rainfall. The average purity coeff. (Q) was lower than that of the precding campaign, and the difference between it and the Q-coeff. of normal molasses was a little lower than it was in the preceding campaign. The content of reducing substances and N decreased, and the content of ash was increased. In comparing the quantity of molasses produced by the individual factories with that of the preceding campaigns, certain factories have every year an excessive production of molasses, while the others show only low losses of sugar in the form of molasses. J. S. B.

Alkalinity and peptisation in defecation of sugar beet juices. V. Tibenský (*Listy cukrovar.* 1965, **81**, 25—29).—It is affirmed that the optimum alkalinity of defecation, expressed by the sum of the alkali metal hydroxide (the natural alkalinity) and of $Ca(OH)_2$ is not a generally significant characteristic of pptn. and coagulation. From the viewpoint of coagulation the natural alkalinity represents a ballasting component of the optimum alkalinity, since for optimum coagulation, as for optimum pptn., only the $Ca(OH)_2$ is decisive. (17 references.) J. S. B.

Composition of glucose syrups. J. L. Martin (*Ann. Falsif., Paris*, 1964, **57**, 105—108).—A brief review covering modifications in composition according to methods of manufacture and industrial uses. P. S. ARUP.

2:3:5-Triphenyl-2H-tetrazolium chloride as a reagent for the determination of sugar mixtures by a differential reaction-rate technique. H. B. Mark, jun., L. M. Backes and D. Pinkel (*Talanta*, 1965, **12**, 27—34).—The rates of reaction of glucose, fructose, mannose, sorbose, galactose, ribose and xylose with 2:3:5-triphenyl-2H-tetrazolium chloride (**I**), were studied at 25° with ratios of sugar concn. to **I** of 1:15 to 1:1. The reaction rates of the sugars differ widely and the reagent is satisfactory for the determination, with relative error of ~ 3·4%, of binary mixtures, by a simple differential rate technique. Exceptions are mixtures of fructose–sorbose, glucose–galactose, glucose–xylose, glucose–ribose and xylose–ribose. Glutathione and creatinine do not react, and do not interfere in the analysis of blood serum sugars, but ascorbic acid does interfere in amounts as small as 1—2%. S. M. MARSH.

Changes in crystalline sugars induced by Röntgen rays. G. Geissler (*Z. LebensmittUntersuch.*, 1964, **125**, 452—457).—The induced effects were discoloration, lowering of the m.p., development of acidity, and losses of glucose, fructose, and sucrose in samples of these sugars. The magnitude of the effects was in proportion to the dosage. No glucose or fructose was formed from glucose. The absorption curves developed in the u.v. spectrum of solutions were of the same type as those described by other investigators. P. S. ARUP.

Influence of certain factors on the fermentation of substrates of molasses and of sucrose by bacteria *Lactobacillus Delbrückii*-70. V. Krumphanzl and J. Dyr (*Sborn. praž. vys. Školy chem. technol. potravin. Technol.*, 1963 [1964], **7**, ii, 15—55).—The influence of bacterial cells, of yeast autolysate, of K_2HPO_4 and the initial concn. of sugar on the rate of fermentation of the substrate of molasses and of sucrose by the above named bacteria is discussed. The quantity of nutritive matter necessary to give precise results of fermentation was examined. Increase in the initial concn. of sugar prolonged considerably the duration of fermentation. With molasses, initial concn. of sugar 15·05% in 6 days % fermented was 18·25; corresponding figures with 10·03% sugar were 84·80% (same no. of bacteria). With sucrose, initial concn. 2·5—10% conversion of sucrose to lactic acid increases with lowering of the initial concn. of sugar. Details are given of the effects of K_2HPO_4, and of favourable conditions for use of bacteria and yeast autolysate. (In Czech, from French summary.) E. M. J.

Detection and characterisation of hydroxymethylfurfuraldehyde in vanilla caramels and its detection in presence of vanillin and ethylvanillin by paper and thin-layer chromatography. S. Stoll and G. Barnier (*Ann. Falsf., Paris*, 1964, **57**, 131—143).—The paper chromatographic spots given by the hydroxymethylfurfuraldehyde (**I**) occurring in caramel (or by pure **I**) simulate that given by ethylvanillin. A method for distinction is found in the observation that the ethylvanillin spot reacts with the Fiehe (resorcinol) reagent, hydrazine sulphate, aniline acetate, or *p*-toluidine much more rapidly and in much smaller amounts than do the spots obtained from **I**. A thin-layer procedure is also described which is entirely free from interference by other known constituents of vanilla pods.
P. S. ARUP.

D-Fructose. G. F. Boehringer & Sohne G.m.b.H. (B.P. 949,923, 9.11.62. Ger., 11.11.61).—This sugar is obtained in excellent yield by base-catalysed isomerisation of D-glucose in aq. solution in which the catalyst is Na or K aluminate. Thus, coarse Al powder is dissolved in 8N-NaOH, any residue is filtered off, and mixed with D-glucose (100) in water (300 c.c.) and kept at 30° for 27·5 h., to give a solution containing D-fructose (67) and D-glucose (10 g.).
F. R. BASFORD.

Fermentation and Alcoholic Beverages

Ion-exchange treatment (H cycle) of white grape juice prior to fermentation. II. Effect on wine quality. C. S. Du Plessis (*S. Afr. J. agric. Sci.*, 1964, **7**, 3—16).—The pH of Riesling and Stein musts was lowered to 3·2—2·8 by passage through a cation-exchange resin, resulting in decrease or retarding of ' soluble ' protein, browning and bacterial growth, diminution in turbidity and loss of bouquet under certain conditions. Wines from treated musts had a sharp acid flavour. The effects of the treatment are mainly the outcome of increased acidity and may be brought about by addition of org. acids, e.g., tartaric or citric acid. It is doubted if the ion-exchange treatment is economically desirable. A. G. POLLARD.

Acacia wood dyes in wine. F. Prillinger (*Mitt. Wein. u. Obstbau, Wien*, 1965, **15A**, 21—24).—Yellow fluorescent dyes were found by paper chromatography to occur in wines that had been kept in acacia wood barrels. Concentrates of the dyes were prepared by adsorption on active C and elution with MeOH containing 10% of NH_3, and purified by two-dimensional paper chromatography. The dyes were probably condensation products with polyphenols extracted from the wood. P. S. ARUP.

Thin-layer chromatographic separation of ascorbic acid from glucose. Determination of ascorbic acid in wine. A. Maurel, S. Rey, and M. Rey (*C.R. Acad. Agric. Fr.*, 1964, **50**, 1081—1084).—The determination of added ascorbic acid (**I**) depends on the oxidation of **I** to dehydroascorbic acid (**II**) with H_2O_2, the pptn. of **II** and the glucose as 2,4-dinitrophenylhydrazones, and the separation of the osazones by two-dimensional chromatography on plates coated with silicic acid and starch. The eluted hydrazone of **II** is determined spectrophotometrically in dil. H_2SO_4 solution at 530 mμ. Recoveries of **I** are ~ 10% low. (14 references.) P. S. ARUP.

Production of yeast-fat during fermentation of sparkling wine. H. Schanderl (*Mitt. Wein u. Obstbau, Wien*, 1965, **15A**, 13—20).—Abnormal development and epidermal erosion of the yeast cells is caused by excessive concn. of SO_4^{2+} and SO_3^{2+} ions in the must. The process is accompanied by the secretion of yeast-oil containing colloidal S. The oily yeast which floats on the surface or forms filmy deposits cannot be removed by disgorging or filtration, but can be churned out by vigorous agitation. (11 references.)
P. S. ARUP.

Production of spirit vinegar by the quick process with a pure culture of *Acetobacter rancens* Beijerinck. H. Suomalainen, A. J. A. Keränen and Jaakko Kangasperko (*J. Inst. Brew.*, 1965, **71**, 41—45).—The identification of the culture used in the manufacture of vinegar at the factories of the Finnish State Alcohol Monopoly is described. The culture was originally isolated from shavings used in a vinegar factory and has been successfully continued for 20 years on 5° Balling unhopped barley wort containing 2·5% ethyl alcohol. According to Frateur's system and Bergey's Manual and biochemical tests, the organism has been classified as *Acetobacter rancens* Beijerinck. Chromatographic tests showed that it does not oxidise glycerol to dihydroxyacetone and has high ability to form 2-ketogluconic acid from glucose. It also gives a positive catalase reaction, forms acid from xylose and arabinose, but forms only traces of fructose from mannitol and shows lack of growth in Hoyer's mineral nutrient solution with NH_3 as sole source of N. No variations in biochemical characteristics were observed in 100 isolated colonies. (22 references.)
J. I. M. JONES.

Determination of sorbic acid in wines. P. Jaulmes, R. Mestres, and B. Mandrou (*Ann. Falsif., Paris*, 1964, **57**, 119—122).—The method of Schmidt (*cf. Anal. Abstr.*, 1962, **9**, 4486) is modified by omitting the preliminary distillation under alkaline conditions in order to remove the EtOH; this object is achieved by the partial evaporation of an (alkaline) aliquot of the distillate that has been obtained under acid conditions. P. S. ARUP.

Water problems in brewing. G. Bianacci and L. Ghiringhelli (*Effluent Wat. Treat. J.*, 1964, **4**, 40—43; 74—79).—Raw waters, effluents, their treatment and re-use after recovery of by-products are discussed. (37 references.) C. V.

Effect of gibberellic acid on the phosphorus metabolism of germinating barley. B. I. S. Srivastava (*J. Inst. Brew.*, 1965, **71**, 21—25).—Barley seeds (100 g. samples) after steeping in tap water for 72 h. were soaked for 4 h., (*a*) in de-ionised water or (*b*) in aq. gibberellic acid (**I**) (5 mg./litre), allowed to germinate in darkness for 96 h., and analysed every 24 h. for dry wt., P fractions (inorg. P, acid-sol.-bound P, nucleic acid P and residual P). Inorg. P, lipid P, RNA and DNA increased during germination but acid-sol.-bound P declined. **I** increased the amount of inorg. P but had no significant effect on the other P fractions or RNA or DNA. During early stages of germination the 6-amino/6-oxo ratio in RNA increased and this process was accelerated by **I** but the differences were not significant. α-Amylase activity increased during germination and was considerably stimulated by **I**. An increase in ribonuclease activity by **I** without change in the amount of RNA compared with controls suggests increased RNA turnover and synthesis of messenger RNA specific for certain enzymes, leading to increased synthesis of specific enzyme protein. (35 references.) J. I. M. JONES.

New varieties of brewing barley recently developed in some European countries. A. Foral (*Kvasný průmysl*, 1965, **11**, 54—55).—The new varieties of spring barley recently developed in some European countries are the result of the intensive selecting activity aimed at introducing more productive varieties of brewing barley. In the Research Institute of Cereals at Kroměříž, (Moravia) a series of comparative tests evaluating the malting properties of the new foreign varieties in comparison with the traditional Czechoslovakian varieties was carried out was the result that the latter are still superior in every respect to all spring barley varieties grown in Europe. J. S. B.

Barley and malt amylases: immunochemical methods. P. Grabar and J. Daussant (*Cereal Chem.*, 1964, **41**, 523—532).—A technique is described for identifying α- and β-amylase among the proteins detected in saline extracts of barley and malt by the immunochemical methods of double diffusion and immunoelectrophoretic analysis. The β-amylase of barley is less sol. in water, and migrates more rapidly at pH 8·2 than the β-amylase of malt, but the two amylases were shown to be immunologically identical. No α-amylase, even in trace amounts or inactive form, was detected in barley. (12 references.) E. C. APLING.

Changes in barley proteins during malting. D. H. Simmonds and J. D. Blake (*Cereal Sci. Today*, 1965, **10**, No. 1, 9—12).—The malting process and changes which barley proteins undergo during malting are reviewed. Extracts of five barley samples in 0·01 M-Na pyrophosphate at pH 7 were subjected to starch-gel electrophoresis in tris-citrate buffer, pH 8. A similarity in protein patterns between varieties was noted. During malting, most of the characteristic

bands disappeared leaving only generalised staining. Acrylamide-gel electrophoresis led to streaking and uneven running of samples. (31 references.) G. W. FLINN.

Flocculation of brewing yeasts. A. Kocková-Kratochvílová (*Kvasný průmysl* 1965, **11**, 25—29).—A simple laboratory method for determining the rate of yeast sedimentation is presented. With the bottom brewing yeasts the sedimentation rate is a factor significant for taxonomy studies, and therefore useful in selection of strains for breweries. The reliability of the method described was verified in the typical strains of *Saccharomyces carlsbergensis*, with the strains forming an interstage to *S. cerevisiae*, and in strains failing to agglutinate on addition of the specific serum used for *S. carlsbergensis*, as in some synonymous and comparative strains. (35 references.) J. S. B.

Estimation of viable *Lactobacilli* in beer and pitching yeasts. I. A rapid method for the estimation of viable brewery *Lactobacilli*. R. G. Ault and J. D. Woodward (*J. Inst. Brew.*, 1965, **71**, 36—40).—An active culture of strongly acidifying *Lactobacillus* sp. on an unhopped wort–gelatin medium gave recoveries of viable cells of 87—108% by a haemocytometer counting chamber compared with the no. of colonies (61—420) developed on plates. A study of factors involved led to the following procedure: unhopped beer is prepared removing the yeast, adding gelatin and sterilising. To the melted medium is added aseptically 1 ml. of the sample to be tested and a drop of the mixture is placed in the counting chamber of a sterile Fuchs-Rosenthal slide, incubated at 30° and examined under low power after 30—36 h. In pure cultures, results agreed with the plate count method. In the presence of yeast, suppression of the yeast growth with 10 μg/ml. of actidione had no effect on the development of *Lactobacilli*, thus enabling use of the method which is suitable for routine control purposes and has the advantage of shortened incubation times compared with conventional methods.

