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SEAWEED EXTRACTS AS FERTILISERS

By G. BLUNDEN, S. B. CHALLEN* and D. L. WOODS

The growth-promoting effect of extracts of *Laminaria saccharina*, *Fucus vesiculosus* and *Ascophyllum nodosum* was due largely to the cations present, but this effect was modified by organic substances in the extracts. The concentrations of amino acids and mannitol in seaweed extracts had little effect on plant growth. The compounds extracted with organic solvents appear to be responsible in part only for the modification of the growth-promoting effect. Alginic acid and its salts were indicated as being the main organic compounds responsible for reducing the effect of the metals with mustard plants. It is suggested that alginic acid competes with the plants by ion-exchange for the metals in the extract.

Introduction

The use of seaweed as a manure is general in coastal areas throughout the world.[1] In addition, extracts derived completely or partly from seaweeds are used commercially in agriculture and horticulture. Brown seaweeds are similar to farm-yard manure[2] in organic matter, nitrogen and potash contents but contain more trace elements.[3,4] Beneficial effects from the use of seaweed and seaweed extracts on crop yields, seed germination and the ability of treated crops to resist fungal disease, insect pests and low temperatures have been reported.[1,5]

Seaweed and seaweed extracts contain several compounds that may be of benefit to plant growth and development, for example, alginic acid has been shown to be an important soil conditioner;[6,7] the presence of indole acetic acid and gibberellins has been demonstrated,[8] and laminaran has been shown to reduce considerably the severity of diseases caused by several soil-borne fungal pathogens.[9]

The work described in this paper was undertaken to determine the effects of different seaweed extracts on growth, and to attempt to relate these effects to the constituents of the extracts.

Experimental

Fresh samples of the seaweeds used were collected around the Portsmouth area. Except where stated, fresh seaweed was used for extraction. Dried samples were prepared by air drying at room temperature for 3 days or by drying in a circulatory air oven at 100° for 16 h.

Preparation of extracts

The preparation of seaweed extracts was based on commercial methods and the published work of Challen & Hemingway.[10] Fresh seaweed (1 kg), cut into pieces 1–2 cm in length, or powdered dry seaweed, equivalent to 1 kg of fresh material, was boiled with 3 l distilled water for 1 h. The hot liquid was strained through fine muslin, the residue was extracted with 3 l cold water for 48 h, and the liquid was again strained. The liquids were combined and concentrated *in vacuo* to 20 wt.-% solids and stored in a refrigerator. The extracts were diluted before use, usually by 1 in 100 wt.-%.

Organic extracts were prepared by macerating 100 g quantities of air-dried seaweed with 3 separate 250 ml quantities of solvent for 1 day each. The combined liquids were concentrated to a volume equivalent to the volume of aqueous extract containing 20 wt.-% solids obtained from the same quantity of seaweed.

Acid extracts of seaweed were prepared by refluxing $\frac{1}{8}$ kg plant material with 1 litre 2 N-HCl for 6 h, filtering and concentrating the filtrate to dryness *in vacuo*. The residue was taken up in water to equal a 20 wt.-% solution. Alkaline extracts of seaweed were made by refluxing $\frac{1}{8}$ kg with 1 litre of water, containing 25 ml 10% NaOH, for 6 h, filtering and concentrating the extract until it contained a percentage solids equal to 20 wt.-%. Both the acid and alkaline extracts were diluted 1 in 100 before use.

Growth test

Multipots* (~100 ml) full of Vermiculite† (17–20 g) were treated with 50 ml test solution or control liquid and sown with 30 mustard seeds (*Sinapis alba* L.). The seeds were lightly covered with Vermiculite and the multipots were covered with glass for 2–3 days until germination occurred. They were then kept in a glasshouse at approximately 18° and treated regularly with either distilled water or mineral solution, which were added to a tray beneath the pots. Three weeks after sowing, the seedlings were harvested by being cut at the top of the roots. Records were made of the number of plants per pot, total wet weight per pot, and total dry weight per pot after the plants had been dried at 105° overnight. Twelve replica pots in each group were used, and the average weight per plant in each pot was used to find the overall mean and standard error. Significance of results was examined using the 't' test at a level of $P = 0{\cdot}95$. An identical procedure was used when test plants other than mustard were used.

The mineral solution used in the tests was the one recommended by Hewitt[11] for brassica crops.

Each organic extract of seaweed (6 ml) was diluted to 100 ml with absolute ethanol and mixed well with enough Vermiculite to fill 12 pots. This was spread out to dry for 30 h before use. The Vermiculite so treated was regarded as containing the organic-soluble fraction of the seaweed in quantities equivalent to that in the aqueous extract.

* Now deceased

* MacPenny's Grimsby, Great Britain

† P.B.I. Ltd., Veri-gro Horticultural Vermiculite

Ashing

The extract (10 g) was evaporated to dryness at 100° and ashed overnight in a muffle furnace at up to 550°; this avoids the loss of volatile salts. The ash was suspended in water, made up to 10 ml and diluted by 1 in 100 before use.

Estimation of metals

Samples of seaweed extracts were ashed, boiled in concentrated HCl, filtered, the filtrate was evaporated to dryness and the residue was taken up in water. These solutions were examined by paper chromatography on Whatman No. 1 paper, using methanol as the developing solvent and locating the metals by spraying with 10% $AgNO_3$ solution, followed by 0·05% fluorescein sodium solution. The spot areas of KCl and NaCl were measured and compared with those of different concentrations of reference solutions on the same sheet of paper. In addition, values of each metal concentration were obtained from the emission lines measured on a Unicam S.P. 900 flame spectrophotometer.

Estimation of amino-acids

To the aqueous extract, concentrated HCl was added to precipitate alginic acid, which was removed by centrifuge. The extract was neutralised with NaOH and then passed through a column of Dowex AG 50W-X4 resin, in the H^+ form. Uncharged substances were removed by the passage of water through the column, and then the amino-acids were eluted with 3 N-NH_4OH. This eluate was concentrated to a small volume and was used for chromatographic examination on air-dried silica gel G (Merck) layers, 250 μm thick. Two-way chromatograms were prepared, using as the solvent for the first direction, chloroform–methanol–17% NH_4OH (2 : 2 : 1 v/v) and for the second direction n-propanol–water (70 : 30 v/v) or n-butanol–acetic acid–water (80 : 20 : 20 v/v) or phenol–water (4 : 1 w/w). After drying, the plates were sprayed with an 0·1% w/v solution of ninhydrin in acetone and the compounds were located by heating in a current of warm air. Individual amino acids were identified by co-chromatography with authentic samples in all the solvent systems listed above. Approximate estimations of the major amino acid concentrations present in the seaweed extracts were determined by comparing different volumes of seaweed extract and different concentrations of reference amino acid solutions on the same thin-layer plate. Five different plates were used for the estimation of each of the major amino acids present.

Estimation of mannitol

Known concentrations of seaweed extract were examined chromatographically on Whatman No. 1 paper using n-butanol–acetic acid–water (5 : 1 : 2 v/v) as the developing solvent. The papers were sprayed with ammoniacal $AgNO_3$ solution and heated at 100° for 15 minutes to locate the mannitol. The spot areas produced by the different concentrations of seaweed extracts were compared with those produced from different concentrations of a reference mannitol solution on the same paper. Five duplicate chromatograms were prepared for each estimation.

Estimation of alginic acid

To 100 ml of 1% w/v seaweed extract was added either 10 ml concentrated HCl or 10 ml 10% w/v $CaCl_2$ solution. The solutions, after standing overnight, were filtered, and the residue was washed with water, dried at 100° for 16 h and weighed. Each gramme of acid-precipitated material was taken to be equal to $\frac{176^*}{194}$ g alginic acid and each gramme of $CaCl_2$-precipitated material was taken to be equal to $\frac{176^*}{215}$ g alginic acid. The mean of the two values obtained was used to calculate the percentage of alginic acid in the extract.

Results

Aqueous extracts of all the seaweeds tested promoted growth in the mustard test, although the amount varied from species to species. In all cases, the increase in the wet weight of the plants, as a percentage of the control, was greater than the increase in the dry weight (Table I).

TABLE I

Comparison of the effects on mustard plants of aqueous extracts (0·2% w/v solids) of different species of seaweed

Species	Average wt. as % of control		No. of tests
	Wet wt.	Dry wt.	
Rhodymenia palmata (L) Grev.	199	147	1
Laminaria saccharina (L) Lamour	144	103	7
Laminaria digitata (Huds) Lamour	131	106	2
Ascophyllum nodosum (L) Le Jol.	171	116	3
Fucus vesiculosus L.	185	110	3
Halidrys siliquosa (L) Lyngb.	313	137	1
Desmarestia viridis (Mull) Lamour	107	94	1

Three brown seaweeds were selected for further work, *Laminaria saccharina* L., *Ascophyllum nodosum* (L) Le Jol. and *Fucus vesiculosus* L, extracts from each species being used in the mustard test at different concentrations from 0·1 to 1·0% solids. The *L. saccharina* extract produced increases in both the average wet and dry weight per plant as the concentration increased over this range, but the *F. vesiculosus* and *A. nodosum* extracts produced most of their increase at the lower concentrations (Fig. 1).

The effect of extracts produced from fresh, air-dried, and oven-dried *L. saccharina* on growth was tested using the mustard test. The extract prepared from fresh weed gave significantly better results than either of the other two extracts, which were not significantly different from each other. However, the ash value was higher from the extract of fresh weed than either of the extracts prepared from air-dried or oven-dried samples (Table II).

The ash values of the different seaweed extracts varied from species to species, the figures obtained, as a percentage of the total solids, being *L. saccharina* 27 to 32%, *A. nodosum* 35 to 40% and *F. vesiculosus* 30 to 37%. An equivalent amount of ash produced greater increases in both the wet and dry weights per plant than did the seaweed extract. Furthermore, when the test was repeated using a mineral solution instead of distilled water, the ash still produced better growth,

* Alginic acid has a theoretical equivalent weight of 176, but oven-drying consistently gives a value of 194, owing to failure to remove 1 molecule of water per equivalent[12]

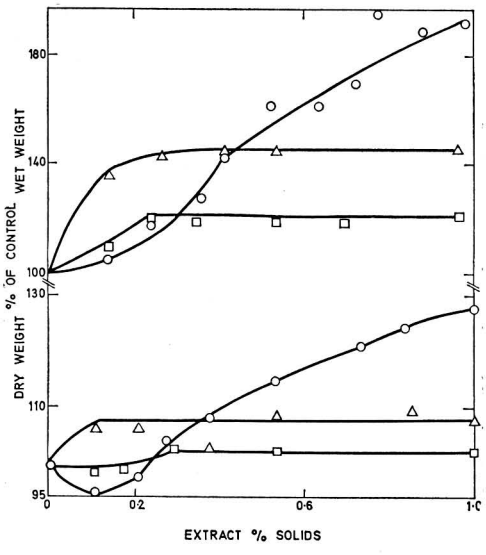

FIG. 1. *Dose-response curves of aqueous extracts of* Ascophyllum nodosum, Fucus vesiculosus *and* Laminaria saccharina

○ = *L. saccharina*
△ = *F. vesiculosus*
□ = *A. nodosum*

TABLE II

Effect on mustard plants of aqueous extracts prepared from fresh air-dried, and oven-dried *L. saccharina*

Extract seaweed	g dry extract per 100 g fresh seaweed	Ash value as % of dry extract	Average wt. as % of control	
			Wet wt.	Dry wt.
Fresh	7·7	29·3	177	105
Air-dried	7·3	27·3	163	105
Oven-dried	8·9	22·9	158	100

whereas the seaweed extracts often produced results not significantly different from the controls, or occasionally results which were lower (Table III). This was not an effect of pH, as there were no significant differences in the results obtained from plants grown with the ash solution or with the ash neutralised with HCl. Moreover, no significant differences in growth were recorded when mustard plants were grown in N/1000 HCl (pH 3), distilled water or N/1000 NaOH (pH 11).

Examination by paper chromatography of the metals present in the seaweed extracts showed that the major differences between the three species tested was that *Laminaria* extracts contained far more potassium than those of the Fucaceae, but the concentrations of sodium were similar. Magnesium was located in all three extracts, but only traces of calcium were detected by this method. In the three week test period, a large proportion of metals available was absorbed by the mustard plants (Table IV), as determined by the flame spectrophotometric method.

The predominant amino acids in all three extracts were α-alanine, glutamic acid, proline, valine and lysine. In *L. saccharina* extracts, α-alanine represented approximately 2% of the total solids, glutamic acid 0·25 to 0·50%, valine 0·25%, proline 0·2% and lysine <0·1%. In seaweed extracts containing 0·2% solids, these amino-acids were present in concentrations which were shown to have no significant effects on the mustard test.

Mannitol was found to constitute 14% of the total solids of the *L. saccharina* extract. Extracts of *A. nodosum* and *F. vesiculosus* both contained mannitol, but in concentrations below 10%. Mannitol concentrations from 0·01% to 0·05%, equivalent to from 5 to 25% total solids of the seaweed extracts, produced no significant differences from distilled water in the mustard seed test.

Alginic acid constituted 1·9 to 5·1% of the total solids of *L. saccharina* extracts, 11·6 to 13·5% of *F. vesiculosus* extracts and 3·6 to 7·8% of *A. nodosum* extracts. In these concentrations, alginic acid was found to have little effect on the growth of mustard or to be slightly inhibitory in both distilled water and in mineral solutions. Sodium alginate, compared with distilled water, caused small increases in the wet weight per plant, and had little effect when the plants were grown in mineral solution. A solution of NaCl, at an equivalent concentration of sodium ions, produced a marked increase in the average wet weight per plant (Table V).

In the mustard test, an HCl extract of *A. nodosum* produced greater growth than an aqueous extract, which in turn produced growth greater than a NaOH extract. The ash

TABLE III

Comparison of the effects on mustard plants of seaweed aqueous extracts and the ash prepared from the extracts, in water and in mineral solutions

Test material	g dry extract per 100 g fresh seaweed	Average wt. as % of control			
		Control water		Control mineral solution	
		Wet wt.	Dry wt.	Wet wt.	Dry wt.
L. saccharina extract	4·6	162	105	102	91
Ash of *L. saccharina* extract		189	113	118	104
F. vesiculosus extract	7·0	156	114	96	93
Ash of *F. vesiculosus* extract		177	125	105	100
A. nodosum extract	6·7	171	120	95	94
Ash of *A. nodosum* extract		177	119	104	102

TABLE IV

Uptake of cations from *A. nodosum* extract by mustard plants. Results using a Unicam SP 900 flame spectrophotometer

	Metal quantity in mg/10 pots			
	K	Na	Ca	Mg
Quantity in applied seaweed extract	8·39	54	2·18	1·04
Yield in test plants (seaweed extract treated)	9·80	63	2·70	2·80
Yield in control plants (distilled water only)	3·84	33	0·64	1·74
Uptake by test plants	5·96	30	2·06	1·06
Uptake as % of applied metals	71	56	94	102

TABLE V

Effects of sodium alginate and sodium chloride on the growth of mustard plants at equivalent sodium ion concentrations

Test material	Average wt. as % of control	
	Wet wt.	Dry wt.
Sodium alginate 0·075% w/v	104	100
0·150% w/v	102	102
0·300% w/v	108	104
Sodium chloride 0·0166% w/v	110	101
0·0332% w/v	114	101
0·0663% w/v	133	110

from the HCl extract produced less effect on growth than the extract itself (Table VI).

Ether, n-hexane and ethanolic extracts of *L. saccharina* were tested on mustard plants. When compared with distilled water, the n-hexane solution showed no significant difference, but the ether and ethanolic extracts produced significant increases in the average wet weight per plant and the ethanolic extract also produced a significant increase in the average dry weight per plant. The aqueous extract, however, gave results which were significantly greater than those with any of the organic extracts. When the organic extracts were tested using a mineral solution in the test instead of distilled water, all three extracts produced significant decreases in the average wet weight per plant, compared with the mineral solution alone. The ethereal extract also produced a significant decrease in the average dry weight per plant (Table VII).

The aqueous extract of *L. saccharina* was also used on spinach beet (*Beta brasiliensis* L.), lettuce (*Lactuca capitata* L.), onion and spring onion (*Allium cepa* L. varieties) leek (*Allium porrum* L.) and wheat (*Triticum sativum* L.). In comparison with distilled water, the *L. saccharina* extract significantly increased the wet and dry weights of spinach beet plants, but decreased both on lettuce. With the other species, the *L. saccharina* extracts tended to reduce the average wet weight of all the plants tested with the exception of wheat, which was not significantly affected. The effect on dry weight varied, the average dry weight per plant being slightly reduced with spring onion and leek and increased with wheat and onion. The ash from the seaweed extract, in all cases, produced significant increases in both the wet and dry weights of the test plants (Table VIII).

TABLE VI

Effect of acid and alkaline extracts of *A. nodosum* on mustard plant growth

Test material	Average wt. as % of control	
	Wet wt.	Dry wt.
HCl extract	269	160
NaOH extract	203	129
Aqueous extract	218	138
Ash of HCl extract	221	144

TABLE VII

Effect on mustard plants of various solvent extracts of *L. saccharina*

Solvent	Average wt. as % of control			
	Control water		Control mineral solution	
	Wet wt.	Dry wt.	Wet wt.	Dry wt.
Water	138	132	100	98
n-hexane	109	94	85	93
Ether	119	99	83	81
Ethanol	126	104	92	87

TABLE VIII

Effect of aqueous extracts of *L. saccharina* on various test plants

Test plant	Average wt. as % of control			
	L. saccharina extract		Ash from *L. saccharina* extract	
Lettuce	87	77	124	104
Spinach beet	145	117	130	105
Wheat	100	110	108	107
Onion	68	110	120	109
Spring onion	71	97	118	106
Leek	85	99	111	105

Discussion

The mustard growth test was designed as a rapid method for evaluating different fertilisers. However, as conditions in the glasshouse varied during the year, results are not strictly comparable between different tests. More reproducible results were observed during the winter, when day lengths were shorter and temperature was maintained more easily at 18°. Weights of a series of controls followed a normal distribution, indicating the suitability of the *t* test in determining the significance of results of individual tests.

Dose response curves showed differences between the extracts of *L. saccharina* and those of the Fucaceae. *L. saccharina* extract, which had the lowest ash value, but the highest potassium content, increased weight with increasing concentration. *A. nodosum* and *F. vesiculosus* extracts, which had high ash values, but low potassium contents, produced dose response curves that levelled out at the higher concentrations. Results obtained with the ash prepared from the seaweed extracts strongly indicated that the growth promoting effect of the extracts was largely due to the inorganic constituents present. The effects of the extracts may be the result of several factors, for example, the inorganic

constituents of the seaweed extracts being unavailable to the plants, or being only available slowly, or inhibitors being present in the extracts. It was shown that the effect was not due to differences in pH. Analysis showed that the mustard plants had taken up a large proportion of the inorganic constituents within the three week test period, although it is possible that the rate of uptake was slower from the extract than from the seaweed ash solution. It appeared probable, therefore, that the seaweed extracts contained growth inhibitors which affected the growth of the plants within the test period. The effect of these inhibitors varied markedly with different test plants, and was very pronounced with lettuce, onion, leek and spring onion. Inhibitors have been reported in seaweed extracts by several workers.[13–17]

A result observed with the use of seaweed extracts on different test plants was the effect on hydration of the plant. With the three dicotyledonous species tested, the seaweed extracts increased the moisture content of the plants when compared with the controls grown using distilled water. However, the monocotyledonous plants tested showed decreases in the moisture content of the plants in comparison with the controls. In all cases, however, solutions of ash prepared from seaweed extracts increased the moisture content of the test plants.

The different effects in the mustard test produced by extracts prepared from fresh, air-dried and oven-dried samples were probably the result of extraction difficulties with the dried seaweed. This was in agreement with the ash values of the different extracts, the extract prepared from fresh seaweed having a higher value than those of the other two extracts.

Amino acids and mannitol, which are known constituents of seaweeds[18–19] and which have been reported to be growth inhibitory,[20–25] were used in the mustard test over considerable concentration ranges. In the concentrations present in the extracts the major free amino acids did not have any noticeable effect on the mustard plants, although with concentrations of seaweed extract in excess of 0·2% solids, some of them, in particular α-alanine, would be in the concentration range that normally has a growth promoting effect on mustard plants. Mannitol was found to be a major component of the seaweed extracts, but in the concentrations present it produced no effect in the mustard test.

Alginic acid is in a soluble form in the seaweed extracts, probably as sodium alginate. Potassium alginate may be present, but the sodium salt is of main interest in this work, as the extract of the seaweed species containing the most potassium, *L. saccharina*, contained only a small quantity of alginic acid. Sodium alginate alone produced a small increase in growth of mustard, this being due probably to available sodium ions, the increase being considerably less than that produced by sodium chloride. Alginic acid alone produced little effect. When the plants were treated with mineral solutions, both sodium alginate and alginic acid produced less growth than the control. It seems likely that the alginate present was acting as an ion-exchange medium and competing with the plants for uptake of cations. It has a theoretical ion exchange capacity of about 515 milli-equivalents per 100 g, whereas most soils have a range from 0 to 100 milli-equivalents per 100 g.[26] The effects of acid and alkaline extraction of seaweed agree with this hypothesis. The acid extract would not contain alginic acid, because this is insoluble, and would break down under these conditions, and the alkaline extract would contain more alginate than the others. The production of better growth by the acid extract in comparison with its ash is probably due to the presence of breakdown products in the extract.

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SUPERFICIAL SCALD, A FUNCTIONAL DISORDER OF STORED APPLES

III.*—Concentration of diphenylamine in the fruit after treatment

By F. E. HUELIN

After treatment of apples with diphenylamine most of it was found in the peelings (1 mm thick) and was distributed approximately as follows: 60% in the cuticle, 20% in the epidermis, and 20% in the hypodermis and cortex (assumed to be predominantly in the hypodermis). Treated apples lost 95% or more of the original diphenylamine during 30 weeks' storage at 1°. A concentration in the peelings of about 0·2 μg/cm^2 appeared to be the minimum required to inhibit scald-inducing reactions.