J. I. M. JONES.

Significance of the use of hops in regard to the biological stability of beer. III. The microflora of hops. R. M. Macrae, Beryl L. Brady and M. Richards (*J. Inst. Brew.*, 1965, **71**, 57—61).—Bacteria were isolated from hops, e.g., green, kilned and sulphured, by pulverising in sterile saline and culturing the liquid in various media. The significance of their effect in brewing was examined by incubating randomly selected pieces of hops in pasteurised pale ale with access of air and under CO_2 and the beers were examined for turbidity and plated on various media. The isolated bacteria were grown on a suitable medium and washed suspensions were incubated with beer under CO_2. Yeasts were detected by shaking with sterile distilled water and plating the liquid on media containing 50 μg./ml. of Aureomycin and 100 μg./ml. of biphenyl to suppress growth of bacteria and mycelial fungi respectively. Gram positive cocci and filamentous bacilli and Gram negative bacilli were isolated with various yeast types but only in one out of 14 samples was an organism (*Candida guilliermondii*) isolated which could grow in beer under normal storage conditions. Kilning with or without addition of S reduces the no. of viable bacteria and fungi. It is concluded that the use of hops in brewing introduces only a negligible degree of potential short term infection. (11 references.) J. I. M. JONES.

Development of volatile constituents of beer during primary fermentation and pre-bottling storage. M. Arbogast, A. C. Maillard, and E. Urion (*Brasserie*, 1965, **20**, 4—7).—Concentrates of these constituents were prepared by partial distillation of the beers and extraction of the distillates with Et_2O–pentane. Details are given of the equipment and techniques used for the gas chromatographic examination of the volatile substances. The concn. of $COMe_2$, EtOAc, n-PrOH, BuOAc, iso-BuOH, and pentanols showed considerable increases during the primary fermentation, and sharp decreases on transference to pre-bottling storage tanks; the decreases were proved to be due solely to entrainment by the escaping CO_2. Slow increases occurred during the subsequent 40 days; slight decreases occurred during bottling. Changes in the relative concn. of the volatile constituents occurred during the storage period. (10 references.)

P. S. ARUP.

Beer diseases. F. Karabec (*Kvasný prumysl* 1965, **11**, 58—61).—Various defects occurring in beer are specified and reviewed. Reasons of colloidal, chemical and biological turbidities and colour of beer are explained and discussed. J. S. B.

Straining and sparging in a continuous brewing process. J. Moštek and J. Dyr (*Kvasný-průmysl* 1965, **11**, 49—53).—By a specially adapted laboratory installation the technological and chemical aspects of continuous straining and sparging were evaluated. With fine-grained malt, or substitutes therefor, very favourable brewing yields under high utilisation of headings were obtained. The time required for preparing sweet wort can be reduced by 75% compared with conventional methods. The continuous process has besides another advantage in that the dry matter content in brewer's grains is 2·5 times higher than in grains from batch sparging in vats. (39 references.) J. S. B.

Presumptive microbiological detection of antiseptics in beer. J. De Clerck and D. A. A. Mossel (*Ann. Falsif., Paris*, 1964, **57**, 109—114).—Precautions recommended in carrying out the Kluyver and De Clerck test include filtration of the sample in order to remove biological infections interfering with the normal fermentation by *Saccharomyces cerevisiae*, dilution of the sample (if necessary) to reduce the content of EtOH to 3·5%, and the addition of powdered yeast extract in order to stimulate fermentation. Excess of free AcOH in beers of the Lambic type should be removed by adjustment of the pH to 4·9 before filtration. (11 references.)

P. S. ARUP.

Malt beverages. Baxter Laboratories Inc. (B.P. 950,128—9, 24.11.61. U.S., 7.3.61. [B] divided out of [A]).—Foam in a malted beverage (beer) is improved by adding [A] Zn ions (5—500 p.p.m.), e.g., as $ZnSO_4$, and optionally a gummy material (gum arabic, propylene glycol aliginate, carboxymethylcellulose, or carboxymethyl hydroxyethyl cellulose); [B] trace amounts of a non-toxic water-sol. Zn salt and at least one of the gummy materials of [A] (the Zn salt comprising 1—70% of the mixture). F. R. BASFORD.

Fruits, Vegetables, etc.

Chemical composition of apple peel. III. Occurrence of mono, di- and trihydroxy fatty acids, an epoxy acid and a dicarboxylic acid in the epidermis of apple peel. C. H. Brieskorn and J. Bösz (*Fette Seif. Anstrichm.*, 1964, **66**, 925—929).—The separation of cutin acids from apple peel is discussed. Thin layer chromatograms of the acids are shown and experimental details are given. The separated acids were juniperinic acid, 10,16-dihydroxypalmitic acid, threo-9,10,18-trihydroxystearic acid, 9,10-epoxy-18-hydroxy stearic acid and thapsic acid. This is the first time that thapsic acid has been detected in cutin acids. (11 references.) W. E. ALLSEBROOK.

Effect of modified atmosphere storage on organic acids and protein metabolism of pears. P. H. Li and E. Hansen (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 100—111).—Storage of Bartlett and Anjou pears at −1·1° in a controlled atm. (2—3% CO_2 and 2—2·5% O_2) resulted, in comparison with storage in air at the same temp., in reduced rate of org. acid loss during storage and subsequent ripening. Malic acid was affected more than other acids, including citric, shikimic, quinic, and tartaric. The rate of protein-N accumulation was also suppressed by controlling atm. storage. Fruit stored in air at −1·1° for long periods did not ripen normally when transferred to 21·1°, whereas fruit stored at the lower temp. in the controlled atmosphere ripened normally when transferred to the higher temp.

A. H. CORNFIELD.

Effect of cooling rate on storage life of pears. S. W. Porritt (*Canad. J. Plant Sci.*, **45**, 90—97).—Delays in placing fruit in cold storage or failure to cool fruit rapidly once in cold storage markedly affected susceptibility to core breakdown, caused accelerated yellowing, and increased respiration rate of fruit in storage but these adverse effects were not necessarily accompanied by accelerated softening.

E. G. BRICKELL.

Relation between malic and citric acids and titratable acidity in peach and nectarine. K. Ryugo (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 154—160).—Malic acid was the predominant acid present in the juice of peach and nectarine fruit. Titratable acidity (*TA*) was more highly correlated with malic than with citric acid. *TA* was less than the sum of malic and citric acids, indicating the presence of some of the org. acids in salt form. In clones low in *TA* most of the org. acid was in the malate form. The *TA*/org. acid ratio increased with *TA* up to 6 mequiv. per 100 ml. of juice and then remained constant. The malic acid/citric acid ratio was similar for progenies from a given genetic cross. A. H. CORNFIELD.

Cranberry colour measurements. E. J. Francis (*Proc. Amer. Soc. hort. Sci.*, 1964, **85**, 312—317).—The Agtron Model H colorimeter was suitable for measuring the surface colour of cranberry fruit. This colour value did not give an accurate index of the pigment content of the fruit for predicting colour of the processed fruit.

A. H. CORNFIELD.

Chemical composition of fruit and fruit juices. R. A. Osborn (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1068—1986).—Numerous representative values for soluble solids, sugars, ash, K_2O and P_2O_5 are tabulated for 22 fruits and their juices, jellies or jams.

A. A. ELDRIDGE.

Quality of fruits and vegetables after holding in nitrogen atmospheres. C. S. Parsons, J. E. Gates, and D. H. Spalding (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 549—556).—Flavour and keeping quality of lettuce were not affected when heads were held for 10 days in 100% N_2 or 99% N_2–1% O_2 at 0·5°. Russett spotting and butt discoloration were reduced in N_2 compared with air. Ripening of green bananas and tomatoes was retarded in N_2 at 15·5°. Strawberries kept well in N_2 at 0·5° and mould growth was reduced. Holding in N_2 retarded decay of peaches, but off-flavours developed over 4 days in N_2 at 15·5°. A. H. CORNFIELD.

Determination of sulphurous acid in vegetables and fruit preserves. A. Meyer (*Conserva*, 1964/65, **13**, 57—58).—Collaborative determinations in dried onions, jam, and beet syrup were carried out in 14 laboratories by the official method of the Institute for preservation and processing of market garden products (I.B.V.T., Rep. 1406, 1964). Statistical analysis of the results confirmed the reliability of the method in the hands of sufficiently experienced analysts. P. S. ARUP.

Analyses of Dutch tinned preserves. II. Vegetable and fruit products. J. J. Doesburg and A. Meyer (*Voeding*, 1964, **25**, 258—301).—Results for the main constituents, minerals (10), and vitamins (10) are given for 12 vegetables and 6 fruit products, comprising 266 samples. Amino-acid compositions of mixed samples of vegetables are also given. (15 references.) P. S. ARUP.

Comparison of vitamin content of deep frozen and tinned vegetables as prepared for consumption. E. Peppler, E. Muskat and H. D. Cremer (*Z. LebensmittUntersuch.*, 1964, **125**, 443—448).—Determinations of thiamine and vitamin C in preserved peas, beans and spinach indicate no reason for preferring one of the techniques to the other. (17 references.) P. S. ARUP.

Sprout inhibition of table stock potatoes with CIPC-treated paper bags. R. E. Nylund and L. C. Ayres (*Amer. Potato J.*, 1964, **41**, 341—348).—Storage of potatoes in paper bags treated with isopropyl *N*-(3-chlorophenyl)-carbamate (CIPC) was effective in reducing the extent of sprouting during storage as well as after removal from the bags. A. H. CORNFIELD.

Effect of storage temperature on reducing sugars, pH and phosphorylase enzyme activity in potato tubers. R. B. Hyde and J. W. Morrison (*Amer. Potato J.*, 1964, **41**, 163—168).—During storage at 4·4° reducing sugars and pH of tubers decreased, phosphorylase enzyme activity increased and chip colour was reduced. Conditioning at 21·1° after storage at 4·4° decreased reducing sugars but increased pH and chip colour. Storage at 21·1° after harvest had little effect on reducing sugars, but reduced pH and increased phosphorylase activity, though to a lesser extent than with storage at 4·4°. Chip colour was still good after 4 weeks at 21·1°, although slightly poorer than at harvest. Varieties differed significantly in phosphorylase activity. The pH of the tuber juice correlated negatively with reducing sugar levels in the tuber and offered an excellent guide for estimating chip quality. A. H. CORNFIELD.

Relationship of specific gravity to total solids of potatoes. W. L. Porter, T. J. Fitzpatrick and E. A. Talley (*Amer. Potato J.*, 1964, **41**, 329—336).—Specific gravity and total solids were studied statistically to test the absolute reliability of the relationship in American potatoes. The variation in the relationship due to tissue air space and possibly other causes throws doubt on the reliability of individual total solids values derived from sp.gr. determinations. A. H. CORNFIELD.

Chemical composition of potatoes. IV. Relationship of the free amino-acid concentration to specific gravity and storage time. E. A. Talley, T. J. Fitzpatrick and W. L. Porter (*Amer. Potato J.*, 1964, **41**, 357—366).—On the moisture-free basis there was in general an inverse relationship between total solids and free amino-acids during storage for 8 months at 3·3°, but few differences were found on the fresh basis. Although proline% tended to increase with storage, other amino-acids showed no consistent changes with storage time. A. H. CORNFIELD.

Soya-bean amylases. A. Gertler and Y. Birk (*Israel J. Chem.*, 1964, **2**, 315).—The purification of soya-bean β-amylase by $(NH_4)_2SO_4$ pptn. followed by column chromatography is described. The enzyme was shown to be homogeneous and its properties were investigated. Comparative assays of this enzyme and an aq. soya-bean extract have shown that the other enzyme present in the latter (z-enzyme) is an α-amylase. S. A. BROOKS.

Determination of aliphatic mono- and di-sulphides in *Allium* by gas chromatography and their distribution in the common food species. A. R. Saghir, L. K. Mann, R. A. Bernhard and J. V. Jacobsen (*Proc. Amer. Soc. hort. Sci.*, 1964, **84**, 386—398).—The proportions of symmetric and asymmetric methyl, n-propyl, and allyl disulphides were determined in the vapours from the common food *Allium* types and discussed in relation to the allicins and the odour of *Allium* species. A. H. CORNFIELD.

Acid-fume peeling of some food products. K. Popper, F. S. Nury and W. L. Stanley (*J. Sci. Fd Agric.*, 1965, **16**, 78—80).—Tests were made on 12 varieties of foods e.g., apple, garlic, onion, pecan, wheat grain, etc. and times of exposure to acid fumes are given. Peeling loss was very low. Anthocyanin pigments of red onion were accentuated. Peeled onion was not lachrymatory but retained typical flavour. Carrots became more orange in colour. E. M. J.

Comparison of assay procedures for aflatoxin in groundnut products. W. T. Trager, L. Stoloff and A. D. Campbell (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 993—1001).—Three published and four other procedures were compared. In the preferred technique SiO_2 gel is used as the determinative step in thin layer chromatography, development being effected with 5% methanol in $CHCl_3$. In some cases the use of Al_2O_3 fails to resolve the individual aflatoxins B and G. The principal steps in all the procedures employed are tabulated. A. A. ELDRIDGE.

Rapid procedure for extraction of aflatoxin from groundnuts, groundnut meal and groundnut butter for bioassay. A. D. Campbell, E. Dorsey and R. M. Eppley (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1002—1003).—The sample (up to 2 kg.) is blended with a mixture of n-hexane and aq. methanol. After centrifugal filtration saturated NaCl solution is added to the extract, the aq. methanol layer is extracted with $CHCl_3$, the solution is partially evaporated and diluted to known vol. An aliquot is evaporated to dryness to indicate the amount of aflatoxin present so as to facilitate the choice of the amount to be administered in the bioassay. A. A. ELDRIDGE.

Mycotoxins. Rapid determination of aflatoxins in groundnuts, groundnut meal and groundnut butter. S. Nesheim (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1010—1017).—A rapid procedure (Nesheim, *et al.*, *ibid* 1964, **47**, 586) with minor modifications was tested in collaborative work. For aflatoxin B, recoveries of 64—137%, average 91%, at the 20—30 p.p.b. level are reported; for G, the recovery was 27% at the 18, 64% at the 115, and 74% at the 30 p.p.b. level (average recovery 58%). The procedure is described and the results are evaluated statistically. A. A. ELDRIDGE.

Milk, Dairy Products, Eggs

Milk-clotting and proteolytic activity. J. Ilany-Feigenbaum, R. Hundert, A. Netzer and J. Leibowitz (*Israel J. Chem.*, 1964, **2**, 317).—The relation between milk-coagulating ability and the proteolytic activity of several enzymic prep. was studied. The enzymes could be divided into two groups according to the dependence of the onset of coagulation on a low or high grade of hydrolysis. Variation in the enzyme concn., pH and age of enzyme solution affected the coagulative ability. S. A. BROOKS.

Chemical and biostatistical investigation of milk from various breeds of cows from the neighbourhood of Sisak. B. Milković (*Bull. sci. Yugosl.*, 1964, **9**, 119).—A thesis concerning the analysis of a large no. of milk samples from 285 cows is summarised. Wt. of dry solid, sp. gr., lacto densitometer measurements and fat content were determined. Solids and fat and sp. gr. and solids showed a high correlation. These characteristics are related to milk yield and time of year. J. B. WOOF.

Determination of functional amino-acid groups in casein. O. Kirchmeier (*Z. LebensmittUntersuch.*, 1964, **126**, 107—110).—Acid casein and rennet casein were analysed for amino-acids by the technique of Spackmann *et al.* before and after treatment with the 2,4-dinitrofluorobenzene reagent by the method of Levy. The whole of the lysine, histidine, and tyrosine, and ~ 20% of the arginine were found to have been removed as the 2,4-dinitrophenyl deriv. from both varieties of casein. Free arginine was, on the other hand, almost totally converted into the above deriv. The probable structure of casein is considered on the basis of these findings with particular reference to the 5-guanido-phosphoric acid linkages of argenine. (26 references.) P. S. ARUP.

Milk phospholipids extracted by the Mojonnier, modified Mojonnier and new silicic acid column chromatographic methods. A. H. Duthie (*Dissert. Abstr.*, 1964, **25**, 2127).—Comparison is made of the original Mojonnier method, a modification in which 1·5% of NaCl was added to the milk prior to a Röse-Gottlieb extraction (thereby increasing the recovery of lipid P by 10—15%) and a chromatographic method using a silicic acid column. Lipids obtained by the three methods were further separated into neutral lipids and phospholipids on a modified Borgström column and still further by thin-layer chromatography into the major classes, lysolecithin, sphingomyelin, phosphatidyl choline and phosphatidyl ethanolamine. The presence of phosphatidyl serine, cerebrosides and unidentified phosphatides is indicated. The modified Mojonnier and chromatographic methods recovered 10·2 and 12·2% respectively, more P than did the standard Mojonnier technique. A. G. POLLARD.

Riboflavin content of mixed milk lots, and its variation with annual rainfall. O. J. M. Barbuto (*Arch. bras. Nutr.*, 1963, **19**, 117—135).—The annual average riboflavin content, found in mixed milk lots as delivered to processing plants in Saõ Paulo city, was 252·5 μg./100 ml. The average concn. for various months differed significantly; autumn-and-winter seasonal values were higher than spring-and-summer; the average monthly value was correlatable to the average monthly rainfall at an interval of two months; the average concn. differed significantly for the breeds of cattle, being higher with the Sindhi; average values for Schwytz and Jersey herds were significantly higher than those for the Buffalo and Red-and-white Dutch, and these in turn exceeded those for the Caldeana and Black-and-white Dutch. There were no significant differences between the pairs. (42 references.) G. F. PENNY.

Accurate method for determination of free air in butter. U. Mennicke (*Z. LebensmittUntersuch.*, 1964, **126**, 93—106).—A definite vol. of butter is taken by a slightly tapered tubular metal probe of known capacity; after having been kept at a definite temp. between 26° and 28°, the surplus butter is levelled off at the ends and the probe and butter are weighed. A formula is given for calculating the vol. of air at 20° and 760 mm. per 100 g. of butter, the moisture and solids-not-fat content being taken into account. The max. error is 0·04% for absolute, and 0·01% for relative determinations. (10 references.) P. S. ARUP.

Cheese making by direct chemical acidification. W. M. Breene (*Dissert. Abstr.*, 1964, **25**, 1139).—Studies were made to determine whether the properties and composition of Cheddar cheese could be reproduced by batch methods that meet the requirements for mechanisation. Three lots of cheese were made from pasteurised whole milk inoculated with 0·25, 1·0 and 2·0% commercial lactic acid starter, acidified at 40°F to pH 5·60 ∓ ·03 and set at 86°F with 100 ml. rennet per 1000 lb. Curd was cut 10 to 15 min. after setting with ¼-in. knives. Cooking treatments of 112°F for 60 min. and 102°F for 90 min. produced curd which was stirred without difficulty after draining. 25 in. vac. of the stirred curd for 50 min. produced a close-textured cheese. F. C. SUTTON.

Milk-like beverages from pulse. K. Fujita and K. Nagasawa (B.P. 950,046, 11.10.62).—There is claimed a process for the production of a milk-like beverage comprising immersing thoroughly washed pulse in a (10—30 wt.-%) aq. solution of an alkali metal- or alkaline-earth metal chloride at a temp. below the b.p. (e.g., at room temp. during 24 h. or at 80° during 20 min.; washing the pulse and swelling it with water until its wt. has about doubled; grinding the swollen product (which may be soya-beans and/or groundnuts) whilst adding water (150—850 wt.-% on dry solids); separating the resulting juice and adding it to an enzyme mixture (0·025—0·1 wt.-% on dry pulse wt.) comprising bacterial enzymes (1), yeast enzymes (1), and mould enzymes (1 pt.); and incubating the mixture during a period long enough (e.g., 6 h. at 30° to 30 min. at 60°) to digest the starch, protein and fat; then rapidly heating the mixture so that the enzymes are deactivated and a homogeneously emulsified bean milk is obtained. F. R. BASFORD.

Edible Oils and Fats

***Trans*-unsaturated fatty acids in animal fats.** R. Guillaumin (*Fette Seif. Anstrichm.*, 1964, **66**, 907—909).—I.r. spectroscopy was used to determine the presence of *trans*-unsaturated fatty acids in animal fats. These acids very rarely occur in non-ruminants. The proportion of *trans*-acids in five types of animal fat from five different cows and five bulls of varying ages was determined. Acids were present to the extent of 2—11%. The acid content varied from animal to animal and different parts, depot and organ fats, of the same animal contained different % contents of *trans*-acid. (13 references.) W. E. ALLSEBROOK.

Paper chromatographic separation of critical pairs of high molecular weight fatty acids using the products remaining after a Margosches determination of iodine value. G. Rankoff and D. Rankoff (*Fette Seif. Anstrichm.*, 1964, **66**, 912—915).—Paper chromatograms of the fatty acids derived from olive oil, sunflower oil and groundnut oil are shown. The *cis*- and *trans*- unsaturated acids have the same R_F value. When unsaturated fatty acids are subjected to a Margosches I val. determination iodinated hydroxyacids are formed. Paper chromatograms of these acids have been prepared and each of these acids has a higher R_F value than the unsaturated fatty acid from which it was formed. (14 references.) W. E. ALLSEBROOK.

Composition of *Bombacopsis glabra* seed oil. J. A. Cornelius, T. W. Hammonds and G. G. Shone (*J. Sci. Fd Agric.*, 1965, **16**, 170—172).—Determination of the fatty acid composition of the oil showed that it contained 34·5% of sterculic acid (known to be physiologically active and toxic, to chickens and rats), 43% of palmitic acid and small quantities of stearic, oleic and linoleic acids. (16 references.) E. M. J.

Formation and removal of steroid devivatives during the refining of edible vegetable oils. H. Niewiadomski and J. Sawichi (*Fette Seif. Anstrichm.* 1964, **66**, 930—935).—I.r. spectrosopy was used to investigate the changes that occur when soya-bean oil is refined. The unsaponifiable matter present in (i) refined soya-bean oil (ii) a solvent extract of the bleaching earth used for refining soya-bean oil and (iii) the condensate formed during the deodorisation of soya-bean oil was examined. Mol. distillation was sometimes used to prepare oil fractions with high unsaponifiable content. The unsaponifiable material was examined by i.r. spectroscopy, thin layer chromatography and colour reactions. The results show that 7-hydroxycholesterol becomes converted into hydrocarbons during refining. W. E. ALLSEBROOK.

Bleaching of refined cottonseed fatty acids. II. Bleaching with alkalis. J. Pokorný, J. Vrána, and J. Zagíc (*Prům. potravin* 1965, **16**, 43—44).—Gossypol is removed from refined cottonseed fatty acids by bleaching in mild alkaline medium. A simple and inexpensive method consists in decomposing it by heating with an excess of soda lye, whereby the mass becomes almost decolorised. The reaction is rapid, and the effect of bleaching is dependent chiefly on the concn. of the lye, and to a minor degree on the temp. of treatment. By a longer heating the soap formed regains the tawny colour. The reaction product differs from that obtained by bleaching with oxidants in that the yellow colorants influencing the significant max. in the yellow zone of spectrum are decomposed. (Cf. *ibid.* **15**, 475). J.S.B.

Thin-layer chromatography of antioxidants. II. [Butylated hydroxy anisole] BHA, [butylated hydroxy toluene] BHT, [nordihydroguaiaretic acid]NDGA, and tocopherol. T. Salo, R. Mäkinen, and K. Salminen (*Z. LebensmittUntersuch.*, 1964, **125**, 450—452).—The previously described procedure applicable to gallic acid and its esters (*cf. ibid.*, 1964, **125**, 167) has been rendered available for the separation of the above antioxidants in the presence of gallic acid and its esters by the substitution of Kieselgel G for acetylated cellulose as the stationary phase. NDGA and propyl gallate have the same R_F values but both compounds can be detected independently by means of a colour reaction and spectrophotometric examination of an eluate of the spot. P. S. ARUP.

Rapid ultraviolet method for the determination of the autoxidation of oils and fats. G. Steinmann and P. Karajanni (*Pharmazie*, 1964, **19**, 704—707).—This is a modification of the method of Palladina and Stepanowa (*Öl- u. Fett-industrie*, 1956, **22**, 16) in which the peroxide value is measured as a function of time while the sample, on filter paper, is irradiated with u.v. light. A. R. ROGERS.

Chemical and nutritional aspects of oxidised and heated fats. C. H. Lea (*Chem & Ind.*, 1965, 244—248).—The effects of thermal polymerisation and oxidative changes during canning are described. The ill effects arising from the consumption of oxidised fats, the substances and mechanisms responsible for toxicity and the hazards in human and animal foods are reviewed. (39 references.) E. C. DOLTON.

Monoglycerides. Heinkel et Cie G.m.b.H. (B.P. 950,667, 19.9.61. Ger., 11.10.60).—Glycerides with high monoglyceride content are obtained by interaction at ~ 260° of a monoglyceride (≮, preferably 3—5 mol.) and a fatty acid (1 mol.) in a medium consisting of glycerol (60—75), monoglyceride (30—20) and di- and tri-glycerides (≯ 15 mol.-%). In an example hydrogenated tallow fatty acids (**I**) (sap. no. 202) (100 pt.), containing 57 pt. by wt. of monoglyceride, are heated at 280° with glycerol (17), **I** (13 pt. by wt.) to give a product containing 79% by wt. of monoglyceride. E. ENOS JONES.

Meat and Poultry

Influence of freezing temperature on some physical, chemical and quality characteristics of beef and on the rate of temperature change in beef. W. J. Costello (*Dissert. Abstr.*, 1964, **25**, 1139—1140).—*Semitendinosus* steaks were frozen at 0, −70, −150, −200, and −320°F., using liquid N_2 as refrigerant. Experimental data included: drip loss; cooking loss; tenderness; juiciness; moisture; expressible fluid; total sol. and insol. protein; non-protein N and colour. Findings and conclusions are given, e.g., freezing temp. did not affect drip or cooking loss. No colour differences were observed in thawed steaks. Freezing beef at −160°F required $\frac{1}{10}$ the time required at 0°F and ⅛ the time required at −60°F. F. C. SUTTON.

Age-associated changes in bovine muscle connective tissue. I. Rate of hydrolysis by collagenase. II. Exposure to increasing temperature. III. Rate of solubilisation at 100°. D. E. Goll, W. G. Hoekstra and R. W. Bray (*J. Fd Sci.*, 1964, **29**, 608—614, 615—621, 622—628).—I. Under conditions of the experiment 8—11% of the protein and 8—21% of hydroxyproline in the residues were solubilised after 12 h. of incubation with collagenase. Groups I, veal, 40—49 days old; II steers 403—495 days old; III cows 3—4 years; IV cows > 10 years ranked I, III, IV and II from fastest to slowest in rate of hydrolysis by collagenase. Larger amounts of lipid associated with II samples shielded collagenase-labile bonds and caused the slow rate. Collagenase digestion of loose connective tissue follows a pattern similar to that reported for skin and tendon collagen. (30 references.)

II. The release of sol. protein, nin-hydrin positive material, and hydroxy proline from collagenous residues into a phosphate-buffered medium (pH 7·0) on incubation at gradually increasing temp. 25—70° was measured. Differences between age groups were marked, at 60° the groups (see preceding abstr.) ranked I, II, III, IV from highest to lowest in amounts of sol. material released. At 70° I samples had released 42% of their hydroxyproline in sol. form compared with 2% from II samples. Thermal shrinkage temp., the temp. at which a sudden release of sol. hydroxyproline occurred, increased with age from near 55° for I to 70° or above for IV. The average mol. wt. of sol. protein released was greater for the younger animals. Results indicate stronger or more extensive cross-linkages in the collagen from older animals. (29 references.)

III. The release of sol. protein and sol. hydroxyproline indicated that collagen from the younger animals was solubilised more rapidly than that from older animals. The average mol. wt. of the sol. protein decreased with increasing age. Results are discussed in relation to those in parts I and II. The no. and strength of cross-linkages in collagen may play an important rôle in meat tenderness. (33 references.) E. M. J.