Concentrations of diphenylamine immediately after treatment and the minimum effective concentrations were estimated in the cuticle, epidermis, and hypodermis. It appeared that the concentration needed to inhibit the scald-inducing reactions was appreciably less than that required for inhibiting respiration, phosphorylation, or the oxidation of carotene.

Introduction

The discovery by Smock[1,2] that superficial scald can be controlled by applying diphenylamine to the surface of the fruit has been confirmed by other workers in the U.S.A., Europe, and Australia. The mechanism of this control has not been established, but diphenylamine is known to affect a number of reactions *in vitro* or *in vivo*. It is an antioxidant for carotene[3] and other unsaturated substances. Diphenylamine also decreases the synthesis of carotene by micro-organisms while the more saturated phytofluene accumulates,[4] and thus appears to inhibit the dehydrogenation of the more saturated polyenes to carotenoids. It has been shown to uncouple oxidative phosphorylation[5] and to inhibit cytochrome oxidase,[6] succinic oxidase,[5,7] and reduced nicotinamide adenine dinucleotide oxidase.[7] Diphenylamine treatment was found to have a variable effect on the ester production of stored apples.[8]

To determine whether any of these effects are relevant to the control of scald it is necessary to know the concentration of diphenylamine in the scald-liable and neighbouring tissue of treated fruit. The cells affected by scald are in the hypodermis below the cuticle and epidermis at depths of 40–150 μ from the surface.[9] This paper reports determinations of diphenylamine in apples immediately after treatment and during subsequent storage. An attempt was made to determine the approximate partition of diphenylamine between the cuticle, epidermis, and underlying tissue.

Experimental

Granny Smith apples from the Bathurst district of New South Wales were treated with diphenylamine and stored at 1°. In 1963 a solution of 0·01 M diphenylamine in ethanol was poured over the apples, which were spread out to dry on stainless steel tables. The apples were stored in 5 cases each containing a bushel, and samples of 10 apples for analysis were obtained by taking 2 apples from each case. In 1964 the apples were divided into comparable samples of 24 before treatment. Each sample was dipped in a solution of 0·01 M diphenylamine in 50% (v/v) aqueous ethanol, dried on a wire tray, and packed in a separate carton. The cartons for each picking were stored in a single stack, and the top carton was taken for analysis. Samples were analysed immediately on removal from 1° and also after a further week at 20°. In 1965 the same sampling and treatment were adopted, and each sample was stored in a gas-tight drum through which air was drawn at 2 or 20 l/h.

Diphenylamine was determined during storage in peelings, flesh, skin, and 'coating', and in air which had passed over the fruit. Peelings of approximately 1 mm thickness were obtained with a vegetable peeler. 'Flesh' included all the remaining tissue. The 'skin' was composed of cuticle and epidermis, and was separated from the peelings with ammonium oxalate.[10] In 1964 and 1965 the 'coating' (probably derived mainly from the cuticle) was extracted by dipping the intact apples in hexane at 20° for 2 minutes. The hexane was purified with fuming sulphuric acid.

The method of Kennett[11] was used for determination. The peelings, flesh, or skin were boiled with water, with the use of a special reflux head[12] containing hexane to extract the diphenylamine from the aqueous condensate before it returned to the flask. With hexane extracts of the coating a 10 ml aliquot was added to the water, which was then heated to boiling point while the solvent distilled into the reflux head. The reflux was continued for 6 hours. The diphenylamine was extracted from the hexane into 18 N sulphuric acid and was determined colorimetrically after oxidation with vanadium pentoxide in 18 N sulphuric acid. The extinction at 580 nm was measured at one-minute intervals until it reached a maximum (usually within 10 min). In 1965 the diphenylamine evaporated from the fruit was determined weekly. Diphenylamine was absorbed from the air leaving the fruit in spiral absorbers containing hexane cooled in a mixture of solid carbon dioxide and ethanol. The hexane solution was

* Part II: *J. Sci. Fd Agric.*, 1964, **15**, 227

separated from the aqueous condensate and the diphenylamine was determined without further distillation.

The concentration of diphenylamine was calculated as μg per cm^2 of apple surface as previously described.[10] Diphenylamine in the coating was added to that found in the peelings after previous extraction of the intact apples to give the total concentration in the peelings.

Results

The results are given in Table I. Rate of air flow had no effect on the diphenylamine left in the fruit in 1965, and the mean figures are given. The same concentration of diphenylamine in the dip gave marked differences in uptake by the fruit. In spite of the analytical and sample variation a general pattern of distribution in the fruit and considerable losses during storage are apparent. The mean ratios of concentrations in peelings, flesh, skin, and coating indicate that the peelings of approximately 1 mm thickness contained 95% of the diphenylamine, only about 5% being found in the flesh. Of the diphenylamine in the peelings 81 $\pm$ 3% was found in the skin composed of cuticle and epidermis and 58 $\pm$ 2% in the coating or cuticle. Hence the diphenylamine in the peelings was distributed approximately as follows: 60% in the cuticle, 20% in the epidermis, and 20% in the hypodermis and cortex to a depth of 1 mm from the surface.

During 30 weeks of storage at 1° the apples lost 95% or more of the original diphenylamine, over half being lost in the first 10 weeks. This loss did not appear to be due to evaporation of diphenylamine from the surface of the fruit. In 1965 the apples lost 1·41 μg/cm^2 in the first 10 weeks and 0·28 μg/cm^2 in the second 10 weeks. During the whole 20 weeks the evaporation of diphenylamine did not exceed 0·01 μg/cm^2. Hence loss by chemical change is indicated.

Discussion

As untreated apples have usually been stored for at least 10 weeks at 1° before the onset of visible scald, it appears that the reactions leading to scald have an incubation period of at least 10 weeks. Hence for treated apples it can be assumed that the concentration of diphenylamine remaining in the fruit should be sufficient to inhibit the scald-inducing reactions until about 10 weeks before visible scald. Differences in development of scald in the four pickings of treated apples were related to the differences in uptake and subsequent loss of diphenylamine. The 1963 apples remained free from scald for over 40 weeks. In 1964, scald was absent from the second picking after 31 weeks but had just appeared in the first picking. The 1965 apples showed a little development of scald in the last 10 weeks. These results are consistent with the view that a diphenylamine concentration of about

TABLE I

Changes in diphenylamine concentration of Granny Smith apples after treatment

Weeks at 1°	Diphenylamine concentration (μg/cm^2)								
	Picked 1 April 1963			Picked 31 March 1964		Picked 27 April 1964		Picked 20 April 1965	
	Peelings	Flesh	Skin	Peelings	Coating	Peelings	Coating	Peelings	Coating
1	3·99	0·09	4·33	2·32	0·87	4·05	2·24	1·73	1·12
2	4·63	0·12	3·20	(1·42)	(0·57)	(2·28)	(1·34)		
3	4·52	0·19	3·35						
4		0·21	2·61						
5	2·91	0·17	2·61						
6	3·36	0·13	2·27			1·60	1·03		
7	2·34	0·16	2·18	0·81	0·30	(0·99)	(0·66)		
8	2·65	0·14	1·91	(0·44)	(0·23)				
9	2·47	0·07	1·53						
10	1·82	0·08	1·93						
11	1·60	0·10	1·68			0·86	0·55	0·32	0·23
12	1·92	0·16	1·48	0·31	0·16	(0·57)	(0·37)		
13	1·76	0·08	1·38	(0·33)	(0·18)				
14	1·19	0·05	1·13						
15	0·93	0·09	1·00						
16	1·08	0·07				0·43	0·26		
17				0·30	0·14	(0·29)	(0·17)		
18				(0·17)	(0·10)				
19									
20	0·89	0·03	0·59						
21	0·60	0·03	0·52			0·27	0·16	0·04	0·02
22	0·51	0·02	0·50	0·22	0·12	(0·21)	(0·14)		
23	0·60	0·01	0·44	(0·16)	(0·10)				
24	0·56	0·02	0·45						
25	0·62	0·04	0·41						
26	0·33	0·04	0·24			0·19	0·12		
27	0·52	0·02	0·33	0·12	0·07	(0·13)	(0·07)		
28	0·43	0·03	0·32	(0·10)	(0·06)				
29	0·26	0·01	0·18						
30	0·17	0·00	0·11						
31						0·12	0·08	0·02	0·01
32				0·10	0·06	(0·08)	(0·05)		
33				(0·06)	(0·03)				

For figures in brackets storage period includes final week at 20°

0·2 μg/cm^2 in the peelings is required to inhibit the reactions leading to scald. The concentration reached this level after about 30 weeks in the 1963 apples, 25 weeks in the second picking of 1964, 20 weeks in the first picking of 1964, and 15 weeks in the 1965 apples.

If it is assumed that the concentration of diphenylamine in the peelings after treatment (1·7–4·5 μg/cm^2) and the minimum effective concentration (0·2 μg/cm^2) are both distributed in the tissues as follows: 60% in the cuticle of thickness 20 μ, 20% in the epidermis of thickness 20 μ, and the remaining 20% predominantly in the hypodermis of thickness 110 μ, the concentrations in these tissues calculated as μg/cm^2 and μg/cm^3 are as given in Table II. Diphenylamine is very soluble in lipid solvents but only sparingly soluble in water (44 ppm at 30°).[13] At equilibrium the concentration of diphenylamine in the lipid phase of the tissue would probably be more than 100 times the concentration in the aqueous phase. It is plausible to assume that the distribution of diphenylamine between cuticle, epidermis, and hypodermis reflects the relative lipid content of these tissues.

TABLE II

Distribution of diphenylamine in outer tissues after treatment and on reaching minimum effective concentration

Tissue	Diphenylamine concentration	
	After treatment	Effective minimum
	μg/cm^2	
Cuticle	1·02–2·7	0·12
Epidermis	0·34–0·9	0·04
Hypodermis	0·34–0·9	0·04
	μg/cm^3	
Cuticle	510–1350	60
Epidermis	170–450	20
Hypodermis	31–82	4

In cell-free systems the concentrations of diphenylamine found to uncouple oxidative phosphorylation[5] and inhibit cytochrome oxidase,[6] succinic oxidase,[5,7] and reduced nicotinamide adenine dinucleotide oxidase[7] were 44, 500, 17–44, and 17 ppm respectively. The concentration given as 500 ppm was that of an aqueous suspension, hence both the aqueous and lipid phases were probably nearly saturated. The concentration in the hypodermis, where scald lesions first appear, was initially in the range for inhibition of respiration and phosphorylation. However, the minimum effective concentration was well below this range, indicating that diphenylamine can still inhibit the scald-inducing reactions when its concentration is less than that required for inhibition of respiration or phosphorylation. This conclusion is consistent with the observation that good control of scald can be obtained with levels of diphenylamine that are insufficient to cause injury. As respiration and phosphorylation are essential metabolic activities, it is inconceivable that they could be inhibited completely without serious injury.

The effect of diphenylamine on the synthesis of carotene by micro-organisms[4] has no relevance to stored apples, in which there is appreciable loss of carotenoids.[8] As an antioxidant for carotene the required concentrations are considerably above those present in treated apple tissues. In a pellet of rice bran mixed with 20% of mineral oil, increased retention of carotene required 5,000 ppm of diphenylamine, which would be concentrated in the oil phase at 25,000 ppm.[3]

It seems unlikely that the control of scald by diphenylamine is exerted primarily through any of the *in vitro* effects so far reported. The effect of diphenylamine on the oxidation of α-farnesene will be discussed in a subsequent paper.

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SUPERFICIAL SCALD, A FUNCTIONAL DISORDER OF STORED APPLES

IV.*—Effect of variety, maturity, oiled wraps and diphenylamine on the concentration of α-farnesene in the fruit

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

During storage of apples at 1° the concentration of α-farnesene in the 'coating' (mainly cuticle) and adjacent cells increased to a maximum and then declined. At the highest maximum farnesene was 15% of the total lipid of the coating. Evidence is presented for a rôle of α-farnesene in superficial scald. More α-farnesene was found in earlier picked apples and more in the scald-liable Granny Smith than in the scald-resistant Crofton variety. During storage α-farnesene moved from the fruit to the oiled wraps until wraps contained more than twice as much as the fruit. Diphenylamine reduced the production of α-farnesene in later picked apples.

Introduction

Earlier papers in this series[1,2] have shown that the natural volatile substance, which may be concerned in superficial scald of apples and which can be partly removed by air movement or oiled wraps,[3,4] is unlikely to be one of the volatile constituents previously identified. For some time attention has been given to the constituent of the natural lipid coating with conjugated diene absorption in the ultra-violet. Davenport (unpublished results) found it in the more volatile vacuum distillates of the unsaponifiable fraction, its lability causing considerable loss on distillation. Huelin (unpublished results) detected it in air which had passed over apples by absorption in purified hexane cooled by solid carbon dioxide. Davenport also found that its concentration in the fruit was affected by treatment with the scald inhibitor diphenylamine. Finally Murray[5,6] isolated and identified this constituent as α-farnesene (2,6,10 trimethyl 2,6,9,11-dodecatetraene). No other hydrocarbon with conjugated unsaturation was detected.

As the preliminary evidence suggested a rôle for α-farnesene in superficial scald, its concentration during storage was determined in apples of two varieties, each picked at two stages of maturity. The scald-liable Granny Smith was compared with the scald-resistant Crofton variety. The effect of oiled wraps and treatment with diphenylamine was also investigated. α-Farnesene is readily determined, as its extinction from 215 to 500 nm gives a single peak at 232 nm with negligible extinction above 260 nm.

Experimental

In 1964 Granny Smith apples from the Bathurst district of New South Wales were picked on the 31 March and 27 April, and Crofton apples from the Batlow district of N.S.W. were picked on the 9 April and the 1 May. They were separated into comparable samples of 24 apples by systematic distribution, so that each sample contained the same number of apples from each tree. Each sample was stored at 1° in a single layer in an 18 × 12 in. carton, and the samples from each treatment were held in a separate stack.

Lots of 15 samples of Granny Smith apples from each picking were stored unwrapped and untreated, or in oiled wraps, or unwrapped but treated with diphenylamine. Each oiled paper wrap contained about 15% of mineral oil. The diphenylamine-treated apples were dipped before storage in a solution of 0·01 M diphenylamine in 50% (v/v) aqueous ethanol and dried on wire trays. The Crofton samples were unwrapped and untreated.

At intervals of 5 weeks two samples of each treatment were removed from storage at 1°. One sample was analysed on removal and the other sample was kept a further week at 20° before analysis. Duplicate determinations were made once for each treatment during storage. Before analysis each sample was examined for superficial scald. The severity of scald on each apple was rated on an arbitrary scale from 0 to 10 and the mean score was calculated for the sample.[7]

All extractions, dilutions, purifications, and ultra-violet measurements were made at approximately 20°. Extraction of the natural coating for determination of α-farnesene was done by dipping intact apples in purified hexane for two minutes. The first apple of the sample was lowered in a wire frame into a beaker of just adequate size and covered with hexane. After two minutes' immersion the first apple was withdrawn and rinsed with a minimum volume of hexane. The remaining apples were then immersed in turn. The extract in the beaker including the combined rinsings was filtered into a 500 ml volumetric flask and made up to volume. As α-farnesene is both labile and volatile, both heat and evaporation were avoided.

The hexane (a petroleum fraction) was purified for ultra-violet measurements by stirring 7 litres with 400 ml fuming sulphuric acid for 2 h. The stirrer blades of stainless steel were located at the interface to ensure adequate dispersion. The hexane was then separated, washed with water and aqueous sodium carbonate, dried with anhydrous calcium chloride, and distilled. Each batch was tested for transparency between 215 and 360 nm.

The natural 'coating' extract made by dipping intact apples is probably derived mainly from the cuticle. For an extract of the underlying cells the previously dipped apples were peeled and the peelings (about 1 mm thick) were cut into small pieces and well mixed. One quarter by weight of the peelings was blended four times with purified hexane in an Omni Mixer. The extracts were decanted through a filter into a 500 ml volumetric flask and made up to volume.

The wraps from an oil-wrapped sample were cut into small

* Part III: Previous paper

pieces and mixed. One quarter by weight was stirred in a beaker with successive volumes of purified hexane. The extracts were decanted through a filter into a 500 ml volumetric flask and made to volume.

The extinction of each extract, either suitably diluted or purified with Florisil and diluted, was recorded from 215 to 360 nm. One g Florisil of 60–100 mesh was packed dry into a 5 mm i.d. glass tube to form a column 10 cm high. An aliquot (3, 4 or 5 ml) of the original or diluted extract was added to the top of the column, and the eluate was collected in a 10 ml volumetric flask. Further small volumes of purified hexane were added until the volume of the eluate was nearly 10 ml. It was then made up to volume. In recording the extinction, hexane similarly treated with Florisil was used as blank. The untreated and Florisil-treated extracts were compared at the same final dilution.

The extinction curves for Florisil-treated extracts were similar to those for pure α-farnesene solutions, and the peak extinction at 232 nm was used for calculating the concentration of α-farnesene. With pure α-farnesene solutions the molar extinction coefficient at 232 nm was estimated as $2{\cdot}774 \times 10^4$. In tests with pure solutions on Florisil columns α-farnesene was recovered quantitatively in the eluate. Diphenylamine was removed by the Florisil. The results, including those for the wraps, were expressed as μg farnesene per cm^2 of apple surface. The method by which surface area was estimated has been described.[8]

An approximate determination of total lipid was made in the extracts, so that the concentration of α-farnesene could also be expressed as per cent of total lipid. For this determination 100 ml extract were evaporated on a water bath and heated at 100° for 2 h before being weighed.

Results

The effects of variety, maturity, and treatment on the development of superficial scald are shown in Table I. The results agreed with previous experience in that the Granny Smith was more liable to scald than the Crofton variety, the 1st picking was more liable than the 2nd picking, and scald was controlled partly by oil-wrapping and more completely by diphenylamine treatment.

In the 1st picking of untreated Granny Smith apples (Fig. 1(a)), α-farnesene in the coating rose from 0·2 μg/cm^2 at picking to 33 μg/cm^2 after 12 weeks at 1° and then declined. α-Farnesene in the cells reached its maximum earlier. α-Farnesene in the coating and cells combined reached 38 μg/cm^2 after 12 weeks at 1°. After removal from 1° there was considerable loss of α-farnesene during the further week at 20°.

In the 1st picking of oil-wrapped Granny Smith apples (Fig. 1(b)), after 12 weeks at 1° α-farnesene in the coating and cells combined reached a maximum of 26 μg/cm^2, which was only 68% of the maximum for the untreated fruit. With longer storage this percentage was reduced to 55. α-Farnesene in the wraps rose at first more slowly than in the fruit, but after 12 weeks at 1° reached 38 μg/cm^2, which was 1·5 times as much as in an equivalent area of the fruit. During the next 5 weeks α-farnesene continued to rise in the wraps but declined in the fruit. As a result the ratio of α-farnesene in wraps to that in fruit increased to 2·6 after 17 weeks at 1°. The ratios after 22, 27, and 32 weeks were 3·0, 2·3, and 1·9, respectively. This movement of α-farnesene into the oiled wraps until there was three times as much there as in the fruit provides evidence for a rôle of α-farnesene in scald. By contrast only a quarter of the non-volatile cuticle oil and only traces of the volatile acids, alcohols, and carbonyl compounds produced by apples have been found in used oil wraps.[2]

Although the oil wraps reduced the α-farnesene content of wrapped fruit below that of untreated fruit, the total α-farnesene in wraps and fruit was increased by wrapping. After 12 weeks at 1° the total α-farnesene retained by the wrapped fruit and oil wraps was 64 μg/cm^2 compared with 38 μg/cm^2 in the untreated fruit. Similar effects were found after longer storage. Restriction of evaporation by the wraps and increased production could both contribute to this increase. There is also evidence (to be presented later) that removal of α-farnesene can increase its production by the fruit.

The curves of α-farnesene per cm^2 for the 1st picking of diphenylamine-treated Granny Smith apples (Fig. 1c) differed little from the corresponding curves for untreated apples. Evidence was obtained from the ultra-violet extinction curves of the whole extracts (not purified with Florisil) that diphenylamine delayed the oxidation of α-farnesene, and this evidence will be presented in a later paper.

The maximum levels of farnesene reached in the 2nd picking of Granny Smith apples (Fig. 2) were appreciably less than in the 1st picking. The coating and cells of the untreated apples reached a maximum of 38 μg/cm^2 in the 1st picking

TABLE I

Effect of variety, maturity, and treatment on superficial scald

Variety	Date of picking	Treatment	Scald score after storage at 1° for different periods, weeks								
			16–17*	21–22	26–27	31–32	11–12	16–17	21–22	26–27	31–32
			On removal from storage				After a further week at 20°				
Granny Smith	31 March 1964	Untreated	0·04	0·17	1·12	1·71	0·87	2·67	3·79	4·33	4·57
		Oil wrapped	0	0·21	0·58	1·25	0·29	1·21	1·87	2·21	2·50
		Diphenylamine	0	0	0	0	0	0	0·08	0·08	0
	27 April 1964	Untreated	0	0	0	0·12	0	0·08	0·08	0·21	0·46
		Oil wrapped	0	0	0	0	0	0·02	0·04	0	0
		Diphenylamine	0	0	0	0	0	0	0	0	0
Crofton	9 April 1964	Untreated	0	0	0	0	0	0	0	0	0·96
	1 May 1964	Untreated	0	0	0	0	0	0	0	0	0

* The earlier-picked Granny Smith apples were removed from 1° after 1,7,12,17,22,27 and 32 weeks. The others were removed after 1,6,11,16,21,26, and 31 weeks. Removals which were free from scald are not included in the table.

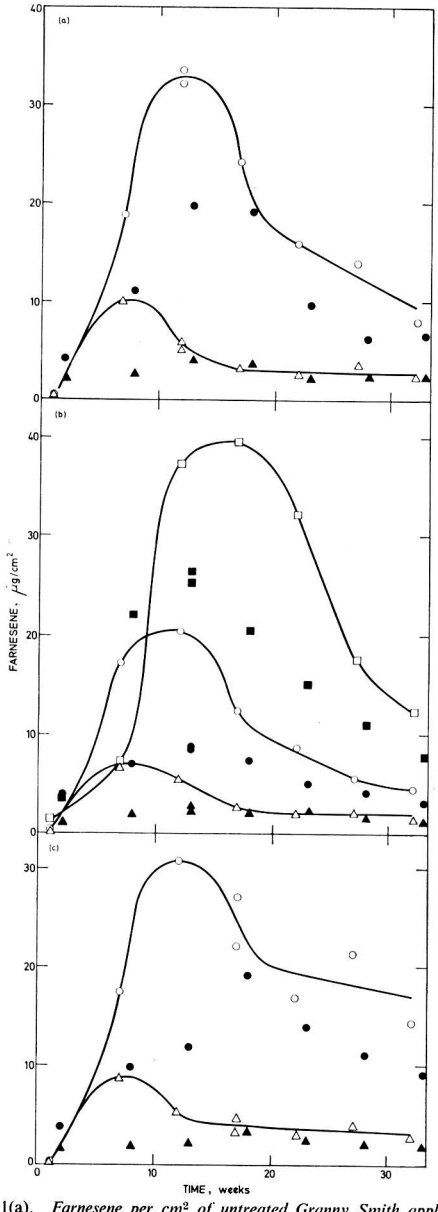

FIG. 1(a). *Farnesene per cm² of untreated Granny Smith apples, 1st pick.*

(b). *Farnesene per cm² of oil-wrapped Granny Smith apples, 1st pick.*

(c). *Farnesene per cm² of diphenylamine-treated Granny Smith apples, 1st pick.*

Farnesene in coating: ○ Storage at 1°; ● storage at 20° for 1 week after storage at 1°.
Farnesene in cells: △ Storage at 1°; ▲ storage at 20° for 1 week after storage at 1°.
Farnesene in wraps: □ Storage at 1°; ■ storage at 20° for 1 week after storage at 1°.

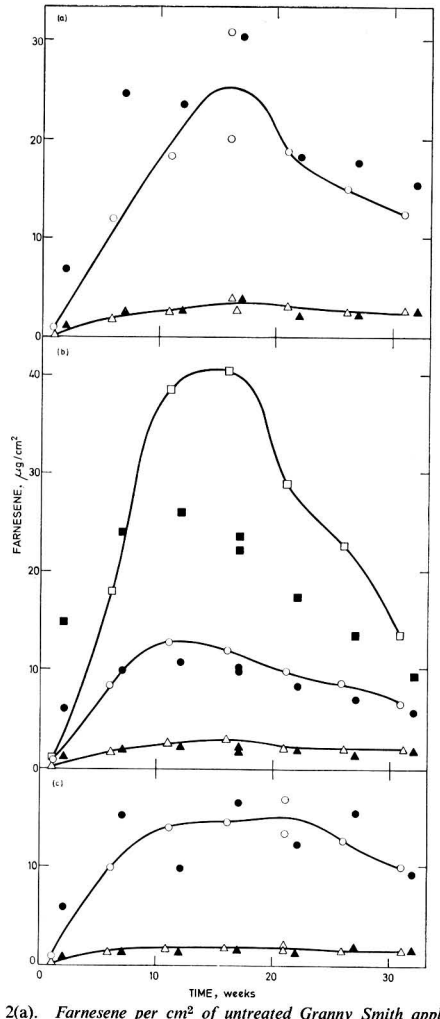

FIG. 2(a). *Farnesene per cm² of untreated Granny Smith apples, 2nd pick.*

(b). *Farnesene per cm² of oil-wrapped Granny Smith apples, 2nd pick.*

(c). *Farnesene per cm² of diphenylamine-treated Granny Smith apples, 2nd pick.*

Farnesene in coating: ○ Storage at 1°; ● storage at 20° for 1 week after storage at 1°.
Farnesene in cells: △ Storage at 1°; ▲ storage at 20° for 1 week after storage at 1°.
Farnesene in wraps: □ Storage at 1°; ■ storage at 20° for 1 week after storage at 1°.

but only 29 μg/cm^2 in the 2nd picking. The corresponding figures for the oil-wrapped apples were 26 and 15 and for the diphenylamine-treated apples 36 and 17 μg/cm.^{2} In the 2nd picking the maximum was reached later than in the 1st picking, and there was not such a marked loss of α-farnesene after removal to 20°. In the 2nd picking oil-wrapping again reduced the level of α-farnesene in the fruit, although it gave greater retention by fruit and wraps combined. The ratio of α-farnesene in wraps to that in fruit reached a maximum of 2·7 after 16 weeks at 1°. In contrast to the 1st picking, diphenylamine treatment of the 2nd picking reduced the production of α-farnesene.

The maximum level of α-farnesene in each picking of Crofton apples (Fig. 3) was less than half that in the equivalent pick of Granny Smith apples. There was a little more α-farnesene in the 1st picking of Crofton apples than in the 2nd picking.

When the concentration of α-farnesene was expressed as per cent of total lipid, the concentration in the coating of the 1st picking of untreated Granny Smith apples (Fig. 4(a)) reached 15% after 12 weeks at 1°. This concentration is about 10 times that expected from earlier ultra-violet measurements of coating fractions, which were extracted and concentrated at the boiling point of the solvent.[8] The high value was only obtained by analysing samples at the peak of α-farnesene concentration and avoiding both heat and evaporation.

The concentration of α-farnesene in the cells passed through its maximum earlier than in the coating. This result is consistent with the production of α-farnesene by the cells and its subsequent transfer to the cuticle. Although the mean concentration in the cells to a depth of 1 mm is less than in the coating, production of α-farnesene may be localised in the epidermis and hypodermis with a total depth of about 130 μm and the concentration in the lipid phase of this tissue may exceed that in the coating. The curves for concentration of α-farnesene in the lipid phases of the 1st picking of oil-wrapped Granny Smith apples (Fig. 4(b)) demonstrate that during storage α-farnesene diffused, as would be expected, from a higher concentration in the 'coating' to a lower concentration

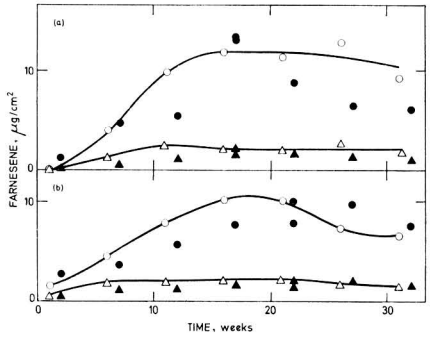

FIG. 3(a). *Farnesene per cm² of untreated Crofton apples, 1st pick.*
(b). *Farnesene per cm² of untreated Crofton apples, 2nd pick.*

Farnesene in coating: ○ Storage at 1°; ● storage at 20° for 1 week after storage at 1°.
Farnesene in cells: △ Storage at 1°; ▲ storage at 20° for 1 week after storage at 1°.
Farnesene in wraps: □ Storage at 1°; ■ storage at 20° for 1 week after storage at 1°.

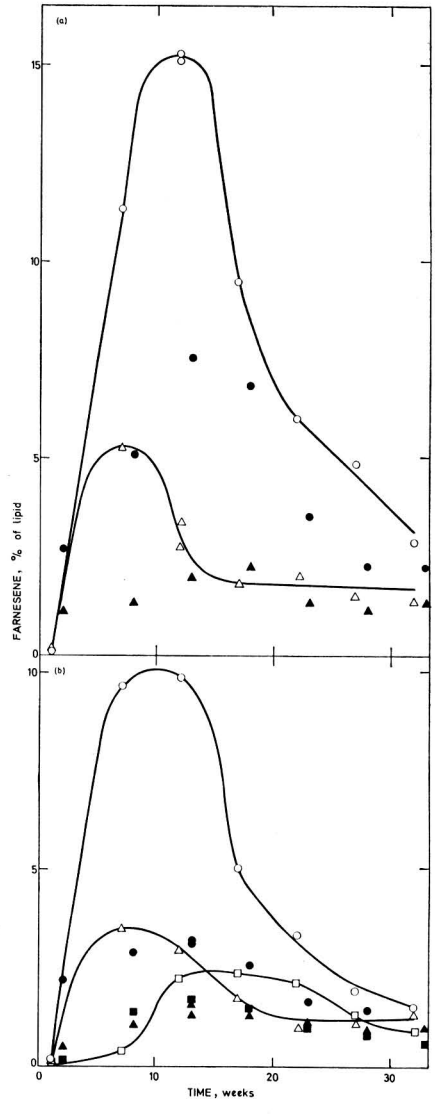

FIG. 4(a). *Farnesene in lipid of untreated Granny Smith apples, 1st pick.*
(b). *Farnesene in lipid of oil-wrapped Granny Smith apples, 1st pick.*

Farnesene in coating: ○ Storage at 1°; ● storage at 20° for 1 week after storage at 1°.
Farnesene in cells: △ Storage at 1°; ▲ storage at 20° for 1 week after storage at 1°.
Farnesene in wraps: □ Storage at 1°; ■ storage at 20° for 1 week after storage at 1°.

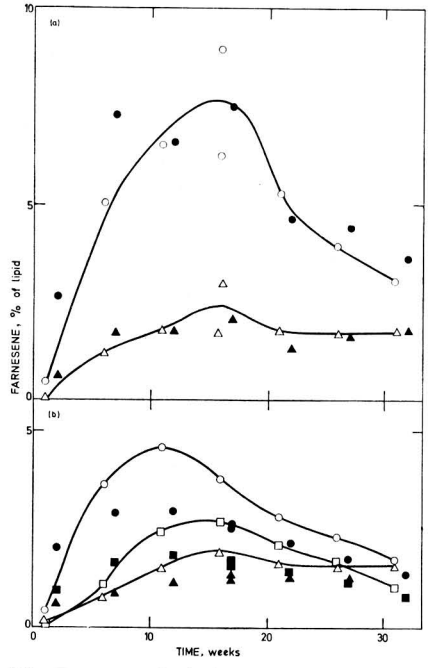

FIG. 5(a). *Farnesene in lipid of untreated Granny Smith apples, 2nd pick.*
(b). *Farnesene in lipid of oil-wrapped Granny Smith apples, 2nd pick.*

Farnesene in coating: ○ Storage at 1°; ● storage at 20° for 1 week after storage at 1°.
Farnesene in cells: △ Storage at 1°; ▲ storage at 20° for 1 week after storage at 1°.
Farnesene in wraps: □ Storage at 1°; ■ storage at 20° for 1 week after storage at 1°.

in the wraps. As previously shown (Fig. 1b) the α-farnesene per cm^2 reached a higher level in the wraps than in the coating, but this difference was reversed for concentration in the lipid phase on account of the much higher lipid content of the wraps. Towards the end of storage the concentration of α-farnesene in the oil of the wraps reached more than half the concentration in the lipid of the coating.

In the 2nd picking of untreated Granny Smith apples the concentration of α-farnesene in the lipid phase of the coating (Fig. 5(a)) only reached about half the maximum concentration for the 1st picking. The 2nd also differed from the 1st picking in that the maximum concentration in the cells was not reached earlier than in the coating. The curves for the 2nd picking of oil-wrapped apples (Fig. 5(b)) show diffusion of α-farnesene from 'coating' to wraps similar to that of the 1st picking.

Discussion

Brooks *et al.*[3,4] obtained considerable reduction of scald by increased air movement and by use of oiled wraps, and postulated that 'apple scald is due to volatile or gaseous substances other than carbon dioxide that are produced in the metabolism of the apple'. Our results have shown that α-farnesene fits such a volatile substance. More α-farnesene was found in the earlier picked apples, which are more liable to scald, and more in the scald-liable Granny Smith than in the scald-resistant Crofton variety. The movement of α-farnesene from the fruit to the oiled wraps provides further evidence. It appears to be absorbed by the wraps to a much greater extent than either the more volatile apple products, which predominate in the air, or the less volatile constituents of the coating. Probably the former are too volatile to be retained by the wraps, and the latter are less volatile and diffusible than α-farnesene.

Although the effects of variety, picking maturity, and oil wraps on the level of α-farnesene in the fruit were similar to the effects on scald, there was no close correlation over the whole range of variation. During storage most of the scald appeared after the α-farnesene maximum and continued to increase while the α-farnesene decreased. It is probable that the production of α-farnesene is only the first stage in the development of scald and that the oxidation products of α-farnesene are the causal agents. Evidence for the inhibition of this oxidation by diphenylamine will be presented later.

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ANALYSIS OF CROPS AND SOILS FOR RESIDUES OF CHLORFENVINPHOS INSECTICIDE AND ITS BREAKDOWN PRODUCTS

By K. I. BEYNON, M. J. EDWARDS, K. ELGAR and A. N. WRIGHT

Field crops treated with chlorfenvinphos insecticide have been analysed for residues of the parent compound and for possible residues of its breakdown products, 1-(2′,4′-dichlorophenyl)ethan-1-ol (**II**), 2,4-dichloroacetophenone (**III**), 2,4-dichlorophenacyl chloride (**VIII**) and conjugates of (**II**).

Residues of (**II**) and (**III**) did not exceed 0·2 ppm in soils within six months of application of chlorfenvinphos at up to 8 lb/ac. Residues of (**VIII**) could not be detected in any of these soils, and the limit of detectability was 0·02–0·04 ppm.

Crops that were grown in soils treated with up to 4 kg/ha (≡ to 3·6 lb/ac) of chlorfenvinphos contained detectable residues of chlorfenvinphos (< 0·01–0·95 ppm) at harvest, and the highest residues were found in radishes. However, residues of the possible breakdown products (**II**), (**III**) and (**VIII**) could not be detected in any of these crops, and the limit of detectability was 0·01–0·05 ppm.

When chlorfenvinphos was applied to the foliage of potatoes there was some evidence for conversion of the *trans* (β) isomer to the *cis* (α) isomer. Both isomers were degraded rapidly and the initial half-life of chlorfenvinphos (*cis* + *trans*) was about 3 days.

Residues of chlorfenvinphos and the breakdown products (**II**, free and conjugated), (**III**) and (**VIII**) could not be detected on potato foliage, potato tubers, or maize grain 13–98 days after foliar application of chlorfenvinphos at up to 1 kg/ha on potatoes and up to 2 kg/ha on maize. The limits of detectability were 0·005–0·05 ppm.

Introduction

The insecticide chlorfenvinphos (Birlane*, diethyl 1-(2′,4′-dichlorophenyl)-2-chlorovinyl phosphate, previously known as SD 7859 and GC 4072) is effective as a soil insecticide, particularly against root flies. It also shows promise as a foliar insecticide for the control of Colorado beetle on potatoes and for seed dressing.

For soil use the recommended dosage level of chlorfenvinphos is 2–6 kg/ha and for foliar use on potato the recommended dosage level is 0·25 kg/ha. The technical material contains about 85% *trans* (β)-chlorfenvinphos and 10% *cis* (α)-chlorfenvinphos.

Procedures have been described for the analysis of soils,[1] crops,[1,2] animal tissues[3,4] and milk[3] for residues of chlorfenvinphos, and results have been reported for residues of the parent compound in soils,[5] in crops grown in treated soils,[5] and in the tissues of sheep[4] and cows[6] that had been sprayed with chlorfenvinphos. Some results have also been reported on possible residues of 2,4-dichlorophenacyl chloride (**VIII**) the *in vitro* hydrolysis product of chlorfenvinphos in crops,[5] soils[5] and in the tissues of sheep[4] and cows.[6]

Studies of the breakdown of ^{14}C-chlorfenvinphos have been reported[7,8] for soils, for crops grown in treated soils, and for crops which had received foliar applications of the pesticide. Soils of different types were treated with 15 ppm ^{14}C-chlorfenvinphos and after four months storage in closed containers the radioactively labelled compounds shown in Table I were detected[7] in the moist soils.

When ^{14}C-chlorfenvinphos was applied directly to the foliage of crops[8] the breakdown was simpler than in soils. There was evidence for some conversion of the *trans* (β) isomer of chlorfenvinphos to the *cis* (α) isomer. The major breakdown product was hydrophilic and was mainly a conjugate or conjugates of (**II**) with traces of desethyl chlorfenvinphos (**IV**).

The breakdown of chlorfenvinphos in soils and plants is a de-toxification process. The breakdown products are not active insecticides and they are much less toxic to animals than is the parent compound. In the present work, however, the extent to which the breakdown products can be formed in crops and soils under field conditions has been investigated. Methods have been developed for the analysis of residues of the breakdown products (**II**, free and conjugated), (**III**) and (**VIII**) and these methods have been applied to field samples. Residue analysis studies were not carried out for desethyl chlorfenvinphos (**IV**) since this is a minor breakdown product and has a low acute oral toxicity to rats, nor was residue analysis carried out for the breakdown products (**V**) and (**VII**).

Results for studies of residues of the parent chlorfenvinphos after its direct application to foliage are included here.

Experimental

Sampling

Crop samples from field trials in the U.K. were extracted within three hours of sampling. Crop samples from Continental trials were received within five days of sampling. These and the soil samples from U.K. trials were stored at −20° for up to four weeks whilst awaiting analysis, the parent chlorfenvinphos and the breakdown products having been shown to be stable in crops and soils under these storage conditions.

Extraction

Soils

(*Analysis for* (**II**), (**III**), (**VIII**) *and the parent chlorfenvinphos*)

Soil samples were extracted with 20 vol.-% acetone in hexane in the presence of sodium sulphate and filtered and the extracts were washed with water to remove the acetone as

* Shell Registered Trade Mark

TABLE I

Component		Range of residues (ppm) in different soils*	LD_{50} acute oral to rats (mg/kg)
Unchanged chlorfenvinphos	(I)	1·0–4·7	*trans* (β) 20–30 *cis* (α)† 33
1-(2′,4′-Dichlorophenyl)ethan-1-ol	(II)	0·06–1·0	>800
2,4-Dichloroacetophenone	(III)	0·1–0·5	>800
Desethyl chlorfenvinphos	(IV)	0·1–0·2	>1000 (as Na salt)
(2′,4′-Dichlorophenyl)ethan-1,2-diol	(V)	<0·03	—
Salts or conjugates of (IV)	(VI)	0·05–0·6	—
2,4-Dichlorophenyl oxirane	(VII)	<0·005	—
2,4-Dichlorophenacyl chloride	(VIII)	<0·005	1450

* As ppm of chlorfenvinphos equivalent to the amount of the component detected
† 86% *cis*, 14% *trans*

described previously[1] for the extraction of chlorfenvinphos alone from soils.

The efficiency of this extraction procedure for the breakdown products was established by preliminary experiments in which a sample of loam was taken from the field at four weeks after treatment with chlorfenvinphos at 4 lb/ac. Separate samples of the soil were extracted with hexane, hexane containing 10, 20 and 30 vol.-% acetone and with acetone as described above. The extracts were analysed by gas–liquid chromatography using the conditions described in a following section. The compound **VIII** was not present in any of the extracts. The amounts of **(II)** (0·70 ppm) and **(III)** (0·17 ppm) extracted by the 20% and 30% acetone–hexane and by acetone were similar and were greater than the amounts (0·5 ppm and 0·12 ppm of the respective compound) extracted by the hexane and 10% acetone–hexane.

Crops

(*Analysis for* **(II)**, **(III)**, **(VIII)** *and the parent chlorfenvinphos*).—Crops were macerated with 30 vol.-% acetone in hexane in the presence of sodium sulphate followed by filtration and washing to remove the acetone as described previously[1] for the extraction of chlorfenvinphos alone from crops.

Conjugates of **(II)**.—A representative portion of the diced or chopped crop (100 g) was macerated for two minutes with ethanol (200 ml). For dry crops such as cereals the crushed crop was extracted by end-over-end tumbling for two hours instead of by maceration. The mixture was filtered, and the residuum was washed with further ethanol until the volume of the filtrate was 350 ml. An aliquot of the filtrate (35 ml ≡ 10 g crop) was concentrated by rotary evaporation at 50–60° using a vacuum of 20 mm Hg until no more ethanol evaporated and about 1 ml of an aqueous solution remained. Aqueous sulphuric acid (10 ml 19 vol.-%) was added and the mixture was heated under an air condenser on a water bath at 95–100° for two hours to hydrolyse the conjugate to free **(II)**. The contents of the flask were allowed to cool and were filtered through a small glass-wool plug into a separating funnel. The condenser, flask, filter funnel and glass-wool plug were washed in that sequence with 4 ml water (twice), 2·5 ml acetone (twice) and 5 ml of hexane, adding all the washings to the separating funnel. A dark precipitate formed on hydrolysis and was considered to occlude some of **(II)** resulting in low recovery values. The precipitate was retained on the glass-wool plug and was subsequently dissolved in the acetone. Although the precipitate was re-formed in the separating funnel it no longer contained the occluded **(II)**.

A further 20 ml hexane were added to the separating funnel and the mixture was shaken for one minute. The organic phase was separated, dried over sodium sulphate and concentrated under an air manifold to 5 ml (≡ 10 g of crop). This extract contained **(II)** formed on the hydrolysis of the conjugate together with any that was originally free in the crop.

The procedure for the extraction and hydrolysis of the conjugates of **(II)** was established using ^{14}C-labelled material in the following way. The leaves of six growing cabbage plants (total weight 240 g above ground) were treated with an acetone solution of ^{14}C-chlorfenvinphos[7,8] (2·4 mg total). After seven days the leaves of the plants were cut off, chopped up finely and mixed to give a single gross sample.

Analysis of the leaves by the methods described previously[8] indicated the following cencentrations of ^{14}C-compounds:

Cis (α)- and *trans* (α)-chlorfenvinphos	5·75 ppm
Conjugates of **(II)**	
acetone-extractable components	1·85 ppm
components not extractable in acetone	0·45 ppm

Separate samples of the leaves were extracted with water, water–acetone mixtures (up to 75 vol.-% acetone), and ethanol. The water and water–acetone mixtures extracted 40–60% of the material that was considered to be conjugate. Absolute ethanol extracted more than 80% of the material and was therefore chosen as the extraction solvent for subsequent work.

Separate samples of the ethanol extract were rotary-evaporated and the watery residue was heated at about 95° with sulphuric acid for 2–20 hours. The percentage of the conjugate (60–65%) that was hydrolysed to **(II)** was similar when the acid concentration in the hydrolysis mixture was 8·5 vol.-% H_2SO_4 or 17 vol.-% H_2SO_4 with reaction times of 2 to 7 hours. Longer reaction times resulted in a decreased yield of **(II)**.