Activity of partially purified bovine catheptic enzymes on various natural and synthetic substrates. C. E. Bodwell and A. M. Pearson (*J. Fd Sci.*, 1964, **29**, 602—607).—The type of proteolytic activity in the fraction of the cathepsins from beef muscle precipitated by 45—55% $(NH_4)_2SO_4$ saturation was assayed on various peptides. Commercially purified serum albumin was hydrolysed. but at a much slower rate than denatured haemoglobin (widely used for assaying catheptic activity). Sarcoplasmic proteins indigenous to the crude extract were readily hydrolysed. (20 references.) E. M. J.

Etiological status and associated studies of pale, soft, exudative porcine musculature. E. J. Briskey (*Adv. Fd Res.*, 1964, [1965] **13**, 89—178).—The following are discussed: (i) post-mortem transformations (*a*) normal musculature, (*b*) dark, firm, dry musculature, (*c*) pale, soft, exudative musculature; (ii) nature of post mortem changes (physiological, biochemical, solubility changes); (iii) histology of pale, soft, exudative musculature (PSE) structural features of PSE muscle and of irregular bands; (iv) incidence of PSE muscle, dependence on various factors, differences between breeds, heritability of PSE muscle; (v) variations in PSE muscle within a carcass, between and within muscles, light and dark portions of *semitendinosus*; (vi) related post-mortem studies, comparison of biopsy and post-mortem samples, glycogen properties vs. glycosis, glycogen structure; (vii) ante-mortem treatment vs. PSE musculature, environment, impact of nutrition, other treatments; (viii) enzymes and metabolites in PSE muscle, phosphorylase, phosphofructokinase, lactic dehydrogenase and associated factors, other aspects of glycolysis, ATPase and related factors; (ix) hormonal considerations, application to PSE musculature; (x) additional related studies in muscle physiology, rigor mortis, differences in red and white muscle; (xi) comparison with muscle diseases, white muscle disease, other muscular dystrophies; (xii) post-mortem handling, chilling, liquid N treatment, related studies; (xiii) processing and monetary value differences, processing differences, monetary estimates; (xiv) prevention; (xv) additional research needs are discussed. (239 references.) E. M. J.

Significance and occurrence of *Proteus* bacteria in animal carcasses. J. Püschner and H. Toepler (*Fleischwirtschaft*, 1965, **45**, No. 1, 41—47).—The organism was found in 1863 cases, ~ 9% of all specimens; the positive findings were specially high in calves, 41%, rising at one period to 65%; infection most often occurred in the liver 40% while the next favoured site was the kidney, 22%. (24 references.) C. V.

Occurrence and significance of lipolytic micro-organisms in salami-type sausages intended for long storage. K. Coretti (*Fleischwirtschaft*, 1965, **45**, No. 1, 21—24).—A review and discussion specially relating to the presence of *Lactobacillus brevis* and, to a lesser extent, *L. fermenti*. (94 references.) C. V.

Protein changes during post mortem tenderisation in poultry meat. A. W. Khan and L van den Berg (*J. Fd Sci.*, 1964, **29**, 597—601).—In chickens 6—12 months old held at 0° after slaughter, during onset of and post rigor ageing, the changes in extractable N resulted from changes in the solubility of myofibrillar proteins. In the non-protein-N fraction some of the amino-acid-containing polymers were removed by interaction or aggregation with proteins. In post rigor tenderisation amino-acids and peptide increased in meat by proteolysis which weakens or breaks the bonds binding myofibrils to the matrix of the muscle. (22 references.) E. M. J.

Dry solid curing salt composition. Griffith Laboratories Ltd. (Inventor: L. Sair) (B.P. 949,287, 28.8.62).—The composition, which is intended to be dissolved in an aq. mass for use in processing meat, contains *inter alia* nitrite curing salt and ⩾ 32 wt.-% of a lactone which slowly hydrolyses to acid when dissolved in water (glucono-δ-lactone). The quantity of lactone is such that, on hydrolysis in the presence of meat, a pH of 5—7 is maintained in the aq. mass in which the composition is dissolved. In addition to a major proportion of NaCl, the composition may also contain a water-sol. salt of an isomer of ascorbic acid, an alkali reserve agent, e.g., $NaHCO_3$, and a sweetening agent (dextrose, in the form of maize sugar). J. M. JACOBS.

Fish

Oxidative changes in the fat of herrings during processing. J. Brendl and S. Klein (*Prům. potravin*, 1965, **16**, 66—68).—Cold marinades oxidise readily, even at low temp. by reason of the highly unsaturated fatty acids present. The oil washed out of the fish oxidises more rapidly than that in the flesh. Boiled marinades have lower content of peroxides. The peroxide content is not a reliable criterion of the changes taking place in fish baking, since the heating (to ~ 160°) decomposes the peroxides, although the acidity is little affected. The tar absorbed during smoking of the fish has a stabilising action against oxidation, but in this process the acidity of the fat increases. J. S. B.

Storage life of vacuum-packed iced trout. I. Influence of packing material. P. Hansen and B. V. Jørgensen (*J. Sci. Fd Agric.*, 1965, **16**, 150—152).—A commercial type packing machine which evacuates and seals plastic bags (each containing one gutted fish) was used. The bags were either of polyamide (**I**) or polyethylene (**II**) (foil thickness 0·04 mm.). The storage life of the fish, kept in wet ice, was > 2 weeks. Trout packed in **I** showed no fat oxidation during storage for 3 weeks, while those in **II** showed slight fat oxidation at the end of the second week. Microbial spoilage occurred in the third week in types of package **I** and **II** and viable counts were higher in **II** than **I**. E. M. J.

Spices, Flavours, etc.

Astringency of fruits and fruit products in relation to phenolic content. M. A. Joslyn and J. L. Goldstein (*Adv. Fd Res.*, 1964 [1965], **13**, 179—217).—The review covers: (i) sensation of astringency; (ii) protein pptn. and protein binding; (iii) analytical methods for tannin and astringency assay; (iv) astringency in fruits, factors influencing in fruits, theories proposed to account for the loss of astringency in fruits. (196 references.) E. M. J.

Aromatic substances of foods. III. Analysis of volatile alcohols of tomatoes. J. Schormüller and W. Grosch (*Z. Lebensmitt Untersuch.*, 1965, **126**, 188—193).—After removal of the carbonyl compounds from the Et_2O-pentane extract of the tomato distillate (*cf. ibid.*, 1964, **126**, 38), the alcohols were esterified with 4′-nitroazobenzene-4-carboxylic acid chloride. A portion of the esters was submitted to paper-chromatographic analysis by the method of Bosvik *et. al.*; the main part was saponified and the liberated alcohols were examined by gas chromatography. The presence of MeOH, EtOH, n-butano-isobutanol, n-pentanol, isopentanol, 2-methylbutanol, and ln hexanol was demonstrated. P. S. ARUP.

Determination of vanillin and ethylvanillin in flavours by paper chromatography. J. Fitelson (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1161—1165).—By the use of a mixture of *cyclo*hexane, ethyl acetate and methanol as mobile solvent, and 10% methylformamide in ether as stationary solvent in a Mitchell chromatographic tank, development is complete in 2 h. The extinction of an aq. Na_2CO_3 extract of the spots is determined at 348 mμ. Good recoveries are reported, but the method does not separate vanillin and *p*-hydroxybenzaldehyde (usually ~ 5% of the vanillin content). A. A. ELDRIDGE.

Linear dependency of scale structure in differential odour intensity measurements. C. S. Ough and G. A. Baker (*J. Fd Sci.*, 1964, **29**, 499—505).—The responses to 2-heptanone by 11 subjects showed that the basic reaction to scaling of odour differences is basically the same. The major differences are the degrees to which they use the

scale range, the location of the central value, and their discriminating ability. Intensity rating gave equally as much information as the paired comparison in a shorter time. (14 references.) E. M. J.

Comparison of normal and stressed-time conditions on scoring of quality and quantity attributes. C. S. Ough, V. L. Singleton, M. A. Amerine and G. A. Baker (*J. Fd Sci.*, 1964, **29**, 506—519).—For judging a quantity difference, tasting under a time stress may be advantageous, but for quality differences, tasting with no time stress is superior. The interpretation of the Brunswick probabilistic theory is generally verified. (11 references.) E. M. J.

Effects of sample sequence on food preferences. J. Eindhoven, D. Peryam, F. Heiligman and J. W. Hamman (*J. Fd Sci.*, 1964, **29**, 520—524).—Hedonic-scale preference tests were run with four types of meat. Each one compared two non-irradiated (' good ') samples with two irradiated (' poor ') samples. When several food samples are to be rated for preference, the first ones presented tend to be liked better than the later ones. When a better sample is served followed by a poorer one, ratings of the poorer sample are further depressed. When a poor sample precedes a good sample, the latter is rated lower. These three effects: position, contrast and convergence are discussed. E. M. J.

Colouring matters

Changes undergone by food colouring matters. H. Lück (*Z. LebensmittUntersuch.*, 1965, **126**, 193—201).—The dyes (18) were tested in 0·01 aq. solutions at pH 7 and pH 3 for resistance to heat, SO_2, ascorbic acid, and H_2O_2 by thin-layer chromatography before and after treatment. At pH 7 all the dyes except Brilliant black BN developed no extra spots after boiling or autoclaving at 120° for 30 min., whilst at pH 3 Brilliant black BN, Yellow 27175 N, Ponceau 6R, and Cochineal red A were the only dyes to be affected. All the dyes except Erythrosin and Indanthrene blue were affected by SO_2, ascorbic acid, or H_2O_2 after 3 days at 20°. In order to extract the dyes for spectrophotometric measurement the samples were mixed with Na_2SO_4 and the mixture was eluted with a 0·5% solution of Kollidon in 50% EtOH. Slight losses of Ponceau 6R and Yellow orange S occurred in marmalade after boiling for 5 or 15 min. (20 references.) P. S. Arup.

Preservatives

Possibilities of chemical influence on vital manifestations of micro-organisms. IV. Mechanism of action of ethyl *p*-hydroxybenzoate and sorbic acid on carbohydrate and protein metabolism of some fungi. R. Springer and F. Schegk (*Z. LebensmittUntersuch.*, 1965, **126**, 178—187).—Previously described methods (*cf. ibid.*, 1964, **125**, 81) are used for the study of the effects of sublethal doses of these preservatives on the enzymic activities of *Aspergillus niger* in surface cultures. The activities of amylase, pectinase, and proteinase are little affected. Aldose activity and the utilisation of pyruvic acid are notably decreased, but considerable stimulation occurs in the activity of glucose oxidase and other carbohydrate-metabolising enzymes. Growth is retarded during the first 5 days but is subsequently stimulated owing to the above-mentioned increased enzymic activity. Concn. of the preservatives greater than those generally used would be necessary for the complete inhibition of growth of the fungus. (16 references.) P. S. Arup.

Chemical preservatives and their modes of action. R. Springer (*Brot u. Gebäck*, 1964, **18**, 197—201).—A brief review of recent advances in knowledge of the effects of chemical preservatives on the metabolism of micro-organisms, with particular reference to the actions of sorbic acid, benzoic acid, *p*-hydroxybenzoic acid esters and formic acid. E. C. Apling.

Preservation of food and sterilisation of medicinal and pharmaceutical products [by radiation]. H. Mohler (*Chimia*, 1965, **19**, 21—34).—After a survey of the main microbial and enzymic sources of food-spoilage and -poisoning, sterilisation and pasteurisation and processing by drying, deep freezing and canning are discussed. The effects of ionising radiation and of various radiation doses are considered, the main examples covering ' convenience ' foods, beef, bacon, fish, fruit, wheat and potatoes. Pilot radiation plants in Canada, U.S.A. and in other countries are reviewed and the sterilisation of medicinal and pharmaceutical prep. is described. The problem of wholesomeness of food, legal requirements and future prospects are discussed. (58 references.) M. Sulzbacher.

Preservatives. H. Soikachi (B.P. 949,738, 8.5.62.)—Vegetable or animal product is protected from contamination with micro-organisms by admixture (during harvesting, storage or processing) with up to 50 p.p.m. of α-(fur-2-yl)-β-(5-nitrofur-2-yl)acrylamide (**I**). The latter may be used as a solution in a non-toxic solvent. Thus, minced meat for bologna sausage is thoroughly mixed with 20 p.p.m. of a mixture of lactose (10) and **I** (1 pt.), stuffed into vinylidene chloride casings, heated at 72° during 30 min., then cooled and stored at 30°. After 6 days, none of the sausages show signs of decomposition (gas) whereas all sausages without **I** do. F. R. Basford.

Nuclear-chlorinated aromatic compounds. N. V. Philips' Gloeilampenfabrieken (B.P. 948,263, 27.4.60. Neth. 28.4.59).—2,6-Dichlorobenzaldehyde is reacted with an alkali metal hydroxylamine mono- or di-sulphonate in acid conditions to form *β-2,6-dichlorobenzaldoxime*, m.p. 175—176°. This is converted into 2,6-dichlorobenzonitrile, m.p. 142—144°, by treatment with $SOCl_2$. The nitrile is useful for preventing the sprouting of potatoes, and also for protection against Colorado beetle. E. Enos Jones.

Pesticides in Food

Determination of DDT in biological material. G. Janíček and J. Davídek (*Sborn. praž. vys. Školy chem. technol. potravin. Technol.* 1963 [1964], **7**, ii, 5—13).—The possibility of application of polarographic methods, after nitration, to determine DDT residues in agricultural products is discussed. With concn. of $\gtrsim$ 1 mg./kg. the extracts containing the DDT should be purified, preferably on Al_2O_3. With this purified extract, very good results were obtained by a new polarographic method. This method of determination of DDT residues in agricultural products although satisfactory is not generally applicable and requires suitable modifications of procedure with individual materials. (18 references.) P. Mekler.

Bromine residues in fresh and dried fruits fumigated with ethylene dibromide. E. Alumot, M. Calderon and A. Bondi (*Israel J. agric. Res.*, 1965, **15**, 27—31).—Bromine residues were determined at different times after fumigation in dried fruits (dates, figs, raisins and peaches) and fresh citrus fruits (oranges and grapefruits) fumigated with various dosages of ethylene dibromide, and in potatoes fumigated with methyl bromide. Only small amounts of residual bromine, lower than the safety limits established by the U.S. Food and Drug Regulations, were found about a week after fumigation. S. A. Brooks.