Untreated crops and soils were extracted and processed in the same way as treated samples and recovery values were determined by adding known amounts of the compounds **(II)**, **(III)** and **(VIII)** to untreated crops and soils at the extraction stage. Recovery values of the conjugates of **(II)** could not be established for field crops since the conjugated material was not available. In these cases recovery values were determined by adding **(II)** itself to the ethanol extract before the hydrolysis.

Clean-up

Procedures for the clean-up of extracts prior to analysis for chlorfenvinphos have been described.[1]

The following procedure was used when necessary for clean-up prior to analysis for the breakdown products (**II**), (**III**) and (**VIII**).

An aliquot of the extract (1 ml) was added to a column (0·8 cm diameter) containing 5 vol.-% water on alkaline alumina (2 g Type H. Laporte) in hexane. Fractions were collected at the rate of 1 drop a second, and the column was eluted further with 15 vol.-% ether in hexane. The first 2 ml of eluate was rejected, (**III**) and (**VIII**) emerged in the next 3 ml. The next 2 ml of eluate was rejected and (**II**) emerged in the next 7 ml of eluate. It is recommended that alumina from different batches or sources be calibrated before use to determine the elution pattern of the different compounds. The fractions were concentrated to 1 ml on an air manifold prior to the final analysis.

Analysis of extracts by gas–liquid chromatography (g.l.c.)

The conditions used for the gas–liquid chromatography of the extracts for determination of the parent compound and the breakdown products are summarised in Table II. Alternative column packings for the analysis of chlorfenvinphos have been described previously.[1]

Results

Blank values and recoveries

The blank values obtained for the analysis of untreated crops and soils are summarised in Tables III and IV together with recovery values. Data are not given for the parent

TABLE II

Operating conditions for g.l.c. for chlorfenvinphos and the breakdown products

Compound	g.l.c. conditions using electron capture detection					
	A 4 ft × 0·125 in Copper or Kunifer 30 column packed with 2 wt.-% cyclohexane dimethanol succinate on 100/120 mesh Gas Chrom Q. 188°c with nitrogen as carrier gas at 150 ml/min		B 2 ft × 0·095 in Copper or Kunifer 30 column packed with 3 wt.-% phenyl-diethanolamine succinate on 100/120 mesh Gas Chrom Q. 112°c with nitrogen as carrier gas at 150 ml/min		C 2 ft × 0·095 in Copper or Kunifer 30 column packed with 3 wt.-% phenyl-diethanolamine succinate on 100/120 mesh Gas Chrom Q. 125°c with nitrogen as carrier gas at 150 ml/min	
	Retention time min	Limit of detectability* ng	Retention time min	Limit of detectability* ng	Retention time min	Limit of detectability* ng
Trans-chlorfenvinphos	7	0·05	>30	—	~30	—
Cis-chlorfenvinphos	6	0·05	>30	—	~30	—
(**II**)	<1	—	5	0·5	2·5	0·5
(**III**)	<1	—	2	0·04	1	0·04
(**VIII**)	<1	—	12	0·1	6	0·1

* Response equal to four times the maximum 'noise' level for standard solutions of the compounds

TABLE III

Blank values and recovery values for the analysis of crops and soils for the breakdown products (II), (III) and (VIII)

Sample	(II)		(III)		(VIII)	
	Blank value with untreated sample	Mean % recovery of compound (ppm) added to untreated sample	Blank value with untreated sample	Mean % recovery of compound (ppm) added to untreated sample	Blank value with untreated sample	Mean % recovery of compound (ppm) added to untreated sample
Medium or heavy loam	<0·10	100 (0·50)	<0·01	98–100 (0·50)	<0·01	96–100 (0·50)
Peat	<0·10	80 (0·50)	<0·01	98 (0·50)	<0·01	92 (0·50)
Potato foliage	<0·10	72 (0·40)	<0·005–<0·01	91 (0·10)	<0·02	84 (0·10)
Potato tubers	<0·10	74 (0·20–0·40)	<0·005–<0·01	86 (0·050–0·10)	<0·02	80 (0·050–0·10)
Maize	<0·10	70 (0·20)	<0·005–<0·01	86 (0·050)	<0·02	74 (0·050)
Carrots	—	—	—	—	<0·01	65 (0·10–0·20)
Onions	<0·05	71 (0·10)	<0·01	90 (0·05)	<0·01	73 (0·05)
Leeks	<0·05	61 (0·10)	<0·01	90 (0·05)	<0·01	77 (0·05)
Radishes	<0·05	82 (0·04–0·10)	<0·01	74 (0·04–0·05)	<0·01	72 (0·05)
Celery	<0·05	50 (0·30)	<0·01	70 (0·02)	<0·01	80 (0·10)

compound chlorfenvinphos since these have been given previously.[1,5]

The compounds (**II**), (**III**) and (**VIII**) could not be detected in the acetone–hexane extracts of untreated soils or crops when chlorfenvinphos (up to 0·5 ppm) was added to the samples immediately before extraction. No (**II**) could be detected in the extracts of untreated crops which had been extracted with ethanol and hydrolysed with sulphuric acid with the addition of up to 0·5 ppm of chlorfenvinphos to the sample immediately before extraction. In such cases, (**VIII**) formed by the hydrolysis of the chlorfenvinphos could, of course, be detected in the final extract.

Analysis of crops and soils from field trials

The results of analysis of soils that had been treated with chlorfenvinphos and crops that had been grown in the treated soils are summarised in Tables V and VI. The analyses of crops that had received foliar applications of chlorfenvinphos are summarised in Table VII. The results in Tables V–VII are not corrected for the percentage recovery.

Discussion

Analytical procedures

The procedures that have been used previously[1] for the extraction of chlorfenvinphos from soils and crops have been applied successfully to the extraction of non-conjugated breakdown products (**II**, **III** and **VIII**). Blank values and recovery values were satisfactory (Table III).

The procedures for the analysis of conjugates of (**II**) were established using cabbages that had been treated with ^{14}C-chlorfenvinphos. The blank values (Table IV) are satisfactory and the recovery values based on the results of the experiments with ^{14}C-labelled materials and the results in Table IV are acceptable for a complex material such as the conjugate.

TABLE IV

Blank values and recovery values for the analysis of crops for conjugates of (II)

Sample	Blank value with untreated sample as equivalent ppm of (**II**)	Mean % recovery of (**II**) added (ppm) to untreated sample
Potato foliage	<0·05	61 (0·20–0·50)
Potato tubers	<0·05	60 (0·50)
Maize	<0·05	68 (0·20–0·50)

Residues in field samples

Soil treatment

There was no evidence for the conversion of the *trans* isomer of chlorfenvinphos to the *cis* isomer in the soils in the present work (Table V) in agreement with previous studies.[5]

(**VIII**) could not be detected in any of the soils within six months of application at 4–6 lb/ac nor within eight weeks of application at 20 lb/ac. In previous studies,[5] however, residues of (**VIII**) were detected in soils, and the maximum concentration was 0·10 ppm in soil at 15 weeks after application of chlorfenvinphos granules at 8 lb/ac.

Residues of (**III**) were detected in soils after application of chlorfenvinphos, and the highest concentration was 0·20 ppm in soil at one month after application of chlorfenvinphos at 8 lb/ac.

Free (**II**) could not be detected in soils within six months of application at 4–8 lb/ac but the limit of detectability was 0·2 ppm. In other experiments using an exceptionally high, and commercially unrealistic dosage level (20 lb/ac), residues of 0·60 ppm of free (**II**) were detected at four and eight weeks after application.

It appears that using dosage levels corresponding to commercial agricultural practice (4–6 lb/ac) residues of (**II**) and (**III**) in the soil are unlikely to exceed 0·2 ppm and the maximum concentrations of (**VIII**) will be lower than this value.

TABLE V

Analysis of soils for residues of chlorfenvinphos and its breakdown products after soil application of chlorfenvinphos

Soil sample	Location of trial	Application	Dosage level lb a.i./ac	Time interval between application and sampling (months)	Residues (ppm) in dry soil**				
					Chlorfenvinphos		(**II**)	(**III**)	(**VIII**)
					trans	*cis*			
Sandy loam	Thursley, Surrey	50% emulsifiable concentrate (e.c.) (incorporated)	8	2	2·1	—*	—	0·15	<0·02
Loam over clay	Alton, Hants	50% e.c. (incorporated)	4	2	2·2	—*	—	0·05	<0·02
Sandy loam	Ampthill, Beds	50% e.c. (incorporated)	6	2	1·7	—*	<0·2	0·02	<0·02
				3	1·7	—*	<0·2	0·02	<0·04
				6	1·2	—*	<0·2	0·03	<0·02
		10% gran. (incorporated)	4	2	1·6	—*	—	—	—
				3	2·0	—*	<0·2	0·02	<0·04
				6	2·3	—*	<0·2	0·03	<0·02
Silt	Holbeach, Lincolnshire	50% e.c. (incorporated)	6	1	2·2	—*	<0·2	0·20	<0·20
				6	0·92	—*	<0·2	0·05	<0·02
		10% gran. (incorporated)	4	6	0·81	—*	<0·2	0·05	<0·02
Clay loam	Bethersden, Kent	25% e.c. in March, 1967 (not incorporated)	20	0	9·0	1·2	—	—	—
				1	4·7	0·63	0·60	0·08	<0·01
				2	2·6	0·28	0·60	0·10	<0·01

* Present in approximately the same ratio to β as in the technical material applied (85% β) (10% α)

** To 0–4 in. depth apart from Bethersden soil which was sampled to 6 in.

Residues of free **(II)**, **(III)** and **(VIII)** could not be detected in crops that were grown in soil that had been treated with 2–4 kg/ha of chlorfenvinphos (Table VI). Residues of the parent compound were present, however, in some crops and the highest residues (1·0–3·4 ppm) were detected in carrots when two applications were made, the second foliar treatment being 3 months after sowing. Such a second application is not recommended commercially. Residues did not exceed 0·35 ppm in carrots which were grown in soil treated once with chlorfenvinphos in accordance with the procedures that are recommended for commercial use.

Foliar application

On the foliage of potatoes (Table VII) the ratio of *cis* isomer of chlorfenvinphos to *trans* isomer increased with time from 0·24 : 1 soon after application to 0·8 : 1 at 24 days after application. Whilst this increase could be due to a difference in the rate of breakdown of the isomers, the *cis* isomer is considered unlikely to be more persistent than the *trans* isomer. During the latter stages of this trial the *cis* and *trans* isomers disappeared at similar rates so that whilst the evidence is not conclusive, the increase in *cis*/*trans* isomer

TABLE VI

Analysis of crops for residues of chlorfenvinphos and its breakdown products after soil application of chlorfenvinphos

Sample	Location of trial	Application	Dosage level kg a.i./ha	Time interval between final application and sampling	Residues (ppm) in crop				
					Chlorfenvinphos		(II)	(III)	(VIII)
					trans	*cis*			
Carrots (root)	Holland (1965)	25% wettable powder (w.p.) applied immediately before sowing	3	3 months	0·03–0·07	<0·01	—	<0·01	<0·01
			4		<0·01–0·13	<0·01	—	<0·01	<0·01
Carrots (root)	Holland (1965/66) on sandy soil	10% gran. applied 3 days before sowing. Single application April 1965	3	11 months	0·20	<0·01	<0·05	<0·01	<0·02
			4		0·35	<0·02	<0·05	<0·01	<0·02
		Application in April 1965 at sowing and to plants on July 1965*	3	7 months	1·0	0·10	<0·05	<0·01	<0·02
			4		1·0	0·10	<0·05	<0·01	<0·02
		25% w.p. applied 3 days before sowing. Single application April 1965	3	11 months	0·20	<0·02	<0·05	<0·01	<0·02
			4		0·10	0·02	<0·05	<0·01	<0·02
		Application in April 1965 at sowing and to plants on July 1965*	3	7 months	3·4	0·40	<0·05	<0·01	<0·02
			4		1·8	0·20	<0·05	<0·01	<0·02
Onions (outer layers removed)	Germany (1965)	10% gran. applied 1 week after sowing	3	4 months	<0·01	<0·01	<0·05	<0·01	<0·01
Leeks (tops of leaves and root removed)	Germany (1965)	10% gran. applied 3 months after sowing	3	5 months	<0·01	<0·01	<0·05	<0·01	<0·01
Radishes (root)	Germany (1966)	10% gran. (August 1966) applied at sowing	2	5 weeks	0·04	—	<0·05	<0·01	<0·01
			3		0·05	—	<0·05	<0·01	<0·01
Celery (var. Late Pink)	UK (Lancs 1966)	10% gran. applied at planting out	2	16 weeks	0·02	—	<0·05	<0·01	<0·01
(var. Lathom)			2	16 weeks	0·20	—	<0·05	<0·01	<0·01

* The use of two applications of chlorfenvinphos in this way is not recommended commercially

TABLE VII

Analysis of crops for residues of chlorfenvinphos and its breakdown products after foliar application of chlorfenvinphos

Sample	Location of trial	Application	Dosage level kg a.i./ha	Time interval between final application and sampling, days	Residues, ppm					
					Chlorfenvinphos		(II)	(III)	(VIII)	Conjugates of (II)*
					trans	*cis*				
Potato foliage	Kent 1966	50% e.c. to foliage (once)	0·25	0	5·9	1·4	<0·10	<0·005	<0·01	—
				6	1·1	0·59	<0·10	<0·005	<0·01	—
				13	0·35	0·26	<0·10	<0·005	<0·01	—
				24	0·09	0·07	<0·10	<0·005	<0·01	—
				65 (Harvest)	<0·01	<0·02	<0·10	<0·005	<0·01	—
Potato tubers	Kent 1966	50% e.c. to foliage (once)	0·25	65 (Harvest)	<0·01	<0·01	<0·10	<0·01	<0·02	<0·05
Potato tubers	Spain 1966	50% e.c. to foliage (once)	0·25	13 (Harvest)	<0·01	<0·01	<0·10	<0·005	<0·01	—
Potato tubers	Spain 1965	50% e.c. to foliage (once)	1	28 (Harvest)	<0·01	<0·01	<0·10	<0·01	<0·02	—
Maize grain	France 1966	50% e.c. to foliage (twice with 7 day interval)	0·60	45 (Harvest)	<0·01	<0·01	<0·10	<0·01	<0·02	<0·05
Maize grain	France 1965	50% e.c. to foliage (once)	1	98 (Harvest)	<0·01	<0·01	<0·10	<0·01	<0·02	—

* As ppm of **(II)**

ratio is likely to be due to conversion of some of the *trans* isomer to the *cis* isomer on the foliage.

On the potato foliage the initial half-life of chlorfenvinphos (*cis* and *trans* combined) was 3 days and residues of the parent compound could not be detected on the foliage at harvest at 65 days after treatment.

Residues of chlorfenvinphos and its breakdown products could not be detected on potato tubers or maize 13–65 days after application of chlorfenvinphos at 0·25–2 kg/ha. The limits of detectability were in the range 0·005–0·10 ppm.

Under the present field conditions the crops and soils contained lower concentrations of the breakdown products than were found in the previous laboratory studies. This was expected for the parent compound and the breakdown products are more persistent under the laboratory conditions since the crops and soils are not exposed to wind and rain as in the field.

Conclusions

Under field conditions chlorfenvinphos can break down in soils to form **(II)** and **(III)** as was shown previously in laboratory studies.[7] However, using dosage levels of chlorfenvinphos close to those expected to be used in commercial agricultural practice (4–8 lb/ac) residues of **(II)** and **(III)** in the soil did not exceed 0·2 ppm within six months of application. Crops grown in soils that had been treated once with up to 4 kg/ha of chlorfenvinphos contained residues of up to 0·35 ppm of chlorfenvinphos but did not contain detectable residues of **(II)**, **(III)** or **(VIII)**.

Breakdown products could not be detected on crops in the field that had received foliar applications of chlorfenvinphos, and the major loss of the pesticide was probably by volatilisation either of the parent or volatile breakdown products.

At present the main outlet for foliar uses of chlorfenvinphos is for the control of Colorado beetle on potatoes. The present results suggest that neither residues of chlorfenvinphos nor its breakdown products in potato tubers are likely to present a toxic hazard to humans or animals that eat the crop.

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PROTEIN QUALITY ASSESSMENT BY THE USE OF WEANLING RATS

1.—Some factors affecting growth measurements

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Interrelationships amongst the factors—protein source, breed group, sex and level of food intake, have been investigated with weanling rats housed in cages with solid or screen floors for a three-week period during which they were fed diets limiting in protein content. The responses were measured in terms of weight gain, FCE (food conversion efficiency), apparent NPU (net protein utilisation) and carcass fatness.

The patterns of responses as measured by bodyweight gain, FCE and app. NPU (carcass) were similar but not identical. Higher values for bodyweight gain, FCE and app. NPU (carcass) were associated with greater carcass fat content. The best diet (that containing Danish herring) led to the most carcass fat and the F_1 crossbreds were the fattest breed group.

Rats on solid floors consumed less food *ad lib.* It is suggested that this may result from more coprophagy in solid floor cages or greater thermal insulation from the bedding.

The evidence indicated that the Norwegian Hooded (N.H.) rats possessed a relatively lower dietary requirement for lysine and methionine than either of the other two breed groups, so that phenylalanine, histidine, leucine and isoleucine may have limited their growth, whilst lysine and methionine were the most limiting amino acids in the three diets for the Albino and F_1 crossbred groups. A valid biological evaluation of proteins for farm livestock, therefore, requires an accurate knowledge of the relative amino acid requirements of the rat strains used. The F_1 crossbred groups gave higher app. NPU (carcass) values than the other two groups and showed slightly greater sensitivity to dietary differences as measured by FCE and app. NPU (carcass). The omission of any adjustment for bodyweight maintenance partly accounted for these higher values.

The interactions of level of food intake and breed with diet as measured by FCE throw doubt on the reproducibility and validity of protein efficiency ratio values. Covariance adjustment of response for variations in *ad lib.* food consumption was inappropriate as the regression may have been influenced by other factors, such as vigour and maintenance requirement of the rat. The use of two dietary protein levels for each test protein allowing an adjustment for bodyweight maintenance, would have overcome only some of the defects mentioned.

Introduction

The purpose of this study was to compare the feeding value for weanling rats of three protein sources as measured by the response criteria of weight gain, bodyweight gain, g/g food consumed and carcass N gain, g/g N fed. A comparison was made of these responses by both sexes in two inbred strains and their F_1 cross, with two cage types in which the diets were given *ad lib.*, or restricted.

It was considered also that responses should be judged on the basis of precision, and sensitivity to small differences in nutritive worth, and they must be readily repeatable and unaffected by changes in food intake levels.

Bender[1] showed that protein efficiency ratio, PER (gain in bodyweight in g/g protein eaten) is highly correlated with food intake because greater intakes lessen the proportion of nitrogen used for maintenance. An allowance for maintenance was made by Bender & Doell,[2] who proposed a modification of the PER method in which the weight lost by rats fed a protein-free diet was added to that of rats fed the test diet. This innovation led to an improvement in the correlation of the results with those of net protein utilisation (NPU). No allowance has been made for maintenance in the food conversion efficiency (FCE) and NPU values to be presented in this paper, and furthermore the alimentary tract was discarded before carcass estimations were carried out. For these reasons the term 'apparent NPU (carcass)' has been used to describe the efficiency measurement carcass N gain as a fraction of N fed.

All three diets were compounded to a level of 9% crude protein in accordance with the finding[3,4] that such a protein level should maximise differences between protein sources of practical significance. Furthermore, the value of the three sources was measured in terms of their supplementary value to barley protein in proportions such that the ratio of the two proteins in each diet was similar to that in a high-protein food compounded for farm livestock.

Experimental

Animals

The rats were weaned at 21 days of age from Norwegian Hooded (N.H.) and Albino (Alb) colonies. The former were brother–sister mated for four and six generations and the latter for six and eight generations. F_1 crossbreds (Xbreds) were derived from the parents of the inbreds. Prior to brother–sister mating the Alb and N.H. colonies had been bred fairly closely.

Diets

The composition of the basal soya diet is given in Table I. In the other two diets two herring meals replaced the soyabean meal; each supplying 5% crude protein and the three diets were equalised as regards crude fat, calcium, phosphorus and

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crude fibre, by adjusting the contents of maize starch, maize oil and arachis oil, filter paper and dicalcium phosphate.

The barley meal supplied approximately 4% crude protein, which had previously been found to be adequate for body-weight maintenance in weanling rats. The chemical composition of the diets is given in Table II.

Sources of protein

The proteins examined in detail were a Canadian extracted soyabean meal, and a Danish and an Icelandic herring meal. English barley meal was common to all three diets. The chemical composition of these ingredients is given in Table III. Together with the mixed diets, they were stored deep-frozen until required.

TABLE I

Percentage composition of the basal soya diet

Barley meal	41·49
Sucrose	39·40
Extracted soya bean meal	10·76
Salt	0·50
Trace minerals and vitamins*	0·12
Maize oil	3·60
Arachis oil	0·60
Filter paper	0·26
Dicalcium phosphate	2·80
Limestone	0·09

* The following trace minerals and vitamins were added (mg/kg diet):

Cobalt	0·5
Iron	40
Copper	10
Manganese	10
Iodine	0·25
Zinc	64
Riboflavine	20
Nicotinic acid	44
Pantothenic acid	28
Folic acid	1·3
Vitamin K	2·6
Vitamin E	30
Thiamine	6
Ascorbic acid	50
Pyridoxine	30
Vitamin B_{12}	0·035
Vitamin A IU/kg	28,500
Vitamin D_3 IU/kg	4,400

Amino acid composition of diets

The amino acid analysis of the proteins was carried out by Spillers Ltd. Central Laboratory, Station Road, Cambridge. The proteins were hydrolysed according to the method of Schram, Dustin, Moore & Bigwood,[5] and separation of the amino acids was carried out on a Technicon amino acid analyser.

Methionine and cystine were determined on a duplicate hydrolysate prepared from part of the sample that had been oxidised to methionine sulphone and cysteic acid. Tryptophan was determined by a colorimetric method after baryta hydrolysis (E. L. Miller personal communication).