Chlortetracycline and oxytetracycline residues in poultry tissues and eggs. W. E. Meredith. H. H. Weiser and A. R. Winter (*Appl. Microbiol.*, 1965, **13**, 86—88).—*Bacillus cereus* 13 was used as the best organism to determine the amount of antibiotic present. The poultry were given 1000 and 200 p.p.m. of CTC and OTC respectively, in the basic feed mixtures. The various methods of cooking and the potentiating effect of terephthalic acid (*TPA*) was also studied. The largest concn. of residual antibiotic were found in liver, then breast and then thigh tissue when assayed for CTC. OTC was seldom found in the thigh tissues. Roasting, frying and autoclaving destroyed all residues even with the potentiating effect of TPA although this compound increased the concn. that was found in all cases. Poaching and scrambling eggs did not destroy the antibiotic in all cases. C. V.

Ion-exchange method for determining paraquat residues in food crops. A. Calderbank and S. H. Yuen (*Analyst*, 1965, **90**, 99—106).—The sample (fruit, vegetable, lentil) is extracted with boiling dil. H_2SO_4 for $\sim$ 5 h., the clear filtrate is diluted and passed through a column of Zeo-Carb 225 containing 8% divinylbenzene; and the paraquat is eluted with saturated aq. NH_4Cl. The paraquat (70—85% recovery) is reduced with 1% alkaline $Na_2S_2O_6$ and the extinction of the blue solution is measured at 396 mμ (4-cm. cell) (mean of readings between 392 and 401 mμ). Base-line calculation corrects for extraneous background absorption from natural plant compounds. Sensitivity is 0·01 p.p.m. and method is also applicable (with slight modification) to grass, straw, sugar-cane juice and water. W. J. Baker.

Thin-layer chromatography for organo-thiophosphate pesticide residue determination. M. F. Kovacs (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1097—1102).—Compounds are resolved and identified on plates of Al_2O_3-G with methylcyclohexane as mobile solvent and *NN*-dimethylformamide in ethyl ether as stationary solvent. With the aid of tetrabromophenolphthalein ethyl ester, $AgNO_3$ and citric acid, 0·05 p.p.m. of diazinon, Trithion, Systox (thiono), malathion and parathion residues can be detected. The method is 20 times more sensitive than paper chromatography. A. A. Eldridge.

Gas chromatographic techniques for the determination of organophosphate pesticide residues. L. Giuffrida and F. Ives (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1112—1116).—Gas chromatography employing a Na thermionic detector and a flame ionisation detector was used to study the efficiency of a clean-up procedure. Using that of Storherr *et al.* (*ibid*, 1964, **47**, 1087) 0·1 p.p.m. of diazinon, molathion, parathion and Trithion could be detected in common foods. Recoveries were 75—100%. A. A. Eldridge.

Determination of organophosphorus pesticide residues by conversion to orthophosphate ion. M. E. Getz (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1103—1105).—The cleaned-up sample is heated at 100° with 0·25M aq. NH_4 persulphate; after being heated with urea solution the mixture is treated with NH_4 molydate and ascorbic acid and the extinction at 660 m.μ is read on a Beckman spectrophotometer. Recoveries of 33—110% at 1 p.p.m. are reported for malathion, parathion, methylparathion, Trithion and diazinon.
A. A. ELDRIDGE.

Determination of herbicides in oils. G. Yip (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1116—1119).—The vegetable oil is extracted with $NaHCO_3$ solution, which is then acidified and the herbicides are extracted with $CHCl_3$. After esterification with diazomethane the residue is analysed by programmed temperature gas chromatography. In the range 0·02—0·08 p.p.m. recoveries of 87—113% are reported for 2,3,6-trichlorobenzoic acid, pentachlorophenol, and various dichlorophenoxy-aliphatic acids. A. A. ELDRIDGE.

Oscillopolarography of DDT and certain analogues. R. J. Gajan and J. Link (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1119—1124).—Under the experimental conditions described *pp'*-DDT, *op'*-DDT, methoxychlor and Kelthane (but not TDE, Perthane, DDE or the TDE olefin) give well defined waves at the dropping Hg electrode of a cathode ray polarograph. Recoveries of 75—90% were obtained from samples of vegetables fortified in the 1—7 p.p.m. range. Measurement of the regular and derivative waves can be used to determine the rates of *pp'*- to *op'*-DDT in a sample.
A. A. ELDRIDGE.

Residues in milk of cows fed rations containing low concentrations of five chlorinated hydrocarbon pesticides. S. Williams, P. A. Mills and R. E. McDowell (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1124—1128).—At feeding levels 0·05—0·3 p.p.m. heptachor epoxide and dieldrin entered the milk in much higher concn. than did endrin and lindane; DDT and its metabolite TDE showed a small transfer effect. A. A. ELDRIDGE.

Food Processing, Refrigeration

Natural convection heating of liquids in unagitated food containers. J. L. Blaisdell (*Dissert. Abstr.*, 1964, **25**, 1138—1139).—The design and use of multipoint thermocouple rods, calorimetry, flow visualisation, model systems, and dimensional analysis techniques are discussed as tools to assist interpretation of experimental heating curves for real systems. Errors in apparatus, temp. sensing systems, and in analysis are treated. The overall problem of transient conduction and convection in canned foods is examined in an analysis of the physical phenomenon and of the food engineering literature.
F. C. SUTTON.

Application of thermal infrared radiation as a heat source in the freeze-drying of liquid food materials. E. B. Lundquist (*Dissert. Abstr.*, 1964, **25**, 1141—1142).—The various wavelength distributions of thermal i.r. radiation were generated from electrically heated nichrome wire elements operated at a constant power output and temp. of 440 to 2147°F. The samples were frozen in ¼ in. thicknesses to an i.r. transparent sample support medium. The differences in the average drying rates of the samples when various heat source temp. were used were attributed to the reflection of radiant energy, the magnitude of reflection being a function of the colour of the sample and the wave length of incident radiation striking the sample. F. C. SUTTON.

Processing of vegetables and fruit. [A] **Influence of preserving on organoleptic quality.** J. J. Doesburg. [B] **Preliminary treatment of vegetables and fruit.** Steinbuch (*Conserva*, 1963/64, **12**, 81—85, 107—111, 136—141, 167—171; 275—280, 305—311, 964, **13**, 7—14, 36—39, 59—63).—[A], [B] Articles forming parts of a series of instructions on preservation of foods. P. S. ARUP.

New methods for examining headspace gases of canned foods. P. W. Board and R. G. P. Elbourne (*Food Pres. Quart.*, 1964, **24**, 25—29).—Full experimental details are given of a method for the examination of headspace gases in canned foods by gas chromatography. The method is rapid and convenient and allows headspace vol., pressure and gas composition to be determined on the same can. S. A. BROOKS.

Bacterial spoilage of processed meats. L. E. Brownlie (*Food Pres. Quart.*, 1964, **24**, 30—35).—The precautions necessary to avoid spoilage and poisoning of meat by micro-organisms during processing are described fully. S. A. BROOKS.

Minimum growth temperatures for food-poisoning, faecal-indicator and psychrophilic micro-organisms. H. D. Michener and R. P. Elliott (*Adv. Fd Res.*, 1964 [1965], **13**, 349—396).—The review covers a study of storage temp. sufficiently low to prevent growth of the above-named micro-organisms in frozen and chilled foods: viz, (i) determination of min. growth temp.; (ii) food poisoning organisms, (*a*) lowest recorded growth temp., (*b*) effect of competing spoilage organisms; (iii) faecal indicators; (iv) psychrophiles, (*a*) definition, (*b*) lowest recorded growth temp.; (v) environmental factors affecting min. growth temp., (*a*) foods are not completely frozen, (*b*) relationship of solutes to min. growth temp., (*c*) effect of added solutes, (*d*) growth on supercooled substrates, (*e*) effect of humidity; (*f*) effect of other specific environmental factors, (*g*) effect of cultural conditions generally; (vi) possible explanations of min. growth temp. Min. reported temp. are: for *Staphylococcus* and *Salmonella* 6·7°; *Clostridium botulinum*, Types A, B and C, 10°; *C. botulinum*, Type E, 3·3°. Growth of faecal indicators on a food does not usually occur below 5°, but has been reported at 0° and −2°. Psychrophiles frequently cause spoilage at above about −7°. (284 references.) E. M. J.

Radiation preservation of meats and seafoods. R. F. Cain and A. F. Anglemier (*J. Anim. Sci.*, 1964, **23**, 572—576).—A review with 26 references. A. G. POLLARD.

Rôle of free and bound water in irradiation preservation: free radical damage as a function of the physical state of water. G. Wedemeyer and A. M. Dollar (*J. Fd Sci.*, 1964, **29**, 525—529).—English sole fillets previously equilibrated with aq. cysteine (0·1%) were dehydrated by three methods to moisture levels ranging from 2 to 72%. Model systems using cellulose in place of muscle tissues were also used. The samples were irradiated at 1 Mrad in air, N_2 or O_2. The destruction of -SH groups was measured and related to the amount and physical state of the tissue water. Destruction steadily increased, as free water was removed, reaching a max. at ~ 20% moisture; destruction decreased markedly at moisture levels < 10%, this being about the level of bound water in the species. Only the free water of the fish muscle appears to be of major importance as a free radical and peroxide source. The model systems give results supporting those of the fish muscle. (24 references.) E. M. J.

Radiation sterilisation of bacon for military feeding. A. Anellis, N. Grecz, D. A. Huber, D. Berkowitz, M. D. Schneider and M. Simon (*Appl. Microbiol.*, 1965, **13**, 37—42).—Sliced, cured bacon (packed in cans and seeded with 6×10^5 and 3×10^6 spores per can of five strains of *Clostridium botulinum*) was irradiated at various dose levels with γ-radiation. Evidence provided by swelling, toxicity and recoverable organisms showed that 4·5 Mrad was more than adequate as a sterilising dose, that the min. effective dose lay between 2·65—2·87 Mrad depending on method of calculation, that some spoilage occurred < 2·0 Mrad and that all visible spoilage within the experimental series, could be attributed to two of the five strains studied. Toxic cans did not always swell nor did swollen cans always produce toxic spoilage. It was also shown that viable *C. botulinum* can exist for at least 8 months at 30° without producing visible or toxic spoilage at doses < 2·0 Mrad. (13 references.)
C. V.

Grain storage by refrigeration. Anon. (*Mod. Refrig.*, 1964, **67**, 1236—1239).—It is generally assumed that grain is adequately protected at 45—50° a temp. at which germination is inhibited without being impaired; mould formation is prevented and insect life reduced even at a moisture content of 22%. Several installations are considered in relation to tonnage stored, temp., and humidity and indications of cost are given. C. V.

Physicochemical changes in some frozen foods. L. van den Berg (*J. Fd Sci.*, 1964, **29**, 540—543).—pH and composition changes were studied in frozen peas and in chicken meat, frozen post- and pre-rigor, stored at −10° for up to 6 months. In peas pH decreased from 6·7 to 6·0 in the first 3 days, increased to 7·0 in the next 2—3 weeks, decreased to 6·4 in another 3 weeks and remained with only slight change at 6·4. In breast and leg meat of poultry, changes (increases and decreases) of a 0·2—0·3 unit in pH occurred in all samples at about the same time. Meat frozen post-rigor increased 0·2—0·3 pH unit during freezing, that frozen pre-rigor changed little or decreased slightly. With salt solutions of composition resembling closely that of the foods tested, and with gelatin solutions pH changes in frozen foods were caused by increased concn. of food components (e.g. proteins), in the unfrozen phase, by pptn. of salts, interaction of proteins with ionic substances and enzymic activity (lactic acid formation) during frozen storage. E. M. J.

Recent advances in the freeze-drying of food products. R. F. Burke and R. V. Decareau (*Adv. Fd Res.*, 1964 [1965] **13**, 1—88).—This review deals with: (i) fundamental aspects of freezing and drying, freeze-drying mechanism, heat transfer, mass-transfer theory, end-point determination; (ii) equipment developments, drying-chamber designs, control systems, evacuation systems, economics of freeze-drying; (iii) applications to foods, (*a*) biological aspects (e.g., enzyme activity in freeze-dried foods, optimum residual moisture content),

(*b*) organoleptic aspects (texture; flavour; colour). 11 research needs relating to processing, five to products and seven to equipment are listed. (155 references.) E. M. J.

Fundamentals of low-temperature food preservation. O. Fennema and W. D. Powrie (*Adv. Fd Res.*, 1964 [1965], **13**, 219—347).—This comprehensive review covers the structure of water and ice, and crystallisation in detail, freezing diagrams of water and simple solutions and of food materials, concn. of non-aq. constituents during freezing, vol. changes during freezing and thawing, rate of freezing, protective chemical additives, effect of freezing, storage and thawing on the physical and chemical properties of food, general aspects of commercial freezing processes. Sub-zero temp. (no change of state) are responsible for little, if any, damage to food quality during freezing. Change of state may cause mechanical damage from change in vol. and damage caused by concn. of non-aq. constituents. During frozen storage, typical physical changes are gelation, diffusion and recrystallisation; and chemical changes are degradation of chlorophyll and ascorbic acid, denaturation of animal-tissue proteins and lipoproteins. Restoration of original properties on thawing is never complete. (380 references.) E. M. J.

Packaging

Fundamentals of protecting foods by wrappings. D. Čurda (*Prům. potravin*, 1965, **16**, 86—92).—Causes of the changes in stored foods are dealt with and classified. The properties of the wrapper as diffusion and thermal barrier are discussed. Permeability of the various wrapping materials for water vapour and permanent gases (CO_2, O_2, N_2) and the formulae derived therefrom for the keeping ability of dried products are given. The effects of the specific permeability of the wrapping material are discussed. Active means of controlling the barrier effect, especially the use of antibiotics and retardation agents are mentioned. (18 references.) J. S. B.

Colours for plastics in foodstuff applications. T. Garlanda and M. Masoero (*Mater. plast.*, 1965, **31**, 52—57).—Continental practice and regulations covering colours for plastics in contact with food are discussed, and details of permitted colours listed. Both org. dyestuffs and mineral products are given. C. A. FINCH.

Measurement of aroma permeability of plastic films by radioactive-labelled substances. W. Hoffmann, H. Krämer and V. Linowitzki (*Chem.-Ing.-Tech.*, 1965, **37**, 34—38).—An apparatus and procedure for the measurement of odour penetration through various commercial packaging plastic films are described, whereby aroma substances such as camphor, eugenol, cineol and cinnamaldehyde, labelled with ^{14}C or tritium, are passed through a permeation cell, dry CH_4 being used as carrier gas, and radiometrically measured. An organoleptic method is also used for orientation. The films must be free of pores. Polyester and acetate films were resistant to penetration. Films of high- and low-pressure polyethylene showed great permeability. The method is also suitable for measurement of water permeability, a mixture of water and T_2O being employed. M. SULZBACHER.

Retail packing of vegetables and fruit. W. C. Boer, J. De Maaker and P. Greidanus (*Zelfbedien. Supermarkt*, 1964, (96—103).—A review covering choice and properties of packaging wraps, mechanical treatment and cooling of vegetables and fruits for packing, refrigeration, economic aspects, and organisation. P. S. ARUP.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Iodine content of foods habitually consumed in Brazil. E. Pechnik and L. R. Guimarães (*Arch. bras. Nutr.*, 1963, **19**, 11—16).—Results are presented on the I content of vegetables and tubers, mostly grown in the State of Rio de Janeiro, as raw material and after cooking in water under standard conditions. The average value for raw material is 13 μg./kg., falling to 11·5 μg.·kg. after cooking. (10 references.) G. F. PENNY.

Indigestible residues in pulse diets. P. V. Subba Rao and H. S. R. Desikachar (*Indian J. exp. Biol.*, 1964, **2**, 234—244).—The feeding of Bengal gram, black gram and soya-bean decuticled split pulses to rats and human beings caused the elimination of larger amounts of faecal matter than with green gram. S. A. BROOKS.

Factors influencing the nutritional value of fish flour. IV. Reaction between 1,2-dichloroethane and protein. A. B. Morrison and I. C. Munro (*Canad. J. Biochem.*, 1965, **43**, 33—40).—Extraction of freeze-dried cod fillets with 1,2-dichloroethane has been shown to cause destruction of cystine and histidine and interfere with the release of cystine, histidine and methionine by pancreatic digestion. The evidence obtained suggests that sulfhydryl groups of protein can be alkylated by 1,2-dichloroethane to produce thioether linkages with a resultant decrease in nutritional value. (19 references.) S. A. BROOKS.

Extraction of bulk proteins from the green seaweed *Ulva rigida*. R. G. Parekh and A. V. Rao (*Indian J. Technol.*, 1964, **2**, 387—388).—*U. rigida* is powdered and extracted with ether–water (1:4) and then N-NaOH. The NaOH extract is precipitated by adding 10% CCl_3COOH at pH 4—5. A 20% overall protein of 56—60% purity recovery is obtained. Other precipitants gave lower yields. (11 references.) E. C. DOLTON.

Modified Udy protein analysis method. M. Wise, E. M. Sneed and W. K. Pope (*Agron. J.*, 1965, **57**, 93—94).—The modification of the method (*Cereal Science Today*, 1962, **7**, 28) permits a 60% saving in time in wheat protein determinations. A. H. CORNFIELD.

Substances accompanying olive oil of dietetic and pharmaceutical interest. J. M. Martinez Moreno (*Fette Seif Anstrichm.*, 1964, **66**, 903—907).—Unrefined olive oil contains squalene, fatty alcohols, triterpene acids, glucosides, tocopherols, inhibitors and traces of metal. The effect of these substances on the stability and nutritional properties of pure olive oil are considered. Little progress has yet been made in determining which substances give olive oil its characteristic odour and taste. (25 references.) W. E. ALLSEBROOK.

Simultaneous oxidation of tocopherol and carotene by oxygen from the air in the presence of saturated and unsaturated fatty acid esters. M. Loncin and B. Jacobsberg (*Fette Seif. Anstrichm.*, 1964, **66**, 910—911).—Atm. oxidation, at 50°, of small proportions, about 500 p.p.m., of α-tocopherol and β-carotene in the presence of methyl stearate and methyl palmitate was studied. β-carotene was oxidised in the presence of both esters but α-tocopherol was stable in the presence of the saturated ester. When traces of α-tocopherol were added to β-carotene/methyl stearate and β-carotene/methyl palmitate mixtures the oxidation of the β-carotene was inhibited. The effect of traces of Fe^{2+} and Fe^{3+} stearate (10 p.p.m.) on the oxidation, at 80°, of α-tocopherol and α-carotene/methyl palmitate mixture is shown. W. E. ALLSEBROOK.

Relationship between vitamin E and polyunsaturated fatty acids. H. Dam (*Fette Seif. Anstrichm.*, 1964, **66**, 899—903).—The oxidation of vitamin E is accelerated in the presence of polyunsaturated acids. Vitamin E deficiency causes foetal resorption, decreasing resistance against oxidative haemolysis and liver necrosis in rats. The development of encephalomalacy in chickens is connected with the presence of linoleic acid. Work on these subjects is reviewed. (48 references.) W. E. ALLSEBROOK.

Separation and estimation of tocopherols in vegetable oils by thin-layer chromatography. M. K. Govind Rao, S. Venkob Rao and K. T. Achaya (*J. Sci. Fd Agric.*, 1965, **16**, 121—124).—A method is described by use of which α, β, γ, δ-tocopherols were estimated in seven vegetable oils. In an artificial mixture, recovery of 97—98% of individual tocopherols is obtained and no pretreatment of the unsaponifiable matter is necessary. β-tocopherol was not found in the oils tested except in neem oil (trace). (21 references.) E. M. J.

Algae nutrient. Boeing Co. (B.P. 950,175, 30.10.62. U.S., 23.1.62).—A chlorophyll-containing alga, suitable for human consumption (e.g., for space travellers), is obtained by exposing the alga, in absence of CO_2, to an intense white light (in excess of 2000 ft. candles), during, e.g., 8—16 h., until substantial bleaching has been effected. Preferred alga is *Chlorella*, which is grown in presence of a substance (e.g., an org. S source) which will combine the alga, to form a nutrient. F. R. BASFORD.

Manufacture and packing of table jelly. A. Bird & Sons Ltd. (Inventor: P. McN. Miln) (B.P. 949,047, 25.5.60).—The method comprises filling a fluid jelly into individual envelopes, which after being sealed, are enclosed in a mould whilst the jelly sets. The envelopes may be made of laminated material, e.g., an inner layer of plasticised PVC and an outer layer of Cellophane. F. R. BASFORD.

Unclassified

Determination of the water activity of some hygroscopic food materials by a dew point method. G. Ayerst (*J. Sci. Fd Agric.*, 1965, **16**, 71—78).—A dew-point apparatus designed to reduce some of the errors inherent in the method is described. The apparatus has been used to measure the R.H. of air in equilibrium with samples of several hygroscopic foods and these measurements have been related to the moisture contents of the samples determined by standard commercial laboratory methods. It has been used successfully to check the water activities of fungal culture media and of humidity-

controlling solutions. The phenomenon of hysteresis and the formulas of Smith and Henderson are discussed. (22 references.)
E. M. J.

Antimicrobial properties of certain glucosamine derivatives. G. R. Gale (*Canad. J. Microbiol.*, 1964, **10**, 887—896).—Forty glucosamine (**I**) deriv. were tested for antimicrobial properties; five showed significant activity against a no. of bacteria, yeasts and fungi *in vitro*. These compounds are: 5-bromosalicylidene-D-**I** (**II**), cinnamylidene-D-**I** (**III**), 3,5-dibromosalicylidene-D-**I** (**IV**) and its oxime (**V**), and 3-nitro-5-chlorosalicylidene-D-glucosamine (**VI**). The acute LD_{50} values for mice were 200, 400, 600 and > 1000 mg./kg. respectively and studies on the mode of action suggest that **II**, **IV** and **V** may cause interference with the glucosamine metabolism in *Candida albicans*, ultrastructural changes having been observed in this organism. **II**, **IV**, **VI** and **III** were phosphorylated by yeast hexokinase and all except **III** depressed the rate of glycolysis in a cell free extract. (11 references.)
C. V.

Physical and chemical characteristics of cellulose ethers used as food additives. III. Examination of commercial cellulose ethers. E. Mergenthaler, S. W. Souci, and F. Crössmann (*Z. LebensmittUntersuch.*, 1965, **126**, 173—178).—Ten commercial samples complied with the standards for composition proposed by the authors (cf. *ibid.*, 1964, **125**, 247, 413). As regards purity the limits for heavy metals were exceeded in three cases.
P. S. Arup.

3.—SANITATION, WATER, etc.

Control of mould during degreening of oranges. D. Leggo and J. A. Seberry (*Food Pres. Quart.*, 1964, **24**, 36—39).—The development of green mould during the degreening of oranges with ethylene can be simply and effectively controlled by NH_3 gas. Fungicidal dip treatment must still be applied subsequently. S. A. Brooks.

Resistance of a Hawaiian strain of the German cockroach to several insecticides. T. Ishii and M. Sherman (*J. econ. Ent.*, 1965, **58**, 46—50).—Nine insecticides were applied topically to chlordane-resistant and -susceptible strains of *Blatella germanica*, and their effect observed over 30 days. The full effect appeared later in resistant strains. The effect of sex on toxicity is discussed. The resistant strain showed cross-resistance to DDT, dieldrin, lindane, malathion, Naled, Bayer 37344 [(4-methylthio)-3,5-xylyl methylcarbamate] and Bayer 39007 (*o*-isopropoxyphenyl methylcarbamate) but not to Kepone. (17 references).
C. M. Hardwick.

Food preservatives as insecticides against *Tribolium confusum*, Du Val., *T. castaneum*, Herbst., and *Lasioderma serricorne* F. D. L. Milne (*S. Afr. J. Agric. Sci.*, 1964, **7**, 79—86).—The insecticidal action of sorbic acid (**I**), its K salt and benzoic acid (**II**) on these insects results from the suppression of certain micro-organisms, normally present in the beetles and having the ability to synthesise vitamins. In absence of these organisms the beetles die from vitamin deficiency. **I** and **II** effectively controlled both *Tribolium* spp.; **II** was moderately effective against the cigarette beetle.
A. G. Pollard.

Malathion for stored product insect control. III. Effect of malathion residues in diets on albino rats. K. Krishnamurthy, S. Godavari and M. K. Krishnakumari (*Indian J. exp. Biol.*, 1964, **2**, 232—234).—When up to 800 p.p.m. malathion was added to a poor rice diet of rats no adverse change in growth rate was noted and no pathological changes were observed in liver, kidney or pancreas or in plasma cholinesterase activity; a slight decrease in erythrocite cholinesterase activity was observed. (18 references.)
S. A. Brooks.

Laboratory evaluation of promising systemic insecticides in guinea pigs, against oriental rat fleas. P. H. Clark and M. M. Cole (*J. econ. Ent.*, 1965, **58**, 83—86). Of 41 compounds tested, 14 systemic insecticides, when given orally to guinea pigs, caused complete mortality of *Xenopsylla cheopis*. Those with the lowest effective dosages were Amer. Cyanamid CL-38064 [*O*-*p*-(ethylsulphamoyl)-phenyl *OO*-dimethyl phosphorothioate] Metasystox-R [*S*-2-(ethylsulphinyl) ethyl *OO*-dimethyl phosphorothioate] and diazinon. (16 references.)
C. M. Hardwick.

Effects of Apholate on a restricted population of houseflies. E. J. Hansens and P. Granett. (*J. econ. Ent.*, 1965, **58**, 157—158).—Populations of caged flies were fed on 2% sugar bait. The addition of apholate caused a smaller population increase in the first 2 weeks followed by a rapid decline, giving a 90% population reduction. Sugar baits were more attractive than maize grits or Quincy granules. These reductions were not considered adequate for fly control in barns.
C. M. Hardwick.

Effects of carbamates on housefly fecundity, longevity and food intake. G. P. Georghiou (*J. econ. Ent.*, 1965, **58**, 58—62).—Topical application of Isolan reduced egg production but did not affect mating, longevity or egg fertility. Reduction in fecundity was significant whether the insecticide was applied before or after mating or before or after access to milk. Egg production was reduced for 19 days after a 0·6 μg dose. Of other carbamates, four caused a reduction in egg production while two did not. Diazinon reduced the no. of eggs by ~ 10%, DDT had no effect and dieldrin stimulated egg production. (13 references.)
C. M. Hardwick.

Hydroxylation as a factor in resistance in houseflies and blow flies. R. D. Schonbrod, W. W. Philleo and L. C. Terriere (*J. econ. Ent.*, 1965, **58**, 74—77).—Methods of tissue prep. are described. The homogenates are identified by paper chromatography. Dosage-response curves for exposures to naphthalene are given. Differences in susceptibility were found between the sexes in the housefly but not in *Phoimia regina*. There was a general correlation between *in vivo* and *in vitro* activity. Older houseflies contain microsomes of greater hydroxylating capacity than do younger houseflies. Flies highly resistant to DDT and dieldrin hydroxylated naphthalene twice as fast as did moderately resistant strains.
C. M. Hardwick.

Selective insecticidal action of isopropyl parathion and analogues. R. L. Metcalf and M. Frederickson (*J. econ. Ent.*, 1965, **58**, 143—147).—The susceptibility of *Apis mellifera* and *Musca domestica* to a series of topically applied substituted phenyl dialkyl phosphorothionates was investigated. Isopropyl parathion was only 0·005 times as toxic to bees as to flies, although the dimethyl-, diethyl- and dipropyl-analogues were equally toxic to both species. The biochemical basis for this was investigated.
C. M. Hardwick.