Available lysine was determined according to the method of Bruno & Carpenter.[6] In the case of extracted soyabean meal a recovery factor of 80% was used. Available methionine was determined using *Streptococcus zymogenes*.[7]

The essential amino acid indices[8] of the three diets were calculated as the geometric mean of the percentage ratios of nine dietary essential amino acids (excluding tryptophan) relative to their content in whole egg protein.

Management

The rats were fed a commercial autoclaved Laboratory Small Animal Diet (Spillers Ltd.) until they were required at 21–25 days of age. Food was then removed and the rats were weighed approximately 24 h later and caged singly in Boots' boxes, 6 × 13 × 4½ in high, constructed of galvanised sheet steel with wire lids or in wire-screen floored Middle Aston cages (E. K. Bowman Ltd., 32 & 42, Holmes Road, London, N.W.). Wood shavings were used as bedding only in the Boots' boxes.

From the time of being weighed the rats were fed either *ad lib.* or restricted to 7 g/rat each morning during the first week, 8 g during the second week and 10 g during the third. Water was available *ad lib.* to rats on both regimes.

The rats under restriction received their last meal 25 h before slaughter; any remaining food offered to the *ad lib.* rats was removed from their cages 18 h before slaughter. Waste food from each cage was collected weekly and weighed at the time the rats were weighed. The room temperature was thermostatically controlled within the range of 21–24°.

TABLE II

Chemical composition of diets (%)

		Danish herring	Soya	Icelandic herring
13 October 1964	Moisture	7·50	8·30	7·70
	Protein (N × 6·25)	9·10	9·00	8·95
	Ether extract	5·25	5·05	5·15
	Crude fibre	2·20	2·35	2·05
	Ash	3·85	4·40	4·90
	Calcium	0·90	1·05	0·85
	Phosphorus	0·75	0·90	0·75
	Iodine value of oil	126·50	111·50	114·00
4 March 1965	Moisture	7·30	7·90	7·20
	Protein (N × 6·25)	8·90	8·80	8·95
	Ether extract	4·95	4·70	4·95
	Crude fibre	1·95	2·05	1·90
	Total lysine	0·66	0·49	0·55

TABLE III

Composition of ingredients (percentage of sample unless otherwise stated)

	Barley meal	Danish herring meal	Canadian extracted soyabean	Icelandic herring meal
Moisture	15·3	5·1	11·0	8·7
Protein (N × 6·25) (average of three determinations)	9·45	69·20	46·20	70·90
Ether extract	1·80	11·90	1·15	9·40
Free fatty acid (as % of oleic acid in oil)	35·1	10·5	14·2	14·7
Peroxide value (m-moles peroxide/g fat)	22·0	16·5	46·5	16·4
Iodine value of oil	122	160	135	130
Ash	2·20	12·35	5·10	9·65
Calcium	0·10	3·15	0·25	2·05
Phosphorus	0·35	2·25	0·60	1·90
Available lysine (May 1964) (mean of two determinations)	—	5·35	2·85	5·20
Available lysine (March 1966)	—	5·10	2·75	4·90
Aspartic acid	0·56	6·45	4·65	6·15
Threonine	0·34	3·20	1·70	2·95
Serine	0·37	2·85	2·15	2·55
Glutamic acid	2·01	8·45	7·55	8·00
Proline	1·06	2·85	2·50	2·85
Glycine	0·39	3·55	2·00	4·45
Alanine	0·41	4·10	1·90	4·25
Valine	0·46	3·80	2·15	3·65
Cystine	0·30	0·70	0·80	0·65
Methionine	0·18	2·20	0·62	2·00
Available methionine (March 1966)	—	1·40	0·55	1·35
Leucine	0·65	5·35	3·55	5·20
Tyrosine	0·33	2·60	1·90	2·20
Phenylalanine	0·45	2·95	2·45	2·80
Lysine	0·34	5·50	3·00	5·30
Histidine	0·21	1·70	1·35	1·65
Arginine	0·51	3·90	3·45	3·80
Isoleucine	0·33	3·40	2·10	3·05

Experimental design

The four factors studied in all thirty-six factorial combinations were:

Diet (protein source)	: Danish herring, soya, Icelandic herring
Breed	: Alb, N.H., crossbred
Sex	: Male, female
Level of food intake	: Restricted, *ad lib.*

Each replicate consisted of six litters, two of each breed, and each litter contained three males and three females receiving the three diets. The interaction of sex and food intake level was confounded with litters so that within a litter the males received one level of food intake and the females the other. The design was, therefore, a split-plot arrangement with two error terms, between and within litter. The same design was used with each of the cage types. Eight replications were completed on screen floors and four on solid floors.

Procedure with slaughtered rats

All rats were killed with diethyl ether. The intestines, stomach, spleen and pancreas were removed and discarded. The remaining carcass was weighed and frozen.

Each carcass was macerated and three portions from the slurry were taken by the method of Hartsook & Hershberger.[9]

A group of twelve male and ten female rats was killed at between 22 and 26 days of age and two portions were taken from each slurry to allow an estimate to be made of the initial N content of the carcasses. Within the range of rat weights used it was found that carcass protein (y) was related to carcass weight (x) by the equation $y = 1{\cdot}51 + 0{\cdot}151\ x$.

Determination of dry matter, oil and N contents of macerated carcasses

One sample was dried in a vacuum oven at 40° and weighed to allow an estimate to be made of the dry matter of the carcass. The dried sample was extracted with petroleum ether (b.p. 40–60°) for 8 h in a Soxhlet unit. The thimbles were weighed after drying in a vacuum oven. The other two samples were analysed for total N by the Kjeldahl method (coefficient of variation of analytical and sampling error was 4·09%).

Results

Dietary intake level

Solid-floored cages

Rats in solid-floored cages consumed *ad lib.* 10·1% less than those in screen floor cages (Table IV). *Ad lib.* feeding in solid floored cages led to a marked increase in weight gain ($P < 0{\cdot}001$) (Table V), protein gain ($P < 0{\cdot}001$) (Table VI) and in carcass fatness ($P < 0{\cdot}05$) (Table VII) without significantly affecting % carcass protein (Table VIII), FCE (Table IX), or app. NPU (carcass) (Table X). The covariance of FCE on food intake of rats fed *ad lib.* was significant ($P < 0{\cdot}01$), higher FCE values being associated with higher intakes (Table XI).

Screen-floored cages

The covariances of FCE on food intake and of app. NPU (carcass) on protein intake of rats fed *ad lib.* were significant ($P < 0.001$ and $P < 0.05$, respectively). Higher intakes were associated with higher values.

When intake for each treatment was adjusted to that of the same breed–sex group fed in restricted quantities, treatment differences were significant, and FCE values especially, in both pen types, were lower than those of their restricted litter-mates (Table XI). Slightly lower FCE values were observed on screen floors despite a slightly lower fat accumulation.

However, carcass weight as a percentage of bodyweight was significantly greater for rats on screen floors compared with those on solid floors ($P < 0.001$), particularly after *ad lib.* feeding ($P < 0.05$) (Table XII).

TABLE IV

Treatment mean food consumption (g/rat/21 days) of rats fed *ad lib.*

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	234	239	238	237	243	250	241	245
N.H.	203	194	184	194	222	223	219	221
Xbred	239	228	228	232	267	270	257	265
		(±7·4)[a]		(±7·7)		(±5·4)[a]		(±7·7)
		(±9·8)[b]				(±8·8)[b]		
Mean	225	220	217		244	248	239	
		(±4·2)				(±3·1)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	238	197	243	226	239	225	265	243
Female	236	190	220	215	251	217	264	244
		(±10·9)		(±6·3)		(±10·8)		(±6·3)
Mean	237	194	232		245	221	265	

[a] within breed
[b] between breed

TABLE V

Treatment mean body weight gain (g/rat/21 days)

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	45·2	41·6	40·9	42·6	43·8	38·7	38·6	40·3
N.H.	37·8	34·8	33·2	35·3	38·3	37·4	34·9	36·8
Xbred	47·4	41·0	42·0	43·5	47·9	42·6	42·0	44·1
		(±1·85)[a]		(±1·62)		(±1·35)[a]		(±1·47)
		(±2·22)[b]				(±1·84)[b]		
Feeding								
Restr.	36·3	32·8	32·4	33·8	34·6	32·7	33·0	33·4
Ad lib.	50·7	45·5	45·0	47·0	52·0	46·4	44·0	47·4
		(±1·51)		(±0·87)		(±1·10)		(±0·64)
Mean	43·5	39·1	38·7		43·3	39·5	38·5	
		(±1·07)				(±0·78)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	44·3	36·3	46·0	42·2	40·3	37·2	45·4	40·9
Female	40·8	34·3	40·9	38·7	40·4	36·6	42·9	39·9
		(±1·51)[a]		(±0·87)		(±1·09)[a]		(±0·64)
		(±1·95)[b]				(±1·65)[b]		
Feeding								
Restr.	33·4	31·9	36·2	33·8	33·1	33·7	33·5	33·4
Ad lib.	51·8	38·6	50·7	47·0	47·5	40·0	54·8	47·4
		(±1·51)[a]		(±0·87)		(±1·09)[a]		(±0·64)
		(±1 95)[b]				(±1·65)[b]		
Mean	42·6	35·3	43·5		40·3	36·8	44·1	

	Male	Female	Mean	Male	Female	Mean
Feeding						
Restr.	34·4	33·3	33·8	34·51	32·38	33·45
Ad lib.	50·0	44·0	47·0	47·40	47·51	47·45
	(±1·88)					
Mean	42·2	38·7		40·9	39·9	

[a] within breed
[b] between breed

TABLE VI

Treatment mean protein gain of carcass (final carcass N × 6·25—initial carcass N × 6·25 g)

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	8·26	6·61	7·22	7·36	8·00	7·02	7·13	7·38
N.H.	6·67	6·12	5·69	6·16	7·04	6·83	6·45	6·77
Xbred	8·97	7·64	7·91	8·17	9·22	8·22	8·12	8·51
		(±0·38)[a]		(±0·35)		(±0·26)[a]		(±0·26)
		(±0·47)[b]				(±0·34)[b]		
Feeding								
Restr.	6·81	5·93	5·95	6·22	6·57	5·72	6·05	6·11
Ad lib.	9·12	7·65	7·92	8·23	9·60	8·99	8·42	9·00
		(±0·31)		(±0·18)		(±0·21)		(±0·12)
Mean	7·96	6·78	6·94		8·08	7·35	7·23	
		(±0·22)				(±0·15)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	7·29	5·77	8·56	7·20	7·51	6·57	8·68	7·58
Female	7·42	6·55	7·78	7·25	7·25	6·98	8·36	7·52
		(±0·31)[a]		(±0·18)		(±0·21)[a]		(±0·12)
		(±0·42)[b]				(±0·30)[b]		
Feeding								
Restr.	5·96	5·83	6·89	6·22	5·98	5·92	6·45	6·11
Ad lib.	8·76	6·48	9·45	8·23	8·79	7·63	10·59	9·00
		(±0·31)[a]		(±0·18)		(±0·21)[a]		(±0 12)
		(±0·42)[b]				(±0·30)[b]		
Mean	7·36	6·16	8·17		7·38	6·77	8·51	

	Male	Female	Mean	Male	Female	Mean
Feeding						
Restr.	6·05	6·40	6·22	6·30	5·92	6·11
Ad lib.	8·37	8·10	8·23	8·88	9·13	9·00
		(±0·41)				
Mean	7·20	7·25		7·58	7·52	

[a] within breed
[b] between breed

TABLE VII

Treatment mean oil content of carcass (g petroleum extract/100 g carcass wet matter)

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	8·66	8·30	9·20	8·72	8·19	7·44	7·09	7·57
N.H.	7·67	7·51	7·57	7·59	7·84	6·55	7·13	7·17
Xbred	8·94	8·56	8·73	8·74	8 63	7·53	8·85	8·33
		(±0·47)[a]		(±0·26)		(±0·40)[a]		(±0·44)
		(±0·47)[b]				(±0·55)[b]		
Feeding								
Restr.	7·84	8·01	8·02	7·96	7·77	6·94	7·16	7·28
Ad lib.	9·00	8·23	8·99	8·74	8·67	7·41	8·22	8·10
		(±0·38)		(±0·22)		(±0·32)		(±0·19)
Mean	8·42	8·12	8·50		8·21	7·17	7·69	
		(±0·26)				(±0·23)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	9·18	8·18	9·10	8·82	7·08	6·80	7·96	7·27
Female	8·26	6·99	8·39	7·88	8·08	7·55	8·71	8·11
		(±0·37)[a]		(±0·22)		(±0·32)[a]		(±0·19)
		(±0·37)[b]				(±0·50)[b]		
Feeding								
Restr.	8·14	7·40	8·34	7·96	7·30	7·15	7·41	7·28
Ad lib.	9·30	7·77	9·15	8·74	7·85	7·19	9·26	8·10
		(±0·37)[a]		(±0·22)		(±0·32)[a]		(±0·19)
		(±0·37)[b]				(±0·50)[b]		
Mean	8·72	7·59	8·74		7·57	7·17	8·33	

	Male	Female	Mean	Male	Female	Mean
Feeding						
Restr.	7·99	7·93	7·96	7·16	7·41	7·28
Ad lib.	9·65	7·83	8·74	7·39	8·81	8·10
	(±0·30)					
Mean	8·82	7·88		7·27	8·11	

[a] within breed
[b] between breed

TABLE VIII

Treatment mean protein content of carcass (N, g × 6·25/100 g carcass wet matter)

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	19·66	19·05	19·55	19·42	20·08	19·94	19·85	19·95
N.H.	20·11	20·09	19·83	20·01	20·21	20·27	20·42	20·30
Xbred	19·87	19·78	19·84	19·83	20·17	20·12	19·85	20·05
		(±0·28)[a]		(±0·23)		(±0·23)[a]		(±0·17)
		(±0·33)[b]				(±0·26)[b]		
Feeding								
Restr.	19·86	19·63	19·37	19·62	20·20	19·71	19·83	19·91
Ad lib.	19·89	19·66	20·11	19·89	20·10	20·50	20·25	20·28
		(±0·23)		(±0·13)		(±0·19)		(±0·11)
Mean	19·88	19·64	19·74		20·15	20·11	20·04	
		(±0·16)				(±0·14)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	19·03	19·55	19·73	19·44	19·89	20·44	19·85	20·06
Female	19·81	20·48	19·92	20·07	20·01	20·16	20·24	20·14
		(±0·23)[a]		(±0·13)		(±0·19)[a]		(±0·11)
		(±0·29)[b]				(±0·21)[b]		
Feeding								
Restr.	19·32	19·87	19·67	19·62	19·76	19·96	20·03	19·91
Ad lib.	19·52	20·15	19·98	19·89	20·15	20·64	20·07	20·28
		(±0·23)[a]		(±0·13)		(±0·19)[a]		(±0·11)
		(±0·29)[b]				(±0·21)[b]		
Mean	19·42	20·01	19·83		19·95	20·30	20·05	

	Male	Female	Mean	Male	Female	Mean
Feeding						
Restr.	19·31	19·93	19·62	19·84	19·99	19·91
Ad lib.	19·57	20·21	19·89	20·29	20·28	20·28
	(±0·27)					
Mean	19·44	20·07		20·06	20·14	

[a] within breed
[b] across breed

TABLE IX

Treatment mean food conversion efficiency (body weight gain, g/food consumed, g)

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	0·225	0·203	0·199	0·209	0·208	0·185	0·188	0·193
N.H.	0·208	0·195	0·186	0·197	0·198	0·192	0·182	0·191
Xbred	0·230	0·207	0·211	0·216	0·216	0·191	0·196	0·201
		(±0·0060)[a]		(±0·0068)		(±0·0035)[a]		(±0·0036)
		(±0·0084)[b]				(±0·0046)[b]		
Feeding								
Restr.	0·217	0·199	0·200	0·205	0·203	0·193	0·195	0·197
Ad lib.	0·225	0·204	0·197	0·209	0·212	0·186	0·182	0·193
		(±0·0049)		(±0·0028)		(±0·0029)		(±0·0017)
Mean	0·221	0·201	0·199		0·208	0·189	0·188	
		(±0·0035)				(±0·0020)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	0·218	0·199	0·222	0·213	0·196	0·190	0·208	0·198
Female	0·200	0·193	0·209	0·201	0·191	0·191	0·194	0·192
		(±0·0049)[a]		(±0·0028)		(±0·0029)[a]		(±0·0017)
		(±0·0076)[b]				(±0·0041)[b]		
Feeding								
Restr.	0·198	0·204	0·214	0·205	0·194	0·201	0·195	0·197
Ad lib.	0·220	0·183	0·218	0·209	0·193	0·180	0·207	0·193
		(±0·3049)[a]		(±0·0028)		(±0·0029)[a]		(±0·0017)
		(±0·0076)[b]				(±0·0041)[b]		
Mean	0·209	0·197	0·216		0·193	0·191	0·201	

	Male	Female	Mean	Male	Female	Mean
Feeding						
Restr.	0·212	0·198	0·205	0·203	0·191	0·197
Ad lib.	0·214	0·203	0·209	0·193	0·193	0·193
	(±0·0079)					
Mean	0·213	0·201		0·198	0·192	

[a] within breed
[b] between breed

TABLE X

Treatment mean apparent net protein utilisation (carcass) (g carcass N/g food N consumed)

	Solid floor				Screen floor			
	Dan. herr.	Soya	Iceld herr.	Mean	Dan. herr.	Soya	Iceld herr.	Mean
Alb.	0·435	0·356	0·385	0·392	0·412	0·364	0·378	0·384
N.H.	0·392	0·373	0·342	0·369	0·385	0·387	0·364	0·379
Xbred	0·469	0·425	0·432	0·442	0·446	0·411	0·413	0·423
		(±0·0152)[a]		(±0·0135)		(±0·0106)[a]		(±0·0095)
		(±0·0183)[b]				(±0·0128)[b]		
Feeding								
Restr.	0·435	0·390	0·387	0·404	0·412	0·372	0·390	0·391
Ad lib.	0·430	0·379	0·386	0·398	0·417	0·403	0·381	0·400
		(±0·0124)		(±0·0072)		(±0·0086)		(±0·0050)
Mean	0·432	0·385	0·386		0·414	0·387	0·385	
		(±0·0088)				(±0·0061)		

	Alb.	N.H.	Xbred	Mean	Alb.	N.H.	Xbred	Mean
Male	0·389	0·340	0·452	0·394	0·399	0·371	0·434	0·401
Female	0·396	0·398	0·432	0·409	0·370	0·387	0·412	0·390
		(±0·0124)[a]		(±0·0072)		(±0·0085)[a]		(±0·0050)
		(±0·0161)[b]				(±0·0112)[b]		
Feeding								
Restr.	0·383	0·387	0·442	0·404	0·381	0·383	0·409	0·391
Ad lib.	0·401	0·351	0·442	0·398	0·388	0·375	0·437	0·400
		(±0·0124)[a]		(±0·0072)		(±0·0085)[a]		(±0·0050)
		(±0·0161)[b]				(±0·0112)[b]		
Mean	0·392	0·369	0·442		0·384	0·379	0·423	

	Male	Female	Mean	Male	Female	Mean
Feeding						
Restr.	0·394	0·414	0·404	0·403	0·380	0·391
Ad lib.	0·393	0·403	0·398	0·400	0·400	0·400
		(±0·0156)				
Mean	0·394	0·409		0·401	0·390	

[a] within breed
[b] between breed

TABLE XI

***Ad libitum* diet mean responses for FCE and NPU and their values adjusted by covariance to the mean restricted intake of the same diet**

	FCE		NPU	
	unadjusted	adjusted	unadjusted	adjusted
Solid-floored cages				
Danish herring	0·225	0·201	Covariance	
Soya	0·204	0·180	not	
Icelandic herring	0·197	0·179	significant	
Screen-floored cages				
Danish herring	0·212	0·199	0·417	0·395
Soya	0·186	0·174	0·403	0·374
Icelandic herring	0·182	0·171	0·381	0·352

TABLE XII

Mean carcass weights as percentage of body weight

	Restricted	*Ad lib.*
Solid-floored cages		
Danish herring	87·8	87·5
Soya	86·7	85·8
Icelandic herring	87·5	87·0
Screen-floored cages		
Danish herring	88·6	89·3
Soya	87·4	88·5
Icelandic herring	88·7	89·4

Breed

Solid-floored cages

Under *ad lib.* feeding the Alb and the crossbred rats gained bodyweight faster ($P<0.001$) than the N.H. rats (Table V) and had a higher FCE ($P<0.01$) and app. NPU (carcass) ($P<0.05$ and $P<0.001$) (Tables IX & X).

With restricted feeding, compared with the N.H. rats, the other two groups showed similar but much less striking differences in weight gain. However, the crossbred rats had a higher app. NPU (carcass) than the other two groups ($P<0.05$) and higher weight of dry matter than the N.H. rats ($P<0.01$).

Screen-floored cages

A similar pattern of breed group responses was observed in these cages under *ad lib.* feeding, except that in all characteristics the crossbreds markedly exceeded the Alb rats as a consequence of their greater food intake. On the other hand N.H. rats suffered a loss of efficiency with *ad lib.* intake.

Sex

Solid-floored cages

Under *ad lib.* feeding the males gained weight faster ($P<0.05$) (Table V) and had a higher FCE ($P<0.05$) (Table IX) than females despite having carcasses much higher in percentage fat ($P<0.001$) (Table VII). There were no overall differences between the sexes in either protein accumulation (Table VI) or app. NPU (carcass) (Table X). This was the consequence, in both cage types, of a large breed $\times$ sex interaction for FCE and app. NPU (carcass) in which crossbred females gave lower values than crossbred males but N.H. females exceeded the app. NPU (carcass) of N.H. males. Other workers have observed sex $\times$ strain interactions.[10]

There were no marked differences between the sexes under restricted feeding although the FCE for males was again higher ($P<0.05$).

Screen-floored cages

In contrast to the observations on rats on solid floors, males possessed carcasses with a lower percentage fat ($P<0.01$), but efficiency characteristics showed a similar pattern.