Titrimetric determination of parathion with chloramine-T. V. Laxminarayana and A. R. Vasudeva Murthy (*Chemist-Analyst*, 1965, **54**, 9—10).—The procedure consists of cleaving the P-S bond in parathion with 0·1N-chloramine-T (**I**) plus 4M-H_2SO_4, with simultaneous oxidation of the S to H_2SO_4, and then determining the excess of **I** by addition of KI and titration with $Na_2S_2O_3$. Eight equivalents of **I** oxidise one mole of parathion, 3·641 mg of which = 1 ml of 0·1N-I. Reproducibility is within ~ 1% and method is applicable to solid samples, emulsion concentrates, and dusts.
W. J. Baker.

4.—APPARATUS AND UNCLASSIFIED

Analysis of solid organic natural products with a field ionisation mass spectrometer. H. D. Beckey (*Z. Anal. Chem.*, 1965, **207**, 99—104).—Examples are given showing that more detailed structural information is obtained by the electron-impact method but that the mol. w. should be determined by the field ionisation method.
P. N. R. Nicholls.

Determination of sulphur in plants. G. Leithäuser and M. Buck (*Z. Anal. Chem.*, 1965, **207**, 113—115).—Earlier procedures (Buck, *ibid*, 1961, **184**, 427, 1963, **194**, 116) are modified to include oxidation with V_2O_5 at 1280° for the determination of total S in the range 0·15—0·20% with a relative error of 5%.
P. N. R. Nichols.

Colorimetric determination of aluminium, iron, manganese, phosphorus, titanium and silicon in liming materials. P. Chichilo (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1019—1027).—Collaborative esults for the determination of Al (with NH_4 aurintricarboxylate), Fe (with 2,4,6-tripyridyl-*S*-triazine), Mn (as $KMnO_4$), P (by a heteropoly blue method), Ti (with Na_2, 1,2-dihydroxybenzene-3,5-disulphonate) and Si (with MH_2 molybdate and 1-amino-2-naphthol-4-sulphonic acid) in limestones, blast furnace slag and cement kiln dust are tabulated. All the methods are considered satisfactory.
A. A. Eldridge.

Evaluation of two modifications of the volumetric ammonium molybdate for [determining] phosphorus. W. M. Hoffman and E. A. Woolson (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1057—1067).—Collaborative studies confirmed the superiority of the quinoline molybdate method (' Changes in methods ', *ibid*, 1964, **41**, 170); the NPFI procedure (Harwell, *et al.*, *ibid*, 1964, **47**, 428) is not sufficiently precise. The TVA method (Brabson, *et al.*, *ibid*, 1964, **47**, 1028) gives slightly lower results than those given by the quinoline molybdate method.
A. A. Eldridge.

Reduction of nitrates in acid medium with Raney catalyst powders. J. A. Brabson and W. G. Burch (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1035—1040).—A powdered Raney catalyst containing Al and Ni is recommended for the reduction of nitrates in acid medium since the Ni provides a catalytic surface, a relatively small quantity of alloy is required, and the resulting salts do not interfere with subsequent steps in the Kjeldahl process.
A. A. Eldridge.

Top reservoir chromatographic column for the determination of squalene by the A.O.A.C. method. W. Y. Ibrahim (*J. Ass. off. agric. Chem., Wash.*, 1964, **47**, 1017—1018).—The glass column used for chromatography of the unsaponifiable matter in the A.O.A.C. method (' Official Methods of Analysis ', 9th Edn., 1960) is provided with a Teflon stopcock, and a top reservoir to increase the no. of samples that can be dealt with simultaneously.
A. A. Eldridge.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1965

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

INDEX OF AUTHORS' NAMES

ABDULEID, H. Z., 3.
Achaya, K. T., 46.
Agnihotri, V. P., 4.
Ahlgren, G. E., 6.
Alexander, T. M. W., 5.
Alumot, E., 42.
American Cyanamid Co., 17, 25.
Amerine, M. A., 41.
Ammerman, C. B., 19, 22.
Anderson, G. C., 23.
Anellis, A., 44.
Angermann, A., 361.
Anglemier, A. F., 44.
ap Griffith, G., 9'
Arbogast, M., 33.
Arrington, L. R., 22.
Asomaning, E. J. A., 12.
Aspinall, D., 5.
Ault, R. G., 33.
Awan, A. B., 8.
Ayerst, G., 46.
Ayres, L. C., 35.

BABOR, K., 28.
Backes, L. M., 30.
Badische Anilin-u. Soda-Fabrik A.-G., 16.
Bailey, R. W., 18.
Baker, D. A., 7.
Baker, G. A., 40, 41.
Baker, R., 13.
Bakermans, W. A. P., 9.
Banerjee, B. K., 4.
Banga, O., 9.
Bardner, R., 13.
Barnes, D. K., 13.
Barnier, G., 31.
Barbuto, O. J. M., 37.
Barth, K. M., 23.
Bath, L. H., 22.
Baxter Laboratories Inc., 34.
Bayfield, E. G., 28.
Baylor, C. E., 20.
Bhatnagar, R. M., 4.
Bhattacharya, K. R., 25.
Bechtel, W. G., 29.
Becker, D. E., 24.
Beítz, D. C., 19, 20.
Bell, A. A., 14.
Bell, T. A., 19.
Bennett, J. H., 7.
Bensadoun, A., 21.
Berkowitz, D., 44.
Bernhard, R. A., 35.
Berry, F. H., 14.
Bianacci, G., 32.
Bird & Sons Ltd., A., 46.
Birk, Y., 35.
Birth, G. S., 10.
Bishop, J., 13.
Blaisdell, J. L., 43.
Blake, J. D., 32.
Bland, B. F., 19.
Blaney, L. T., 11.
Blessin, C. W., 27.
Blinn, R. C., 15.
Block, S. S., 19.
Blosser, R. O., 4.
Board, P. W., 43.
Bodwell, C. E., 39.
Boehringer Ingelheim Ltd., 17.
Boehringer & Sohne G.m.b.H., G. F., 31.
Boeing Co., 46.
Boer, W. C., 377.
Bösz, J., 34.
Bolin, D. W., 21.
Bolling, H., 26.
Bondi, A., 42.
Boskma, K., 8.
Bowling, D. J. F., 5.
Bowman, T., 13.
Boyd, J. E., 15.
Brabson, J. A., 5.
Brady, B. L., 33.
Braude, R., 24.
Bray, R. W., 39.
Brecher, J. D., 27.
Breene, W. M., 37.
Brendl, J., 40.
Bretz, T. W., 14.
Brieskorn, C. H., 34.
Briskey, E. J., 39.
Broadbent, L., 11.
Brokovec, A. B., 48.
Brooks, G. T., 48.
Brown, D. A., 2.
Brown, N. A., 20.
Brown, R. E., 19, 20.
Brown, W. H., 23.
Brown, W. O., 21.
Brownlie, L. E., 43.
Bruns, V. F., 15.
Buckner, A. J., 48
Burch, W. G., 5.
Bureš, J., 30.
Buriánek, J., 30.
Burke, R. F., 44.
Burt, R. L., 5.
Bushuk, W., 29.
Butler, J. D., 9.
Byers, J. H., 20.

CAIN, R. F., 44.
Calderbank, A., 42.
Calderon, M., 42.
Campbell, A. D., 36.
Campbell, A. G., 20.
Cannon, H. B., 9.
Caro, J. H., 4.
Carvalho, A., 12.
Celanese Corp. of America, 25.
Cerning, J., 29.
Chang, S. C., 48.
Chao, T. T., 2.
Chapman, H. D., 4.
Chen, L. L., 26.
Chepil, W. S., 1.
Child, R. D., 22.
Clark, K. G., 5.
Clark, P. H., 47.
Cluskey, J. E., 27.
Cole, M. M., 47.
Compton, D. C., 11.
Conibear, D. I., 12.
Coombe, B. G., 7.
Coretti, K., 39.
Cornelius, J. A., 37.
Costello, W. J., 38.
Cotton, R. H., 29.
Coup, M. R., 20.
Cox, J. L., 24.
Cremer, H. D., 35.
Cronin, E. H., 15.
Cropley, R., 11.
Crössman, F., 47.
Cunningham, R. K., 10.
Curda, D., 45.
Curtis, G. T., 9.

DAINTY, J., 6.
Dam, H., 46.
Daniels, T. L., 25.
Danielson, R. E., 8.
Daussant, J., 32.
Dávid, A., 28.
Davídek, J., 42.
Davidson, R. L., 20.
Davis, C. L., 19.
Davis, G. K., 22.
Davison, K. L., 20.
Davis, R. E., 22.
de Bruyn, J. W., 9.
Decareau, R. V., 44.
De Clerck, J., 34.
Dedolph, R. R., 11.
DeFigueiredo, M. P., 29.
Dekker, A. J., 6.
De Maaker, J., 45.
Dent, J. W., 18, 19.
Desikachar, H. S. R., 25, 45.
Deutsche Gold-u. Silber-Scheide-anstalt, 17.
Devine, J. R., 9.
DeWit, C. T., 2.
Dijkstra, N. K., 20.
Dimler, R. J., 27, 28.
Dinusson, W. E., 21.
Doesburg, J. J., 35, 43.
Dollar, A. M., 44.
Donoho, C. W., jun., 14.
Dorsey, E., 36.
Du Plessis, C. S., 31.
Dury, K., 16.
Duthie, A. H., 36.
Dyr, J., 31, 33.

Eaks, I. L., 11.
Eclipse Peat Co., Ltd., 5.
Edwards, R. S., 7.
Eindhoven, J., 41.
Elbourne, R. G. P., 43.
Elgindi, D. M., 3.
Elliott, R. P., 43.
Enyi, B. A. C., 8.
Eppley, R. M., 36.
Erickson, C. J., 10.
Erwin, F., 15.
Estrada, M. G., 14.
État Française, 16.
Evans, E. J., 6.

FANG, S. C., 2.
Farbenfabriken Bayer A.-G., 16, 17, 18.
Feldberg, C., 26.
Fennema, O., 45.
Fenster, C. R., 1.
Findlay, W. I., 8.
Finney, E. E., 8.
Fischer, A., 16.
Fitelson, J., 40.
Fites, R. C., 14.
Fitzpatrick, T. J., 35.
Foral, A., 32.
Fox, R. L., 3, 10.
Francis, E. J., 34.
Frans, R. E., 14.
Frederickson, M., 48.
Freeman, J. F., 2.
Frere, M. H., 2.
Freyer, R. H., 13.
Friedman, S. J., 15.
Friedrich, K., 15.
Fujita, K., 37.
Fulton, B. E., 2.
Fulton, J. M., 8.
Furmidge, C. G. L., 12.

GAJAN, R. J., 43.
Gale, G. R., 47.
Garlanda, T., 45.
Garrett, W. N., 23.
Gates, C. E., 20.
Gates, J. E., 35.
Geissler, G., 30
Georghiou, G. P., 47.
Gertler, A., 35.
Getz, M. E., 15, 43.
Ghiringhelli, L., 32.
Gilles, K. A., 27.
Gillingham, J. T., 6.
Gillis, J. A., 29.
Ginterová, A., 28.
Giskin, M. L., 1.
Giuffrida, L., 15, 42.
Glessner, A. S., 12.
Godavari, S., 47.
Goldstein, J. L., 40.
Goll, D. E., 39.
Goodman, D., 1.
Gorbach, G., 29.
Gordon, J. G., 23.
Gosnell, J. M., 12.
Gouws, L., 23.
Govind Rao, M. K., 46.
Grabar, P., 32.
Gramlich, J. V., 14.
Granett, P., 47.
Grant, R. S., 24.
Grecz, N., 44.
Greenhill, W. L., 19.
Greenwood, D. A., 21.
Greenwood, D. J., 1.
Greidanus, P., 45.
Griffith Laboratories Ltd., 40.
Griffiths, D. C., 13.
Grosch, W., 40.
Guillaumin, R., 37.
Guimarães, L. R., 45.
Gulyás, F., 3.

HACKMAN, J. T., 16.
Halevy, A. H., 7, 11.
Hall, C. W., 8.
Hamman, J. W., 41.
Hammerton, J. L., 7.
Hammonds, T. W., 37.
Handert, R., 36.
Hansel, W., 20.
Hansen, E., 34.
Hansen, P., 40.
Hansens, E. J., 47.
Hanson, A. A., 10.
Hanson, J. B., 14.
Hardy, G. W., 12.
Harmon, B. G., 24.
Harpstead, D. D., 7.
Harris, C. E., 18.
Harris, L. E., 21.
Harrison, A., 48.
Harthoorn, P. A., 16.
Harwood, M. E., 2.
Hays, V. W., 24.
Hazen, T. E., 24.
Heald, W. R., 2.
Hedden, O., 13.
Heiligman, F., 41.
Heinemann, W. W., 23.
Heinkel et Cie G.m.b.H., 38.
Heinly, N. A., 4.
Helton, A. W., 14.
Hemingway, R. G., 20.
Hlynka, I., 29.
Hoekstra, W. G., 39.
Hoffmann, W., 45.
Holaday, C. E., 27.
Holmes, M. R. J., 9
Holtschmidt, H., 18.
Hommers, H. J., 30.
Hooker Chem. Corp., 16.
Horan, F. E., 25.
Houston, D. F., 27.
Huber, D. A., 44.
Hudman, D. B., 24.
Hughes, F. J., 25.
Hutcheson, T. B., jun., 2.
Hutton, J. B., 18.
Hyde, R. B., 35.

ILANY-FEIGENBAUM, J., 36.
Ingle, T. R., 28.
Irvine, G. N., 29.
Ishii, T., 47.
Ives, F., 15, 42.
JACKSON, R. D., 1.
Jacobsberg, B., 46.
Jacobsen, J. V., 35.
James, D. W., 2.
Janatková, O., 28.
Janíček, G., 42.
Jaulmes, P., 32.
Jenkins, W. R., 13.
Jensen, A. H., 34.
Jezeski, J. J., 20.
Johnson, B. C., 21.
Johnson, D. D., 3.
Johnson, D. E., 21.
Johnson, F. J., 5.
Jones, D. I. H., 9.
Jones, J. R., 24.
Jørgensen, B. V., 40.
Joslyn, M. A., 40.
Joy, K. W., 7.
Jury, K. E., 18.
Juska, F. V., 10.