Diet

Solid-floored cages

There were significant differences between diets in protein gain (Table VI), FCE (Table IX), app. NPU (carcass) ($P<0.001$) (Table X) and weight gain ($P<0.01$) (Table V). At both levels of feeding Danish herring meal was superior to the other two proteins, which did not differ significantly in any characteristic.

Screen-floored cages

Again Danish herring meal under *ad lib.* feeding sustained a greater weight gain, FCE ($P<0.01$) and protein gain ($P<0.05$) than the soyabean meal, and a greater app. NPU (carcass) than the Icelandic herring ($P<0.01$).

Despite the higher FCE for the Danish herring, more carcass fat was found on that diet than on the soya ($P<0.01$) (Table VII).

Restricted, as opposed to *ad lib.* feeding, led to similar but smaller differences in favour of the Danish herring. The Icelandic herring meal was not significantly different from the soya, but both fish meals under all situations tended to promote fatter carcasses.

Screen-floor interactions involving diet

Diet $\times$ intake level

There were significant diet $\times$ level interactions for both FCE ($P<0.001$) (Table IX) and weight gain ($P<0.05$) (Table V). The differences between the Danish herring and the other two proteins widened with *ad lib.* feeding.

Diet $\times$ breed $\times$ intake level ($P<0.01$)

The Danish herring led to a 4·4% greater FCE under *ad lib.* feeding, whereas the other two diets on average depressed FCE by 5%. This average effect varied with breed, as crossbreds increased their FCE by 6% with *ad lib.* feeding, while the N.H. decreased theirs by 10%.

Precision of individual breed–sex groups

Individual analyses of variances were conducted for each breed–sex group within food intake level. Percentage coefficients of variation for Alb, N.H. and crossbreds were on average 11·4, 14·6 and 9·4 for gain, 10·4, 9·7 and 7·6 for FCE and 15·9, 15·2 and 9·7 for app. NPU (carcass).

Discussion

Comparison of the FCE measurement with other efficiency measurements

An inadequate number of diets precluded conclusive evidence from being adduced as to whether the various criteria of dietary quality were equivalent. Nevertheless, treatment mean liveweight gain correlated well with protein gain, but the herring meals tended to produce fatter carcasses, expecially when given *ad lib.* Bender & Doell[2] did not consider this to be a serious disadvantage, and no significant correlation occurred between carcass fat and N in the present experiment. The correlation between the efficiency measurements FCE and app. NPU (carcass) was $r = 0.85$ between treatment means, representing differences in diet, breed and pen type. For similar FCE values the NPU values of crossbreds were higher than those of the other breed groups. The most serious disadvantage of the criterion, FCE was the interaction of diet $\times$ level and diet $\times$ breed $\times$ level, indicating that the ranking of proteins is affected by food intake level and strain of rat. Henry[3] and Bender,[1] on the other hand, concluded that protein efficiency ratios (for equal protein intakes PER is the ratio of test to control FCE values) were correlated reasonably well with NPU, even though a number of dietary factors influence PER.[11–13]

The diet $\times$ breed $\times$ level interaction in FCE is not explained by changes in carcass fatness, as crossbreeds were more efficient with *ad lib.* feeding yet fatter than the other groups. Likewise, the Danish herring diet produced as great an increment in fat with *ad lib.* feeding as did the other diets. Bender & Doell[2] have made a similar observation. Furthermore, it is unlikely that the fatness of crossbreds resulted from reduced activity.[14]

In an unpublished study with rats the correlation coefficients for PER with the available lysine[15] *Streptococcus zymogenes*[7] and GPV (chick) assays were 0·68, 0·79 and 0·68 for thirty proteins; however, the last two methods were

correlated with available lysine to the extent of 0·80 and 0·88. Furthermore, the estimated standard deviations per determination for the other three methods were 9·5%, 10·5% and 14·3% compared with 16·5% for the PER method*.

Other evidence on similar relationships has been reported previously.[16]

Amino acid composition

There was no significant overall difference between the soya and Icelandic herring for both FCE and app. NPU (carcass) but a marked superiority of Danish herring. However, essential amino acid indices (EAAI)[8] of the three diets were: Danish herring, 78·6; soya, 76·1; and Icelandic herring, 74·7 (77·9, 73·6 and 73·9 excluding arginine,[17]). In comparison there was a tendency for app. NPU (carcass) of the Alb and crossbred rats to under-rate the value of the soya and for that of the N.H. rats to overrate it. The total lysine, available lysine and total methionine contents of the Danish herring diet were adequate for growth and their concentrations in both herring meals were higher than in the soya diet. It was inferred that Alb and crossbred rats responded mainly to the concentration of these two amino acids rather than to the overall amino acid level.

The relatively good performance of the N.H. rats receiving the soya diet, in which methionine and lysine were growth-limiting amino acids, is more difficult to explain. It was anticipated that their requirement might reflect a lower rate of hair growth. The regrowth of hair over a patch in the lumbar region was measured in 7-week-old female rats. It was found that Alb rats exceeded N.H. rats in this respect ($P < 0{\cdot}01$) (2·15 as opposed to $1{\cdot}73 \pm 0{\cdot}07$ mg hair N/cm^2 of pelt/3 week). This difference, in addition to the lower bodygrowth rate and muscle mass of N.H. rats, may have reduced their requirement for methionine and lysine relative to that of other amino acids.

In comparison with the herring meals the soya protein contained high concentrations of leucine, isoleucine, histidine, tyrosine and phenylalanine. It is possible that the requirement of N.H. rats for these amino acids is relatively high. The pigmented coat of N.H. rats may augment the metabolism of phenylalanine–tryosine in melanin synthesis so that their requirement is in excess of estimates published for Albino rats.[18,19] The crossbred rats are also pigmented, but greater muscle growth may have increased the relative importance of dietary lysine. The overall efficiency of N utilisation (app. NPU, (carcass)) of crossbred rats was about 17% greater than that of the N.H. rats. Differences between breed groups in amino acid requirements may, therefore, explain in part the diet × breed × level interaction in FCE.

Cage type

The increased consumption *ad lib.* by rats on screen floors may have been partly the consequence of screen floors offering less thermal insulation[20] and also restricting the incidence of coprophagy.[21,22] However, the patterns of FCE and app. NPU (carcass) responses to the three diets were similar in both cage types, indicating that a difference in the incidence of coprophagy had no effect on either the efficiency of methionine metabolism,[23,24] or N retention. Nevertheless, an 8% higher fat deposition on solid floors is consistent with the effect of an increase in coprophagy on PER measured in those cages.[25]

Variability and sensitivity

The variation of crossbreds in app. NPU (carcass) particularly, was on average less than that of the other groups. Other workers have found F_1 crosses between highly inbred strains to be less variable in physiological characteristics than parent groups,[27–29] but it is not possible to generalise about the extent of phenotypic variation when inbreds are compared with F_1 crosses.[10,29]

Food intake

From the point of view of significant differences between diets of a consistent and rational nature, *ad lib.* feeding of male rats on screen floors would seem to be the preferred system for FCE and app. NPU (carcass) determinations. However, *ad lib.* feeding is even more removed from the ideal of equalised responses in biological assays[30,31] than is restricted feeding. Better proteins tend to be more palatable, and under *ad lib.* conditions the differences in weight gain and FCE can be exaggerated, as was found in this study. Even so, some workers consider gain to be a useful measure of protein quality when the diet is fed *ad lib.*[32] The increase in treatment differences brought about by *ad lib.* feeding[32,33] introduces systematic errors which some investigators consider to be partly avoided by comparison of growth increments per unit of food.[34] However, such biases are more effectively controlled by an allowance for maintenance. Covariance adjustments of FCE and app. NPU (carcass) for food and protein intake respectively allow for some of the distortions associated with variable *ad lib.* intake. In this study the adjusted FCE values were generally lower than those of the comparable restricted groups, apparently because the more efficient rats had better appetites or restricted rats possessed lower maintenance requirements. This suggests that the regressions used were inappropriate. Furthermore, covariance adjustment does not necessarily remove all the biases between responses that can arise through a variation in food intake.

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PESTICIDES RESIDUES IN FOODSTUFFS IN GREAT BRITAIN*

VI.**—Mercury residues in rice

By N. A. SMART and A. R. C. HILL

Analyses of samples of rice from United Kingdom importers and from retail sources show that levels of mercury are often negligible, although they may rise to 0·01 ppm and occasionally to 0·015 ppm.

Introduction

Organomercury fungicides are used on rice as seed dressings and also at tillering for control of rice blast (*Piricularia oryzae*). Investigations into the mercury content of harvested rice have hitherto been mainly carried out in Japan where extensive use is made of mercury fungicides in rice culture.

Furutani & Osajima[1] compared the mercury contents of rice dusted with mercury fungicides and undusted rice. The concentration of mercury in unhulled undusted rice was about 0·3 ppm and that in polished rice about 0·2 ppm. The mercury content increased to 0·55 ppm in unhulled rice and 0·3 ppm in polished rice after they had been dusted at the flower primordia stage and to 0·9 ppm and 0·6 ppm, respectively, after a dusting at the heading stage. No reduction of the mercury content was found after cooking. The same workers[2] suggested that the mercury content of undusted rice may be largely related to the concentration of mercury in the paddy field soil; for example, a well drained soil containing 0·3 ppm mercury (dry weight) gave rice containing 0·3 ppm while a poorly drained soil containing 1·4 ppm mercury yielded rice containing 0·8 ppm. The mercury content of wheat grown in a paddy field containing 0·3 ppm mercury in the soil (dry weight) was much lower, being about 0·01 ppm. Tomizawa *et al.*[3] used neutron activation analysis to investigate the mercury contents of rice from treated paddy fields and found levels mostly in the range 0·1–1·0 ppm although more than 1 ppm was found in two cases. Control samples of undusted rice contained 0·227–0·238 ppm mercury. Tomizawa also found more mercury in raw rice than in polished rice. Goto & Sato[4] gave mercury contents of unpolished rice as 0·077–0·185 ppm and for polished rice 0·056–0·065 ppm.

Epps[5], working in the U.S.A., has reported some analyses of rice treated experimentally in the field with phenylmercury acetate. Rice endosperm by-product from control plots contained no mercury, while endosperm from treated plots contained 0·2 ppm and endosperm by-product from treated plots contained 0·1 ppm mercury, but the method of analysis was sensitive only to the nearest 0·1 ppm so that these figures are of limited value.

Several countries have set maximum allowable limits for mercury residues in foodstuffs: in the Benelux countries it is 0·03 ppm, in Brazil 0·05 ppm and in the U.S.A. zero.

** Part V: *J. Sci. Fd Agric.*, 1968, **19**, 119

Some other countries have figures of about this order for certain foodstuffs and these are used as a guide to an acceptable level of mercury. It was considered desirable to investigate the levels of mercury in edible rice imported into the United Kingdom to see whether they approached those reported by these workers and to provide a basis for assessing any possible consumer hazard. The results have been submitted to the Panel on Residues of Pesticides in Foodstuffs[6] and are reported here.

Experimental

Samples of about 500 g rice were obtained from importers, retail shops and through the Ministry of Overseas Development, Tropical Products Laboratory. 25 g sub-samples were taken for each analysis.

25 ml water were added to each sub-sample in a 1 litre flask and the analyses were carried out by the method recommended by the Joint Mercury Residues Panel[7] using an additional 60–70 ml nitric acid to prevent carbonisation during digestion.

Results

The standard deviation of the analytical method at the 0·00–0·01 ppm level was $3{\cdot}5 \times 10^{-3}$ ppm. The recovery of phenylmercury compound added at 0·2 ppm was 93% with a standard deviation of 3×10^{-2} ppm. All samples were analysed in duplicate and the mean of the results, uncorrected for recovery, is quoted. The results above 0·005 ppm are given to the nearest 0·005 ppm; below 0·005 ppm they are not significant.

Twenty-six samples of rice from Argentina, Australia, Burma, China, Hungary, Korea, Pakistan, Thailand and the U.S.A. obtained directly from importers were examined. Seventeen of these samples gave a mercury content of less than 0·005 ppm; these samples did not, therefore, show statistically significant traces of mercury when regarded individually. However, a statistical analysis of the results for these samples treated as a group showed a mean level of 0·001 ppm with a standard error of $\pm 0{\cdot}0004$ ppm revealing a significant trace of mercury in the majority of importer's rice. Of the other nine samples of importer's rice one contained 0·005 ppm mercury. Milled 'Begmi' (medium width, long) from Pakistan, milled 'Shiao-chan' and 'Soonan' (both round, short) from China, milled 'Emata' (medium, long) from Burma and milled 'Bluebonnet' (slender, long) from the U.S.A. contained 0·01 ppm mercury, and milled (bold, middling) from the U.S.A., unmilled 'Shiao-chan' and milled 'Yujien' (medium, middling) both from China contained 0·015 ppm.

Two out of six retail samples of rice did not contain significant traces of mercury; two contained 0·005 ppm and two 0·01 ppm mercury.

Six samples of rice from field experiments, the details of which are not known, in Australia, Nigeria, Trinidad and the U.S.A. were also examined. The Australian and American samples did not contain significant traces of mercury. The Nigerian milled rice contained 0·01 ppm mercury and Nigerian unhulled and parboiled rice contained 0·02 ppm mercury. One sample of Trinidad paddy contained 0·095 ppm mercury.

Discussion

These results show that residues of mercury in rice imported into the United Kingdom are often negligible although they may rise to 0·01 ppm and occasionally to 0·015 ppm.

The levels found in Japanese rice by workers in Japan are much higher than this even in 'control' samples; it is possible that the 'control' samples came from paddy fields that had received organomercury compounds in earlier years. Japan exports negligible quantities of rice.[8]

Although 0·02 ppm was found in some samples of Nigerian rice and 0·095 ppm in Trinidad paddy, it should be pointed out that these were not normal commercial samples and cannot be given the same consideration as the importer's samples. Trinidad and Nigeria do not export rice.

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FREEZE-DRYING OF RAW BEEF
I.—Influence of some raw material variables

By O. BENGTSSON and N. E. BENGTSSON

The effect of raw material variables, such as breed, sex, age, fat content of muscle and ageing time, upon the quality of the reconstituted product were studied in multifactorial experiments of a preliminary nature. The effects observed were in fair agreement with general experience with unprocessed raw beef and with the limited published data for reconstituted freeze-dried raw beef.

Introduction

No systematic investigation of the effect of different raw material variables on quality of freeze-dried raw beef has been found in the literature, and none regarding the variables of breed, animal category, age or intramuscular fat content. On the other hand several workers have made more limited freeze-drying studies on different muscles, cuts, *rigor* stages or pre-slaughter injections. Comprehensive studies have been made on fresh raw beef and it has been suggested that a certain degree of correlation with freeze-dried quality might be expected.

Zhuravskaya[1] studied the effect on freeze-dried quality of *rigor* state and concluded that *post-rigor* conditions were advantageous to quality.

Within a freeze-drying study regarding the effect of pH changes induced before slaughter, Penny *et al.*[2] compared the muscles *semimembranosus* and *longissimus dorsi* of young bulls of Aberdeen Angus × Jersey breed. They noticed a tendency towards higher tenderness score for *longissimus dorsi* but no difference in juiciness.

That increased time of ageing or hanging has a positive effect on tenderness in fresh raw meat is a general experience. Olenev & Livsic[3] studied the effect of ageing before freeze-drying, comparing ageing times of 2, 5 and 11 days at 0°. They reported a marked improvement in tenderness, juiciness and reconstitution with increased time of ageing. Hamdy & Deatherage[4] reported that water-holding ability was increased through ageing of the meat but that this effect did not survive freeze-drying.

For fresh raw meat a number of studies comparing different muscles have concluded that overall tenderness and juiciness is higher for *longissimus dorsi* than for *semimembranosus*. Ritchey & Hostetler[5] found that results were affected by the cooking temperature used, with no difference between the two muscles at lower cooking temperature.

A study of the literature on quality differences in fresh raw beef led the authors to some general conclusions: the tenderness of meat from steers is greater than that from bulls; tenderness decreases with age; juiciness is unrelated to either of these factors or to animal category; intensity of feeding is an important factor affecting juiciness and tenderness; these two qualities increase with intramuscular fat content.

The objective of the present investigations has been to study more systematically the effects of various raw material variables, in the context of a larger study on factors important to quality in freeze-dried raw beef.

Experimental

Experimental plan

Two separate multifactorial studies of raw material variables were made, designated below as series A and B.

In series A, raw meat from different muscles of young bulls from one breed and two crossbreeds were compared for quality after storage in the freeze-dried state. Sensory evaluation was made on a boiled preparation.

In series B, *longissimus dorsi* muscles from one breed were compared for animals of different age, sex and intramuscular fat content, and for different ageing and frozen-storage times before freeze-drying. Sensory evaluation was made on a pan-fried preparation.

Raw material

For series A, meat from *longissimus dorsi* (posterior end), *supraspinatus* and *semimembranosus* muscles from young bulls of S.R.B. breed (Swedish red- and white-coloured cattle) and crossbreeds with S.L.B. (Swedish lowland cattle) and Hereford were used. The animals were raised under very well defined conditions at the W.I.A.D. Institute of Animal Husbandry, and were slaughtered, hung and dissected under controlled conditions.

For series B posterior ends of *longissimus dorsi* muscles from animals of the S.L.B. breed were used. Muscles from young bulls and young and old cows of low and high intramuscular fat content were selected at a slaughterhouse by experts from the W.I.A.D. Institute. Muscles from calf and steer were also included in part of the experiment.

In both series all cuts were wrapped in plastic film and shipped to the S.I.K. Institute overnight under cooled transport at +3°

Treatments

Preparation for drying

After slaughter the carcasses were tempered at +3° and the muscles were dissected after 1–3 days in cold storage, vacuum-packed in plastic pouches and stored together at +3° for a total of 6 days in series A, and 6 or 12 days in series B.

The different muscles were trimmed after ageing and frozen in plastic pouches in a freezer at −30° at an average freezing rate of 0·5 cm/h. The frozen muscles were sawn at −7° into discs 10 mm (series A) or 13 mm (series B) thick across the direction of the fibre.

Freeze-drying

A small pilot unit equipped with electrically heated radiant heating shelves and gear for measuring and recording chamber pressure, weight change and temperatures was used. The average working pressure was 0·2 torr, and maximum temperatures for heating shelves, product surface and ice front were +120°, +40°, and −25°, respectively. Drying time was approximately 9–10 hours at 10 mm thickness and 10–12 hours at 13 mm thickness, and residual moisture content was between 0·9 and 1·5%.

Packaging and storage

The vacuum was broken with nitrogen, and the product was exposed to ambient air (r.h. 30–40%) for up to ½ hour before packaging. Meat slices were individually packed under nitrogen in gas-tight cellophane aluminium foil polyethylene at a residual head-space oxygen content of 0·6–1·3% by volume (corresponding to an oxygen partial pressure of 4·5–10 mm mercury). Head-space volume averaged 125 ml, and the weight of the dried meat samples 15 g.

In series A, samples were evaluated after storage for 1–2 weeks, 6 months and 1 year at +3 and at +30°. In series B, evaluations were made after 3 weeks' storage of the dried samples at +3°. No zero-time evaluations were made because of the practical difficulties involved.

Analytical methods

Water content

For the raw material this was determined from the weight change in freeze-drying, and for the dried material from further drying of powdered material at +70° in a vacuum drying oven, using a procedure suggested by Matheson[6] to avoid moisture pick-up.

Fat content

This was determined directly on powdered freeze-dried material by Soxhlet extraction using diethylether.

Headspace oxygen

This was determined using a Beckman 777 oxygen analyser.

Water uptake or degree of reconstitution

This was evaluated by letting a 15 g sample float in a 600 ml beaker half filled with water at room temperature, turning the sample upside down after 15 min for another 5 min soaking, and draining it for 2 min before weighing it. Reconstitution was expressed as reconstituted weight as % of original wet weight.

Centrifugation loss

As a measure of water-holding ability this was determined according to a method of Aitken *et al.*[7] and expressed as percentage weight loss.

Surface colour

In series A no objective colour determination was made. For series B a Hunter Color and Color Difference meter was made available, and a slight modification of a method given by MacDougall[8] was used for colour determination on reconstituted samples.

Sensory evaluation

Boiled product—Series A

Samples were prepared for testing using the above mentioned reconstitution method, and were cooked in 1% salt solution at atmospheric pressure for 18 minutes (after having been heated to a centre temperature of 98°–100° as measured by a thermocouple inserted into the geometric centre of the piece. For comparison, pressure cooking at 120° for 4 minutes was tested but showed no apparent quality difference. Evaluation was made by an 8-member panel for tenderness, juiciness and taste, using a 7 grade scale ranging from 7 = very tender, through 4 = neither tender nor tough, to 1 = very tough. As reference frozen-stored (not freeze-dried) standard samples were used.

Pan-fried—Series B

Preliminary work, in which preparation for sensory evaluation by boiling and by pan-frying were compared, suggested advantages for the frying method in terms of stronger meat flavour, better discrimination between samples and higher general acceptability to the panel and it was decided to adopt pan-frying for the organoleptic evaluations in this series. A frying time (in margarine) of 5 minutes at a pan temperature of 195° set by a thermostat was used. Since no important differences in flavour were expected in these series (no storage) the testing was sensitised for aspects of meat texture, using a specially selected and trained expert panel of 4 people and judging for fibrousness–mealiness, chewiness, juiciness and overall tenderness by 9 grade scales. These ranged from 9 = extremely tender to 1 = extremely tough, ranged with no verbal descriptions for the intermediate scale divisions. The technique used was similar to that given by Cover, Ritchey & Hostetler.[9]

Results

Series A

Difference between breeds

Results from a factorial experiment comparing 3 young bulls of each of the breeds S.R.B., S.R.B. × Hereford and S.R.B. × S.L.B. are given in Table I together with the results of the analysis of variance. Muscles from two animals of each breed were analysed after 1½ weeks of freeze-dried storage, while muscles from the third animal of each breed were analysed after 6 and 12 months of storage.

Overall an advantage for the crossbreeds over the S.R.B. breed is indicated in tenderness and taste. For the third animal of each breed stored for 6 and 12 months, a significant quality difference remained after 1 year's storage at +3° but not after 6 months at +30°. After 1 year at +30° a general deterioration in quality was noted, especially for the animal of the S.R.B. breed. Particularly the reconstitution ability was very poor for all three muscles of the S.R.B.

Differences between muscles

The results in the same factorial experiments from comparisons between the three muscles *semimembranosus*, *longissimus dorsi* and *supraspinatus* are listed in Table II together with the results of the analysis of variance. After 1–2 weeks' storage, statistically significant differences were noted in tenderness and degree of reconstitution. *Semimembranosus* and *supraspinatus* were more tender than *longissimus dorsi*, *supraspinatus* giving the highest degree of reconstitution. These differences remained with little change

TABLE I

Comparison between animals of three different breeds for tenderness, juiciness, taste, degree of reconstitution and centrifugation loss
(Pooled data for 3 different muscles. Overall means)

Property	Storage time (months)	Storage temp. °C	Breed or Crossbreed			Analysis of variance Significance level[1]	Number of replicates
			S.R.B. × Hereford	S.R.B.	S.R.B. × S.L.B.		
Tenderness	1/3	+30	4·03	3·14	3·91	0·1%	48
	6	,,	3·08	3·09	3·50	n.s.	24
	12	,,	—	2·45	2·90	n.s.	24
	12	+3	4·33	3·62	4·05	5%	24
Juiciness	1/3	+30	—	—	—	—	—
	6	,,	4·84	4·71	4·88	n.s.	24
	12	,,	—	3·83	4·40	n.s.	24
	12	+3	4·88	4·76	4·69	n.s.	24
Taste	1/3	+30	—	—	—	—	—
	6	,,	4·83	4·42	5·00	n.s.	24
	12	,,	—	2·50	3·48	0·1%	24
	12	+3	4·52	4·19	4·36	n.s.	24
Degree of reconstitution %	1/3	+30	97·2	94·6	97·3	n.s.	18
	6	,,	91·0	93·8	93·9	n.s.	9
	12	,,	—	38·9	79·6	0·1%	9
	12	+3	89·6	96·9	98·6	1%	9
Centrifugation loss %	1/3	+30	29·9	31·5	34·8	1%	18

[1] A separate analysis of variance was made for each storage time because of missing values n.s. = no significance

TABLE II

Comparison between three different muscles for tenderness, juiciness, taste, degree of reconstitution and centrifugation loss
(Pooled data for 3 breeds and 1 or 2 animals)

Property	Storage time (months)	Storage temp. °C	Muscles			Analysis of variance, significance level	Number of replicates
			semimembranosus	*long. dorsi*	*supraspinatus*		
Tenderness	1/3	+30	4·05	3·03	4·00	0·1%	48
	6	,,	3·15	2·46	4·04	0·1%	24
	12	,,	2·60	2·03	3·40	1%	16
	12	+3	4·50	2·97	4·52	0·1%	24
Juiciness	1/3	+30	—	—	—	—	—
	6	,,	4·21	4·88	5·33	1%	24
	12	,,	4·07	3·93	4·39	n.s.	16
	12	+3	4·53	4·93	4·88	n.s.	24
Taste	1/3	+30	—	—	—	—	—
	6	,,	5·06	4·52	4·67	n.s.	24
	12	,,	2·75	2·90	3·22	n.s.	16
	12	+3	4·26	4·45	4·36	n.s.	24
Degree of reconstitution %	1/3	+30	94·5	94·1	100·5	0·1%	18
	6	,,	89·9	92·3	96·5	0·1%	9
	12	,,	51·6	63·4	62·4	0·1%	6
	12	+3	94·8	97·3	93·0	n.s.	9
Centrifugation loss %	1/3	+30	32·8	31·1	32·4	n.s.	18

after 1 year of storage, except that *semimembranosus* gave a sharper decrease in degree of reconstitution than the others. The generally low value in this property after 1 year of storage is caused by the sharp quality drop in the S.R.B. animal.

Differences between animals

Statistically significant differences between animals within breed were found in fat content, in centrifugation loss and, for the S.R.B., in degree of reconstitution, but the differences were quite small.

Series B

Differences between animal categories

Results of a multifactorial experiment comparing animal category for animals of S.L.B. breed are summarised in Fig. 1.

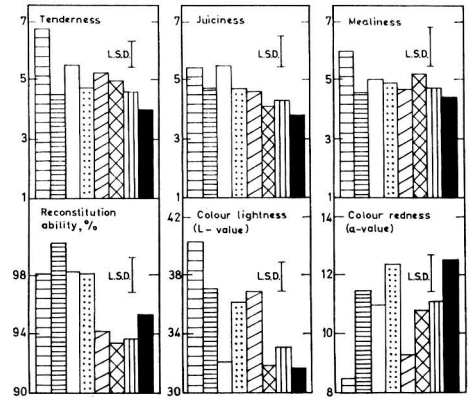

FIG. 1. *Comparison between different animal categories for tenderness, juiciness, mealiness, degree of reconstitution and colour*

Least significant difference is indicated in each diagram

— Calf
▤ Steer
□ Fat young bull
⁚⁚ Lean young bull
⁄ Fat young cow
× Lean young cow
▥ Fat old cow
■ Lean old cow

Significant differences were obtained in tenderness, juiciness, mealiness, degree of reconstitution and surface colour. Meat from the calf was considerably more tender and mealy than from the other categories; and steer, young bull and calf had the highest degree of reconstitution.

Differences for age, fat content, ageing time and frozen storage

In Table III data are presented to demonstrate the specific effects of the variables of age, fat content, ageing time and frozen storage together with the results of analysis or variance.

In regard to age, young animals were significantly more tender and lighter in colour. Meat with higher fat content was more tender and juicy but paler in colour. Longer ageing time gave significantly more tender and mealy meat.

Frozen storage up to 3 months before freeze-drying showed no negative effect on quality compared with freeze-drying immediately after freezing.

Discussion

Difference between breeds

The significant difference obtained in tenderness (and taste) should not be generalised since only three animals of each breed were included in the whole investigation and the differences noted were relatively small. The marked difference in degree of reconstitution after 1 year of storage is surprising in view of the lack of a corresponding difference after 6 months at $+30^\circ$ or 1 year at $+3^\circ$.

Variation in fresh meat quality even between animals of the same category and breed are generally considered to be rather large, but only small differences between animals were noted with the well defined raw material used in this experiment, where all animals had been raised under almost identical conditions. Such well defined raw material would certainly not be generally available for commercial use, but was a necessity in this experiment with its very limited number of test animals.

Difference between animal categories appear to be in good agreement with published data for fresh raw beef.

Differences between muscles

The observed advantage for *semimembranosus* over *longissmus dorsi* in tenderness is in disagreement with observations generally reported in the literature. Ritchey &

TABLE III

Effect of age, fat content, ageing time and frozen storage
(Overall mean values and levels of significance for difference)

Property	Age			Fat content			Ageing time			Frozen storage before freeze-drying		
	3 years	6 years	Levels of sig. for differences	1·5%	4·0%	Levels of sig. for differences	6 days	12 days	Levels of sig. for differences	3 days	12 weeks	Levels of sig. for differences
Tenderness	5·14	4·31	0·1%	4·58	5·15	1%	4·80	5·36	0·1%	4·92	5·24	5%
Juiciness	4·41	4·09	n.s.	4·37	4·89	0·1%	4·65	4·85	n.s.	4·76	4·74	n.s.
Mealiness	4·89	4·55	n.s.	4·85	4·01	n.s.	4·49	5·02	0·1%	4·81	5·10	n.s.
Chewiness	4·89	4·25	1%	4·45	5·00	1%	4·50	4·64	n.s.	4·77	4·76	n.s.
Reconstitution ability %	93·9	94·6	n.s.	95·7	95·5	n.s.	97·4	95·7	0·1%	96·6	96·4	n.s.
Centrifugation loss %	32·5	31·7	n.s.	32·5	31·8	n.s.	32·7	31·9	10%	30·9	33·9	0·1%
Lightness (L value)	34·4	32·4	0·1%	33·2	34·0	n.s.	34·6	35·2	10%	34·9	34·9	n.s.
Redness (a value)	10·1	11·8	0·1%	11·9	10·5	0·1%	11·2	10·2	0·1%	11·6	10·2	0·1%

Hostetler[5] reported, however, that preparation temperature has a different effect on tenderness for the two muscles, for which reason complementary experiments with young bulls were made (with frozen and freeze-dried raw material) comparing the muscles at different degrees (time–temperature) of boiling and pan-frying. In these experiments reversions in tenderness between *semimembranosus* and *longissmus dorsi* were observed, but could not be correlated with cooking procedure or degree of boiling and pan-frying. Lewis[10] reported that different muscles can react differently to stress before slaughter, *quadriceps femoris* becoming more tender and *longissmus dorsi* unaltered or less tender. This finding might suggest slaughter conditions as a possible reason for the relative tenderness disadvantage observed for *longissmus dorsi*.

Differences due to ageing time

The results obtained seem to support the opinion of Olenev & Levsic[3] in that improved tenderness with increased time of ageing was obtained. However no improvement in juiciness or degree of reconstitution was noted.

Conclusions

The following conclusions of potential general interest for freeze-drying of raw beef can be drawn, bearing in mind the limited number of animals and limited replication involved in the present investigation.

Different crossbreeds and different muscles showed statistically significant quality differences in tenderness and degree of reconstitution. Meat from young animals was found to be significantly more tender and of lighter colour than that from older animals. Higher fat content resulted in improved tenderness, juiciness and water-holding ability, while prolonged ageing time showed improved tenderness. The quality differences seem to agree fairly well with published data for fresh or frozen (non-freeze-dried) raw beef, except that *longissimus dorsi* was found less tender than *semimembranosus*.

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ESTIMATION OF *TRITICUM AESTIVUM* IN PASTA FLOURS: INTERSPECIFIC LIMITS FOR SITOSTERYL PALMITATE CONTENT*

By R. GARCIA-FAURE, F. GARCIA-OLMEDO and J. M. VALLEJO-ACEVEDO

Sitosteryl palmitate (SP) content of flour was shown to be not significantly affected by normal variations in milling yield. Since the distribution of fat in a wheat kernel does not follow the same pattern as does SP, it is preferable to consider SP content on the basis of DM content of the flour. A survey of 46 *Triticum aestivum* and 24 *T. durum* flours showed that the latter contained SP, but its level did not exceed 1·5 mg/100 g. *T. aestivum* varieties show a two-peak distribution, with the maxima at approximately 4 mg/100 g and at 12 mg/100 g, respectively. Three *T. aestivum* flours were within the *T. durum* range and three others were close to it. Limits for sitosteryl palmitate content in *T. aestivum* and *T. durum* were tentatively established at 16·5 mg/100 g and 1·5 mg/100 g respectively. Based on these limits, a method is proposed for the estimation of the minimum amount of *T. aestivum* in a mixture.

Introduction

Detection and measurement of *Triticum aestivum* endosperm, flour or semolina, in pasta products have become of interest from the point of view of quality and market control. Among the physical and chemical differences between *T. aestivum* and *T. durum* endosperms proposed, sitosteryl palmitate content seems to be the most general and significant.

Although cholesterol-like substances had been previously reported in wheat flour,[1,2] Walde & Mangels[3] were the first to observe that a precipitate that did not appear in acetone extracts of *T. durum* was formed at 0° in acetone extracts of *T. aestivum*. The precipitate was tentatively identified as a sterol ester,[3] and confirmed as a mixture of sterol palmitates by Spielman.[4]

Matweef,[5] following Walde & Mangels, checked the occurrence of sitosteryl palmitate in a number of *T. aestivum* and *T. durum* varieties and proposed its gravimetric or colorimetric determination as a means of quantification of *T. aestivum* products in macaroni. Some improvements of Matweef's procedure have been suggested by different authors.[6–8] The remaining problem appeared to be poor recovery of the products. Gilles & Young[9] reported a t.l.c. estimation of sitosteryl palmitate, and finally an accurate method was developed in this laboratory.[10]

In the present paper a survey of sitosteryl palmitate (SP) content in a wide number of varieties, as well as in milling fractions, is reported, and the minimum proportion of *T. aestivum* in a mixture is tentatively established as a function of the SP level.

Experimental

Wheat varieties

Forty six *T. aestivum* varieties and 24 *T. durum* varieties were used in this study. These were of diverse origin but were grown either commercially or experimentally in Spain (Crops of 1965 and 1966), with the exception of six *T. durum* samples grown in the U.S.A.

Three varieties, Magdalena and Aragón O3 (*T. aestivum*), and Hibrido-D (*T. durum*), were employed in the fractionation experiment.

Milling fractions

Samples of 2 kg each were normally milled in a Buhler experimental mill, to give three break flours and three reduction flours plus bran and shorts. Bran and shorts were pooled and run through the mill again to give three re-milled fractions: flour, bran and shorts.

Analytical methods

Sitosteryl palmitate content was determined essentially as described previously:[10] 1 g of *T. aestivum* or 3 g *T. durum* product was extracted with diethyl ether in a Soxhlet apparatus. The extract was then fractionated by preparative scale t.l.c. on a 5% $AgNO_3$ silica gel layer using carbon tetrachloride for development. The lipid was applied as a band 3 cm long. Sitosteryl palmitate was detected under u.v. light (sodium fluoresceinate spray) as the strongest of two bands appearing between the application line and the solvent front. The fainter one was tentatively identified as sitostanyl palmitate and had the higher R_f. An SP standard might be run when this procedure is first applied. The adsorbent zone containing SP was transferred with suction to a small column, and this substance was eluted with chloroform until 3 ml were collected. Evaluation was carried out by Tchugaeff colour reactions: 1 ml zinc chloride reagent (melted $ZnCl_2$, 40 g, in glacial acetic acid, 150 ml) and 1 ml acetyl chloride were added to the eluate, the mixture was heated at 65° for 15 min and read in the colorimeter at 525 nm. Sitosteryl palmitate synthesised in the laboratory was used as a standard. Alternatively, cholesterol palmitate (Fluka A. G. purum) can be used with due correction for the difference in molecular weight.

Sitosteryl levels as low as 1 mg/100 g can be measured with good reproducibility (variation coefficient, $100\,\frac{S}{X} = 4{\cdot}7\%$).

Ash by the I.C.C. method[11] and fat by the A.A.C.C. method[12] were determined in all milling fractions.

* Some of the results given in this paper were presented at the 3rd Conference on International Problems of Modern Cereal Processing and Cereal Chemistry, Potsdam

Results and Discussion

The dependence of SP content on milling yield and fat content has been studied in connexion with the setting of tentative limits for SP level in the endosperm of *T. aestivum* and *T. durum*.

Table I summarises the values obtained for ash, fat, and SP content of flour, re-milled flour, shorts, and bran in three wheat varieties, one *T. durum* and two *T. aestivum*. In both species, lower SP levels seem to be present in the outer parts of the kernel (pericarp and seed coats), compared with the endosperm. Although a greater proportion of this substance (2 to 3-fold) has been found in hand-dissected germ, it is not high enough to affect markedly the SP content of bran and short (Table I), where germ is mainly included as a minor component.

In Fig. 1, nine milling fractions from each of the above varieties have been arranged in order of ash content from low to high, and the average values for ash and SP content have been plotted against milling yield.

As the milling yield increases greater amounts of particles from the outer layers of the endosperm and from bran are incorporated into flour. Variations in the content of a particular substance in flour due to variations in milling yield will be more noticeable with greater differences in its level relative to the endosperm and the other fractions. Data in Fig. 1 show a fairly even distribution of SP from the inner to the outer layers of the endosperm. A variation of milling yield between 75% and 80%, which implies a sharp increment in ash, does not significantly change SP content. The total variation intervals for SP amounted to 21% of the flour values in Magdalena, 12% in Aragón 03, and 10% in Hibrído-D. Even so, variability due to maximum changes in milling yield is considerably smaller than intervarietal difference within species, as will be seen later.

In Fig. 2, the same arrangement of milling fractions of Fig. 1 has been kept, fat and SP being similarly plotted. Since fat content is greatly dependent on milling yield and its distribution in the kernel does not parallel that of SP, more

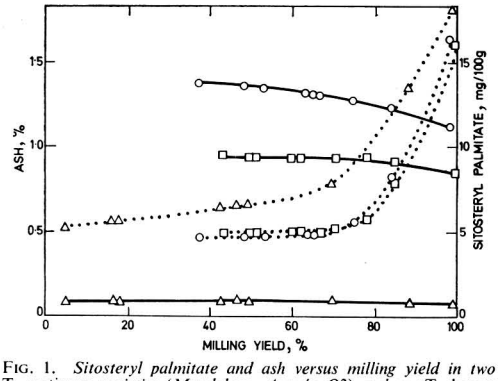

FIG. 1. *Sitosteryl palmitate and ash versus milling yield in two* T. aestivum *varieties (Magdalena, Aragón O3) and one* T. durum *(Hibrido-D)*

○ Magdalena □ Aragón-O3 △ Hibrido-D
·····Ash ——— Sitosteryl palmitate

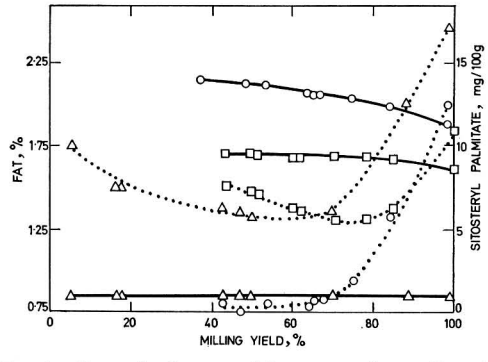

FIG. 2. *Sitosteryl palmitate and fat versus milling yield in the same varieties of Fig.* 1

○ Magdalena □ Aragón-O3 △ Hibrido-D
·····Fat ——— Sitosteryl palmitate

TABLE I

Distribution of sitosteryl palmitate in milling fractions†

Name of Product	Fraction, %			Ash, %			Fat, %			Sitosteryl palmitate mg/100 g		
	A	M	D	A	M	D	A	M	D	A	M	D
Flour*	69·9	68·0	49·6	0·57	0·55	0·72	1·23	1·05	1·33	9·4	13·1	0·9
Re-milled flour	8·0	7·9	20·2	1·01	1·14	1·12	1·45	1·85	1·45	9·1	11·3	1·0
Shorts	8·6	9·0	18·5	2·88	3·16	3·60	2·90	4·60	4·60	6·9	8·2	0·5
Bran	13·3	14·3	10·8	6·94	6·41	5·59	4·15	5·90	5·80	4·7	4·1	0·5
Whole wheat**	99·8	99·2	99·1	1·65	1·68	1·87	1·78	2·08	2·45	8·5	11·1	0·8

† All data refer to dry matter
* Pooled break and reduction flours
A, Aragón O3; M, Magdalena; D, Hibrido-D
** Sum of previous fractions

reproducible results will be obtained by referring SP to dry matter. In view of these results, the SP content of normally milled flours has been adopted in these studies.

Fig. 3 (a) shows the results obtained for SP content of flour in a survey of 46 *T. aestivum* and 24 *T. durum* varieties. The *T. aestivum* distribution seems to show two maxima at about 4 mg and 12 mg respectively. All *T. durum* varieties are included in the 0·1–1·5 mg/100 g of flour interval. Only 3 *T. aestivum* varieties are actually included in this interval and 3 more are included in the next interval (1·6–3·0 mg/100 g). The conclusion to be drawn from these results is that in flour SP levels above 1·5 mg/100 g indicate the presence of *T. aestivum* endosperm in pasta products. Since there are three *T. aestivum* varieties included in the *T. durum* interval, SP contents lower than 1·5 mg/100 g do not guarantee purity. For a given SP level, the minimum percentage of *T. aestivum* present can be calculated in terms of the maximum SP content found for this species. This minimum is shown in Fig. 3 (b) as a function of SP content. The higher the SP content the closer the estimated minimum will be to the true *T. aestivum* percentage of the mixture.

Although the existence of some *T. aestivum* varieties with SP values similar to those of *T. durum* implies that this difference cannot be used alone to solve the problem, the test is useful because it allows detection of most *T. aestivum* varieties. Since other interspecific biochemical differences are bound to show similar problems, i.e., intraspecific variability and some exceptions, more than one test will probably have to be used not only for qualitative identification, but for a better quantitative estimation of the whole range of *T. aestivum* varieties. In this connexion, several other interspecific differences have been found at this laboratory, and are being confirmed at present.

Although a high number of wheat varieties were used, and these were diverse in origin, the limits proposed for this interspecific difference are only tentative and many more varieties should be tested in other countries.

It has been shown that high SP content is associated with the D genome of *T. aestivum*[10] and is not due to an interaction of this genome with genomes A and B, which are present in both *T. durum* and *T. aestivum*. The distribution of the two maxima in Fig. 3 (a) suggests a simple genetic control for this biochemical characteristic. Therefore, it should be pointed out that as a result of breeding programmes new varieties can make this or other tests obsolete, so that then new interspecific differences should be available to cope with these changes.

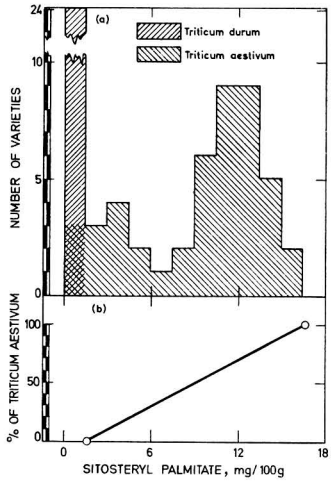

FIG. 3 (a). *Distribution of sitosteryl palmitate in the* T. aestivum *and* T. durum *species.* (b) *Minimum proportion of* T. aestivum *in a mixture versus sitosteryl palmitate content*

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IDENTIFICATION OF ETHYLENE IN GIBBERELLIC-ACID-TREATED POTATOES

By P. A. POAPST, A. B. DURKEE, W. A. McGUGAN and F. B. JOHNSTON

Internal ethylene was evacuated from cold-stored (39° F) Kennebec potato tubers and identified by means of mass spectrometry, and gas and paper chromatography. The ethylene content of normal tubers after 7 months' storage was $0{\cdot}7 \times 10^{-3}$ μg/g fresh weight and increased when tubers were treated with gibberellic acid.