KACAR, B., 3.
Kaláč, V., 28.
Kangasperko, Jaakko, 32.
Karabec, F., 33.
Karajanni, P., 38.
Katz, S. E., 25.
Kelley, J. D., 11.
Kemmerer, H. R., 9.
Keogh, J. L., 12.
Keränen, A. J. A., 32.
Kerr, H. D., 15.
Kerr, J. A. M., 21.
Kesler, E. M., 20.
Kester, E. B., 27.
Khan, A. W., 39.
Khan, D. H., 12.
Kidd, J., 16.
Kischmeier, O., 36.
Kistner, A., 33.
Klages, M. G., 9.
Klein, S., 40.
Klopfenstein, T. J., 22.
Koch, R. B., 25.
Kocková-Kratochvílová, A., 33.
Kohnke, H., 1.
Kommedahl, T., 7.
Kovacs, M. F., 42.
Krämer, H., 45.
Krishnakumari, M. K., 47
Krishnamurthy, K., 47.
Krook, L., 20.
Krumphanzl, V., 30.
Krusberg, L. R., 14.
Kuníori, T., 26.
Kunkel, H. O., 22.

LABRECQUE, G. C., 48.
Lage, D. P., 10.
Lancaster, E. B., 27.
Lancaster, J. L., jun., 25.
Larkin, P. J., 20.
Larson, R. A., 11.
Laxminarayana, V., 48
Lea, C. H., 38.
Leasure, J. K., 15.
Leatherwood, J. M., 18.
Leggo, D., 47.
Lehmann, R., 22.
Leibowitz, J., 36.
Lennon, E. A., 14.
Letey, J., 1.
Levy, R., 16.
Lewis, D., 23.
Li, P. H., 34.
Limburg, A. M., 48.
Link, J., 43.
Linowitzki, V., 45.
Lipps, R. C., 10.
Lister, R. M., 14.

Lockard, R. G., 12.
Loncin, M., 46.
Lorenz, K., 29.
Löttage, W., 15.
Luce, W. G., 24.
Lück, H., 41.
Lundquist, E. B., 43.
Lyman, C. M., 22.
Lyon, M. R., 24.

MABEY, G. L., 19.
McDonald, C. E., 26.
McDowell, R. E., 43.
McEntee, K., 20.
McIntyre, M. L., 11.
McLaren, G. A., 21, 23.
Macrae, R. M., 33.
Maes, E., 26.
Magnoux, C., 16.
Maillard, A. C., 33.
Mäkinen, R., 38.
Malz, H., 18.
Mandrou, B., 32.
Mangold, D. W., 24.
Mann, L. K., 35.
Maples, R., 12.
Marek, C. J., 29.
Marešová, D., 30.
Mark, H. B., jun., 30.
Martin, J. L., 30.
Martin, J. P., 2.
Martinez Moreno, J. M., 46.
Masoero, M., 45.
Mason, J. L., 10.
Matsumoto, H., 26.
Maurel, A., 31.
Maurer, C. L., 13.
Mayfield, E. D., 21.
Meade, R. J., 24.
Melles, J. L., 17.
Mennicke, U., 37.
Menoret, Y., 16.
Menzies, J. A., 8.
Meredith, W. E., 42.
Mergenthaler, E., 47.
Mestres, R., 32.
Metcalf, R. L., 48.
Meyer, A., 35.
Michener, H. D., 43.
Milborrow, B. V., 6.
Milburn, J. A., 7.
Milford, R., 18.
Milković, B., 36.
Miller, R. D., 3.
Millikan, D. F., 10.
Mills, P. A., 43.
Miln, P. McN., 46.
Milne, D. L., 47.
Minson, D. J., 18.
Mitterhauszerova, L., 28.
Mochrie, R. D., 18, 19.
Mohamed, H. A., 13.
Mohammad, Ali, 27
Mahammed, K., 23.
Mohler, H., 41.
Monaco, L. C., 12.
Moorby, J., 6.
Morgan, P. B., 48.
Morrison, A. B., 45.
Morrison, J. W., 35.
Moss, P., 2.
Mossel, D. A. A., 34.
Moštek, J., 33.
Mukherjee, S., 4.
Munro, I. C., 45.
Muskat, E., 35.
Moulton, K. J., 27.

NAGASAWA, K., 37.
Neales, T. F., 6.
Nesheim, S., 36.
Netzer, A., 36.
Nielsen, H. M., 21.
Nielsen, K. F., 10.
Niewiadomski, H., 38.
Nowakowski, T. Z., 10.
Nummerman-Vaupel, C., 4.
Nury, F. S., 36.
N.V. Philip's Gloeilampenfabrieken. 15, 42.
Nylund, R. E., 35.

OERTEL, G., 18.
Ohman, J. H., 7.
Olsen, K. L., 10.
O'Neal, J. M., 5.
Orbán, E., 28.
Osborn, R. A., 34.
Oteifa, B. A., 3.
Ough, C. S., 40, 41.
Owens, E. L., 4.

PAGE, E. R., 6.
Page, N. R., 6.
Paladines, O. L., 21.
Paleg, L. G., 5.
Parekh, R. G., 46.
Parks, P. F., 22.
Parsons, C. S., 35.
Patrick, W. H., jun., 3.
Paulsen, T. M., 35.
Pearce, G. W., 48.
Pearson, A. M., 39.
Peart, J. N., 20.
Pechnik, E., 45.
Penman, H. L., 1.
Penny, A., 9.
Peo, E. R., jun., 24.
Peppler, E., 35.
Perry, A. S., 48.
Peryam, D., 41.
Pettyjohn, J. D., 18.
Philleo, W. W., 48.
Phillips, R. E., 2.
Pickett, E. E., 10
Pinkel, D., 30.
Pitman, M. G., 6.
Pitts, D. W., 29
Podrazký, V., 26, 29.
Pokorný, J., 38.
Pommer, E.-H., 16.
Pond, W. G., 24.
Ponte, J. G., jun., 29.
Pope, W. R., 46.
Popper, K., 36.
Porritt, S. W., 34.
Porter, W. L., 35.
Posnette, A. F., 11.
Powrie, W. D., 45.
Pratt, P. F., 42.
Prescott, J. H. D., 23.
Price, C. A., 14.
Prillinger, F., 31.
Purser, D. B., 22.
Puschner, J., 39.
Putnam, C. A., 22.
Putnam, P. A., 22.

RALEIGH, R. J., 21.
Ranjhan, S. K., 24.
Rankoff, D., 37.
Rankoff, G., 37.
Rao, A. V., 46.
Rathbone, C. B., jun., 12.
Ray, M. L., 22.
Raymond, W. F., 18.
Regula, E., 29
Rehm, H.-J., 4.
Reicheneder, F., 16.
Reid, J. T., 21.
Reimer, D., 24.
Rey, M., 31.
Rey, S., 31.
Richards, M., 33.
Richards, S. J., 2.
Rieme, M. E., 22.
Riley, P. W., 23.
Ritchie, N. S., 20.
Roberts, A. N., 11.
Roberts, E. C., 10.
Robinson, D. W., 23.
Robocker, W. C., 15.
Rohm & Haas Co., 17.
Rook, J. A. F., 22.
Rose, D. A., 1.
Rose Innes, R., 19.
Rowell, J. G., 24.
Roy, A. C., 12.
Roy, A. K., 4.
Roy, H., 4.
Rubin, J., 1.
Ruppel, E. G., 13.
Ryerson, D. E., 9.
Ryugo, K., 34.

SABINE, J. R., 21.
Saghir, A. R., 35.
Sair, L., 40.
Salminen, K., 38.
Salmon, R. C., 3.
Salmon-Legagneur, E., 24.
Salo, T., 38.
Sanchez, F. F., 48.
Santiago, A., 13.
Sawichi, J, 38.
Scaletti, J. V., 20.
Schanderl, H., 31.
Schegk, F., 41.
Schlesinger, J., 27.
Schneider, M. D., 44.
Schonbrod, R. D., 48.
Schormüller, J., 40.
Schrader, G., 16.
Schuman, L. M., 20.
Sciuchetti, L. A., 7.
Seberry, J. A., 47.
Seckinger, H. L., 28.
Seidel, D., 13.
Sergeant, E. P., 14.
Seymour, E. W., 24.
Shank, D. B., 7.
Shaw, W. C., 14.
Shell Internationale Research Mij N.V., 17.
Shell Research Ltd., 16, 17.
Sherman, M., 47, 48.
Sherrod, L. B., 21.
Shirakawa, T., 11.
Shirley, R. L., 22.
Shone, G. G., 37.
Short Milling Co., J. R., 30.
Shoub, J., 11.
Shupe, J. LeG., 21.
Siddoway, F. H., 1.
Simco, J., 25.
Simon, M., 44.
Simmonds, D. H., 32.
Šimová, J., 26.
Singleton, V. L., 41.
Slack, C. R., 6.
Slife, F. W., 14.
Sluijsmans, C. M. J., 4.
Smart, W. W. C., jun., 19.
Smith, J. H., 3.
Smith, Kline and French Laboratories, 25.
Sneed, E. M., 46.
Sohns, V. E., 27.
Soikachi, H., 41.
Souci, S. W., 47.
Spahr, S. L., 20.
Spalding, D. H., 35.
Spanswick, R. M., 5.
Speer, V. C., 24.
Spicher, G., 29.
Spock, J., 25.
Springer, F., 26.
Springer, R., 41.
Srivastava, B. I. S., 32.
Stammbach, K., 15.
Stanley, N. W., 19.
Stanley, W. L., 36.
Stauffer Chem. Co., 16.
Steinbuch, —., 43.
Steinhardt, R., 1.
Steinmann, G., 38.
Stoddard, G. E., 21.
Stoll, S., 31.
Stoloff, L., 36.
Stolzy, L. H., 1.
Storherr, R. W., 15.
Stroud, J. W., 19.
Stull, J. W., 23.
Stummeyer, H., 16.
Subba Rao, P. V., 45.
Subrahmanyan, V., 25.
Sudia, T. W., 6.
Suess, M. J., 3.
Suomalainen, H., 32.
Szczepanowska, A. D., 28.
Szegi, J., 3.

TALLEY, E. A., 35.
Tango, J. S., 12.
Tayler, J. C., 19.
Teich, A. H., 8.
Terriere, L. C., 48.
Tessem, B. M., 25.
Thomae, G.m.b.H., K., 17.
Thompson, N. R., 8.
Thresh, J. M., 11.
Tibenský, V., 30.
Tiblárik, K., 28.
Tillman, A. D., 21.
Timm, L. A., 29.
Titcomb, S. T., 29.
Toepler, H., 39.
Trager, W. T., 36.
Tyznik, W. J., 22.

UGOCHUKWU, E. N., 15.
Uhl, D., 27.
Unger, P. W., 8.
Unrau, A. M., 5.
Urion, E., 33.
U.S. Borax & Chem. Corp., 18.

VALORAS, N., 1.
van den Berg., L., 39, 44.
van der Hey, D., 8.
van Niekerk, B. D. H., 21.
van Niekerk, P. E. le R., 4.
van Schuylenborgh, J., 1.
Vasudeva Murthy, A. R., 48.
VEB Farbenfabrik Wolfen, 15.
Vengrio, J., 14.
Venkob Rao, S., 46.
Veselý, Z., 26.
Voneš, F., 26.
Vrána, J., 38.

WAAG, T., 15.
Wagner, K., 18.
Wain, R. L., 15.
Wallnöfer, P., 4.
Walters, R. J. K., 9.
Wasserman, T., 27.
Watson, D. P., 11.
Watson, M., 14.
Watts, R. R., 15.
Weatherley, P. E., 5.
Wedemeyer, G., 44.
Weinhold, A. R., 13.
Weiser, H. H., 42.
Welch, J. A., 23.
Whanger, P. D., 23.
Whistler, R. L., 28.
Whittington, W. J., 6.
Widdowson, F. V., 9.
Widstrom, N. W., 7.
Wiersum, L. K., 1.
Williams, M. C., 15.
Williams, R. J. B., 9.
Williams, S., 43.
Williard, J. W., 5.
Wilson, J. D., 13.
Winchester, J. A., 14.
Windel, H., 16.
Winter, A. R., 42.
Wise, M., 46.
Wolf, M. J., 28.
Wolfenberger, D. A., 11.
Woodruff, N. P., 1.
Woodward, J. D., 33.
Wren, J. J., 28.
Wright, M. J., 20.
Wyatt, R., 3.

YATES, R. J., 20.
Yip, G., 43.
Yong, F. C., 5.
Young, D. A., 9.
Young, W. E., 28.
Youngs, V. L., 27.
Yuen, S. H., 42.

ZAGIC, J., 38.

J. Sci. Fd Agric., 1965, Vol, 16, July

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

CONTENTS

PAGE

The effect of heat in drying on the nutritive value of wheat for animal feed 369
By C. K. Milner and J. Woodforde

Inhibition of soya lipoxidase 373
By J. A. Blain and G. Shearer

Sarcomere length of free and restrained bovine muscles at low temperature as related to tenderness 379
By H. K. Herring, R. G. Cassens and E. J. Briskey

Fusel oils in ciders and perries 384
By A. Pollard, Margaret E. Kieser, Pauline M. Stevens and O. G. Tucknott

Further studies on the limiting amino-acids in differently processed groundnut meals 390
By Hans Fisher

Factors influencing the pyruvic acid content of wines 394
By B. C. Rankine

The reaction of wheat proteins with sulphite. I.—Sulphitolysis of the proteins of flour with cuprammonium sulphite 399
By E. E. McDermott and J. Pace

Quantitative changes in the polyphenols during the processing of tea leaf and their relation to liquor characters of made tea 408
By I. S. Bhatia and M. R. Ullah

The determination of steam-volatile fatty acids in rumen liquor, blood plasma and milk fat .. 417
By J. E. Storry and D. Millard

Fumigation of agricultural products. XIX.—Methyl bromide fumigation of narcissus bulbs infested with stem nematode, *Ditylenchus dipsaci* (Kühn) 421
By R. E. Purnell and N. G. M. Hague

Abstracts i-1—ii-48

Printed in Great Britain by Unwin Brothers Ltd., Woking and London (B3735)