Introduction

The rôle played by ethylene in the dormant period of the potato tuber is uncertain, and experiments have been described in which applied ethylene stimulated or inhibited sprouting.[1–5] Burton[6] has suggested that concentrations of growth-inhibiting volatiles (including ethylene) in the cell sap may have important implications in an explanation of dormancy. Ethylene might also have stimulating effects near the end of, or after, this period. Ethylene has been detected in the atmosphere above potato stores, and it was suggested that it emanated from the tubers.[7] While this was a reasonable suggestion, it is known that ethylene can originate from many sources, including strains of ubiquitous *Penicillium digitatum*[8–10] and from various other fungi.[11–13] In any case, it would be difficult to ascertain cell sap concentrations of ethylene from measurements of atmospheric concentration. It therefore appeared necessary as a prelude to subsequent studies on the influence of endogenous ethylene on dormancy in the potato tuber, to establish unequivocally its presence in the tissues, and to determine concentrations by analysis of the tissues.

Experimental

Potato tubers (*Solanum tuberosum* cv. Kennebec) stored for 4–7 months at 39° F were used. These were washed, treated with 42 ppm aqueous solutions of gibberellic acid (GA_3) (Fisher Scientific), kept for 3–6 days under warm and humid conditions (78° F and 90% relative humidity), washed again, sliced and then refluxed in 500 g amounts as described by Nelson.[14] Evacuated volatiles were washed with mercury perchlorate[15] which was then retained at 32° F for qualitative tests. Absorbed volatiles were liberated with 2 N-HCl. Three methods were used to determine the presence of ethylene:

Ascending paper chromatography

The relative mobilities of the mercuric acetate addition products formed with the volatiles previously liberated by HCl hydrolysis and the mercuric acetate addition product formed with authentic ethylene were compared.[9] Dilutions prepared from the extracts of 1000 g of tissue were transferred to Whatman No. 1 paper. Diphenyl carbazone and ultraviolet light were used to detect the addition products.

Gas chromatography

Retention times of perchlorate-absorbed volatiles (representing 2–20 g of tissue) were compared with authentic ethylene on a silica gel column (Perkin–Elmer column J) using a Perkin–Elmer Model 154 D equipped with a hydrogen flame ionisation detector.

Mass spectrometry

The mass spectra of potato volatiles (representing 500 g tissue) were compared with those from a perchlorate reference sample containing 31 μg ethylene, using a Bendix Model 12 'time-of-flight' mass spectrometer operating at 70 eV. A reaction vessel and sampling tube were evacuated to 10^{-3} torr (approx.). The perchlorate solution was admitted to the reaction vessel, followed by 2 N-HCl. The volatiles liberated were allowed to diffuse into and condense in the sampling tube, which was cooled in liquid nitrogen. The sampling tube was fitted to the mass spectrometer inlet system and evacuated to 10^{-6} torr (approx.) while held in liquid nitrogen. The tube was allowed to warm gradually, while spectra were obtained of the volatilising condensate. A small correction made to the *m/e* (mass/charge) 28 peak to remove the contribution of N_2 was based on the intensity of the O_2 at *m/e* 32.

To clarify further the concept that ethylene is an endogenous constituent of potatoes, and that its presence in extracts cannot be attributed to abnormal metabolism or to the mild boiling procedures used, gas samples were removed from 3 kg of pared central tissues of tubers stored continuously at 39° F. The tissue was placed in a flask connected to a trap at −112° F (dry ice–ethanol), a perchlorate solution trap at 32° F, and a mechanical pump. Volatiles were removed from the tissues at a pressure of 10 torr. The amount of ethylene recovered from the perchlorate trap was estimated by comparisons with known amounts of authentic ethylene using gas chromatography.

Some observations were made on the ethylene content of potatoes stored for 7 months at 39° F, and then wetted with 0, and 42 ppm GA_3 solutions 4 days before removal; or removed and wetted with 0, and 42 ppm GA_3 solutions, then kept for 3 days at 78° F. Gas samples collected from 200 g of tissue[14] immediately after the tubers had been removed from storage were examined by gas chromatography without prior absorption in mercury perchlorate solution. Concentrations were assessed by comparison with known dilutions of authentic ethylene. Samples were injected in 2·5, 5·0 and 30·0 ml amounts into the column. Data pertaining to the largest injection were increased by one fifth, to correct for non-linearity of response.

Results and Discussion

That ethylene was one of the constituents evacuated from the internal tissues may be seen in Tables I and II and Fig. 1. Good agreement was observed between the mobilities of authentic ethylene and the test samples in paper and gas chromatography, and between the mass spectra.

Examination of the samples removed by vacuum pump indicated ethylene concentrations slightly greater than $0{\cdot}04 \times 10^{-3}$ μg/g fresh weight. While this figure is un-

TABLE I

Ascending paper chromatography R_f values of mercuric acetate addition complexes of authentic ethylene, and of unknown substance from gibberellic acid-treated potatoes

Solvent system	Respective proportions (v/v)	R_f of complexes	
		unknown	ethylene
Propan-1-ol Ammonium carbonate (12% w/v) Ammonium hydroxide (20% v/v)	5 : 1 : 1	0·34	0·36
Butan-1-ol Ethanol (95% v/v) Ammonia (28% w/w)	8 : 1 : 3	0·45	0·44
Propan-2-ol Ammonium carbonate (6% w/v) Ammonium hydroxide (20% v/v)	2·5 : 1 : 1	0·61	0·61

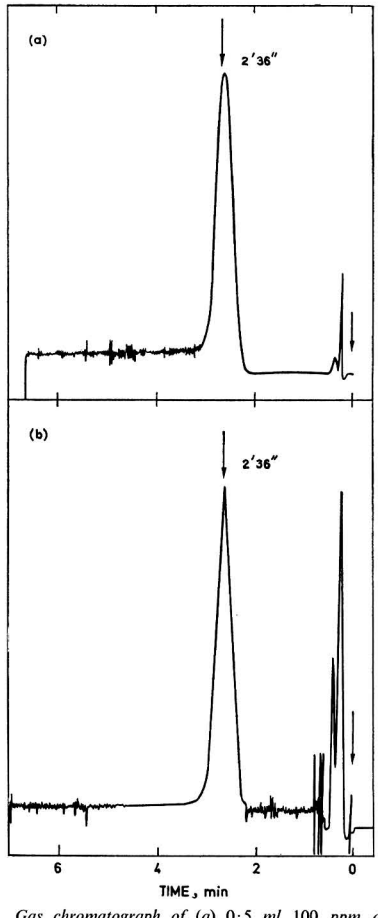

FIG. 1. *Gas chromatograph of (a) 0·5 ml 100 ppm authentic ethylene, (b) 5·0 ml of mercury perchlorate-trapped volatiles from 15 g gibberellic acid-treated potatoes*
Ethylene peak is shown

TABLE II

Mass spectrum data for authentic ethylene and unknown substance from gibberellic acid-treated potatoes compared with Dow data[16]

m/e	Relative Intensities		
	Dow	Reference (authentic C_2H_4)	Unknown substance
28	100	100	100
27	62	63	61
26	53	54	53

realistically low (attributable to incomplete evacuation of the fresh tissues), there appears to be little doubt that ethylene gas is a naturally occurring internal constituent of potatoes.

Some analyses of whole tubers may be seen in Table III. Under the conditions of the experiment, GA_3-treated tubers contained more ethylene than the control tubers. The stimulating effect of added GA_3, combined with exposure to higher temperature, was observed early in this study; and this treatment was incorporated into the experimental procedures used because ethylene concentrations in mid-winter had declined to very low levels. In the following year, the stimulating effect of GA_3 on ethylene accumulation in potatoes stored for 7 months at 39° F, then treated and kept at 39° F, was confirmed with a distinctly different technique of ethylene collection; details of this method and associated findings will be published later.

Various applied plant hormones, including GA_3, have been reported to increase the evolution of ethylene from several plant tissues.[17–19] This prompted consideration by Abeles & Rubinstein of an earlier hypothesis[19] that part of the effect of hormone application may be traced to the accompanying production of ethylene. There is accumulating evidence of hormonal interactions with ethylene; however support for this hypothesis is perhaps more apparent in certain plant organs and physiological mechanisms than in a general way. Ethylene, like gibberellin, can break dormancy in a variety of plant organs;[2] but the latter is known to have a much more positive effect on potato tubers. Although the results in Table III may give some support to this contention, it would be of further interest to know the concentration of ethylene in gibberellin-treated potatoes during a considerable interval, and also to compare this concentration with the rate of sprout development.

TABLE III

Ethylene concentrations in Kennebec tubers treated and untreated with gibberellic acid at the end of 7 months' storage

Treatment	Ethylene ug/g fresh wt. × 10^3
39°F storage	0·7
39°F storage + GA_3	2·2
39°F storage + 3 days at 78°F	1·7
39°F storage + GA_3 + 3 days at 78°F	2·5

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DIGESTIBILITY *IN VITRO* AND AVAILABLE LYSINE CONTENTS OF INDIAN OILSEED MEALS

By N. VENKATESAN and D. V. REGE

Experiments with untreated and autoclaved meals of mowra (*Bassia latifolia*), pisa (*Actinodaphne hookeri*) and undi (*Calophyllum inophyllum*) are reported. The three meals were digested by pepsin to approximately the same extent as casein. While pisa and undi meals were fairly rapidly degraded by trypsin, mowra meal was cleaved slowly, but did not possess any trypsin inhibiting activity. Destruction and inactivation of amino acids were caused by autoclaving the meals. The presence of naturally occurring trypsin-resistant bonds in mowra meal protein is inferred. The nutritional significance of the amounts of FDNB-reactive lysine units in these meals is discussed.

Introduction

Work is in progress in this laboratory on the possible utilisation of three oilseed meals, namely, mowra (*Bassia latifolia*), pisa (*Actinodaphne hookeri*) and undi (*Calophyllum inophyllum*), which are reported to be available in large quantities in India.[1] Earlier investigations[2,3] on the protein value of these meals showed fairly good correlation between composition of essential amino acids and rat growth assay for pisa and undi meals but not for mowra meal. The importance of the ease of digestibility of proteins, in addition to their content of essential amino acids, in determining the overall nutritive value has long been recognised. The extent of breakdown *in vitro* of proteinaceous materials by purified enzymes often gives valuable information on the availability

of amino acids to the host *in vivo* and the presence of any enzyme inhibitors as well as the effects of processing conditions. The digestibility *in vitro* of untreated and heated meals of mowra, pisa and undi was therefore studied.

Substrate specificity requirements for proteolytic enzymes have been recognised.[4-6] It has also been shown that lysine units with the ε-amino group blocked are not nutritionally active,[7-9] and only those lysine units having free ε-amino groups are available. In the present communication, the available lysine contents of the three meals and the effects of heat on these values are also reported.

Experimental

Materials

Expeller-pressed mowra, pisa and undi meals were further extracted with petroleum ether (boiling point range 66–69°) in a Soxhlet extractor to remove residual fat and then the solvent was removed in a blast of air at temperatures below 50°. Since the untreated mowra meal could not be used in biological trials owing to its intense bitter taste, it was de-bittered by being extracted with 5 volumes of 70% ethanol for 1 h at room temperature (28–32°) and filtered; the residue was washed free of the traces of bitter principle with the solvent. This meal was then dried at temperatures below 50°.

Heat-treated meals were obtained by autoclaving the untreated meals at 120° for 1/2 h.

The enzymes used for *in vitro* digestion studies were pepsin (1 : 3,000 BP 1953, A. Gostantino & C. Favria, Italy) and trypsin (approx. 20,000 Fuld-gloss units per g; E. Merck A.-G., Darmstadt).

Methods

Nitrogen determination

The nitrogen contents of untreated and autoclaved meals were estimated by micro-Kjeldahl digestion and Nesslerisation.[10]

Analyses for total and available lysine

10 g portions of the meals were hydrolysed with 100 ml 6N-HCl under gentle reflux for 20 h, decolorised by being shaken for 30 min with Norite (2 g/100 ml), made up to 250 ml after filtration and analysed for lysine by the microbiological assay method,[11] in which *Leuconostoc mesenteroides* was used as the test organism. In recovery experiments it was observed that about 3% of the lysine was lost on the Norite.

Available lysine was estimated by the 2,4-dinitrol-1-fluorobenzene (FDNB) method of Carpenter.[12] Loss of ε-DNP-lysine during hydrolysis was not estimated.

Enzyme digestion procedure

Known amounts of substrate were suspended in 100 ml of the medium (at optimum pH) containing the enzyme at the required concentration, and the whole was incubated at 37° with occasional shaking. Toluene was used as the preservative. 0·2 M phosphate buffer (pH 8·0) and 0·05 N-HCl were the media for trypsin and pepsin hydrolyses, respectively. At known intervals of time, 8 ml of the digest were treated with 2 ml 50% trichloacetic acid to precipitate the proteins; these were centrifuged, and the supernatant was analysed for amino nitrogen by the method of Pope & Stevens.[13]

For the sequential pepsin and trypsin digestion experiments, the volume of the pepsin digestion system was 50 ml; after pepsin digestion the pH of the solution was brought to 8·0 by the addition of NaOH; 0·2 M phsophate buffer (pH 8·0) containing trypsin was added to a final volume of 100 ml and digestion was continued. In all the experiments, suitable enzyme blanks and casein controls were used. The results reported in Tables II–IV have been corrected for enzyme blanks.

Total amino nitrogen

Known amounts of substrates (10 g of meal or 3 g of casein) were refluxed gently with 150 ml 6 N-HCl for 20 h, filtered and made up to 250 ml. Portions of these hydrolysates were analysed for amino nitrogen. This acid treatment was assumed to hydrolyse the protein completely. Percentage hydrolysis was calculated from the amino nitrogen in the acid hydrolysate and that in the enzyme digest at the start of the digestion and after a known period of incubation, as suggested by Melnick & Olser.[14]

Preparation of extracts of mowra meal

The extracts of de-bittered untreated mowra meal used in experiments on trypsin inhibitor activity (Table IV) were prepared by suspending 10 g of the meal in 100 ml of the extractant at 4° overnight followed by 2 h of mechanical agitation at room temperature (30°) and filtration. The extracts were adjusted to pH 8·0 and then added to the digestion mixture.

Results

The protein contents of the meals were: mowra 17%, pisa 39%, and undi 21%. The total and available lysine contents of untreated and autoclaved meals are presented in Table I. Autoclaving did not destroy lysine in the three meals under study, since the same amounts were obtained by acid hydrolysis and microbiological response for both the untreated and heated meals. Only a small proportion (29–37%) of total ε-NH_2 groups present in proteins in untreated mowra and pisa meals reacted with FDNB, whereas from the undi meal protein 80% of the total ε-NH_2 groups reacted with FDNB. After autoclaving, the proportion of FDNB-reactive ε-NH_2 groups of lysine was unaffected in the case of pisa meal protein, but increased in mowra meal protein and was reduced in undi meal protein.

Table II summarises the results of pepsin, trypsin, and combined sequential digestions of casein, and of untreated and heated meals. The three untreated meals were susceptible to pepsin attack to about the same extent as casein. Mowra meal was very slowly digested by trypsin whereas the other two meals were about as susceptible to trypsin as casein. There was no percpetible improvement in the digestibility of mowra meal with trypsin after preliminary degradation by pepsin or as a result of heat treatment.

When mowra meal and casein together were exposed to trypsin (Table III), it was observed that there was a slight decrease in the amount of casein degraded. Extracts of mowra meal prepared with different reagents appeared to have little influence on the trypsin digestion of casein (Table IV).

Discussion

Autoclaving the meals had no effect on the amount of lysine recoverable by acid hydrolysis, showing thereby that no destruction has occurred. However, different situations were encountered as regards FDNB-reactive ε-NH_2 groups.

The fall in the number of FDNB-reactive groups when undi meal was heated, may be attributed to the interaction of ε-NH_2 groups with —COOH groups of other amino acid residues and/or the reducing groups of accompanying carbohydrates, to form enzyme-resistant linkages, a phenomenon generally referred to as inactivation.[15] Heat treatment exposes some ε-NH_2 groups for FDNB action in the case of mowra meal.

The proportion of FDNB-reactive ε-NH_2 groups is small for mowra and pisa meals. All the free ε-NH_2 groups present in different haemoglobins have been found to react with FDNB.[16] On the other hand, 12 out of 31 lysine ε-NH_2 groups per molecule of untreated β-lactoglobulin and 23 out of 80 of native horse serum pseudoglobulin do not react with this reagent.[17] On denaturation by acid, heat, alcohol or guanidine treatment, almost all of the ε-NH_2 groups of lysine in β-lactoglobulin reacted with FDNB.[17] The reactivity of ε-NH_2 groups to different reagents may vary. Thus, in β-lactoglobulin, while only one-third of ε-NH_2 groups were found to react with FDNB, all of them could be acetylated by ketene or acetic anhydride. In contrast, in native serum pseudoglobulin the same number of ε-NH_2

TABLE I

Total and available lysine contents of untreated and autoclaved meals

Meal		Total lysine (Acid hydrolysis) g/16 g N	Available lysine (FDNB-reactive) g/16 g N	% ε-NH_2 groups (FDNB-reactive)
Mowra meal				
	untreated	4·6	1·7	37
	autoclaved	4·5	2·2	49
Pisa meal				
	untreated	4·7	1·3	29
	autoclaved	4·7	1·3	29
Undi meal				
	untreated	3·3	2·6	80
	autoclaved	3·2	1·9	60

Total lysine was assayed microbiologically[11] in an aliquot of the acid-hydrolysed sample after decolorisation with Norite. Available lysine was determined by the dinitrophenylation method of Carpenter[12]

TABLE II

Proteolysis in vitro of casein and untreated and autoclaved meals

Enzyme	Hours of digestion	Casein		De-bittered mowra meal 4 g		Pisa meal 4 g		Undi meal 4 g	
		1g	2 g						
				untreated	autoclaved	untreated	autoclaved	untreated	autoclaved
Trypsin (200 mg)	48	24·2	29·8	4·6	3·7	30·4	26·8	28·9	42·1
	120	28·9	33·4	9·2	7·4	38·2	35·7	31·2	45·3
Pepsin (200 mg)	48	19·6	16·5	17·6	13·8	20·2	18·8	20·2	27·1
	120	25·8	20·2	18·9	14·7	23·8	19·4	27·6	32·2
Pepsin (500 mg)	48	41·1	30·8	29·8	18·6	39·3	35·8	38·7	46·2
+									
Trypsin (500 mg)	48	10·7	13·2	3·1	2·1	14·2	13·3	11·2	15·3

Amino nitrogen was determined according to the method of Pope & Stevens[13] in de-proteinised enzyme-digested mixture as well as acid-hydrolysed samples. Percentage hydrolysis was calculated by assuming acid hydrolysis to be 100%

The sets of figures opposite pepsin (500 mg) and trypsin (500 mg) represent percentage hydrolysis with pepsin alone and additional hydrolysis with trypsin.

TABLE III

Effect of de-bittered mowra meal on trypsin digestion of casein

	1	2	3	4	5	6	7
Casein (g)	3	3	3	3	3	1·5	—
Mowra meal (g)	—	1	2	3	6	4·5	9
% hydrolysis in 24 h	14·9	14·4	14·7	11·2	11·5	14·0	3·7

The system contained 400 mg trypsin; amino nitrogen of mowra meal was not taken into account in calculating % hydrolysis for Experiments 1–6

TABLE IV

Effect of extracts of de-bittered mowra meal on trypsin digestion of casein

Addition	% hydrolysis in 24 h
1. Nil	15·1
2. Water extract	15·3
3. 10% NaCl extract	15·2
4. 0·05 N-HCl extract	15·1
5. 0·5 N-HCl extract	15·0
6. 0·2% NaOH extract	14·9

The system contained 3 g casein, 400 mg trypsin and 10 ml neutralised extract equivalent to 1 g de-bittered mowra meal in a total volume of 100 ml. Other details about preparation of extracts and calculation of % hydrolysis are given in the text

groups (i.e. 23) do not respond to FDNB as to these acetylating agents. The increased response to the different reagents on denaturation was almost identical.[17] In intact soft tissue collagen, only about two-thirds of the ε-NH_2 groups could react with FDNB;[18] however, the concentrations of reactive ε-NH_2 groups of hard tissue collagens increased from very low levels to almost the value that is theoretically possible at complete calcification.

These observations show that some of the ε-NH_2 groups of lysine residues may lie within the protein molecule and thus may be unable to react with chemical agents, but they can be exposed by suitable treatment. The response of individual proteins to these physico-chemical treatments may, however, vary, depending on the nature of the protein environs.

In addition to this inherent chemical reactivity of ε-NH_2 groups in a particular protein, the validity of the method must also be considered. Large losses of ε-DNP-lysine occurred during hydrolysis of DNP-pelastin, and even with specially modified conditions only 65% of the added compound is recovered.[19] However, Carpenter[12] found his method gave consistently reliable results for a number of animal protein foods. Rao *et al.*[20] showed that most of the ε-NH_2 groups of cottonseed, groundnut, soyabean and sesame meals were free to react with FDNB. The protein environs and the nature of carbohydrate materials of these meals might differ from those of the test meals.

The effect of heat treatment experienced by the meal proteins during the expeller treatment of the seeds should also be considered. Losses in lysine attendant upon the processing of sesame[21] and groundnut[22] seeds have been noted. When heat is avoided in the processing of cottonseed, all of the lysine in the meal is available.[23]

The resistance of mowra meal to trypsin attack prompted a search for the presence of any possible inhibitor. That heating the meal did not increase the ease of digestibility, indicated the absence of any heat-labile trypsin inhibitor. Further support for this conclusion comes from the absence of inhibition of trypsin digestion of casein by the untreated meal or the different extracts of the meal. Extracts of untreated soyabeans at pH 4·2 have been shown to contain the inhibitor which retards the *in vitro* activity to trypsin.[24,25] The slight decrease in the trypsin digestion of casein in the presence of appreciable amounts of mowra meal is presumably due to the lower availability of trypsin to attack casein in the presence of meal of low digestibility. There is an appreciable decrease on autoclaving the meals, in the total amino nitrogen recovered by acid hydrolysis, (11·7% for pisa, 17·0% for mowra and 34·0% for undi meals). This large decrease could be due to the appreciable amounts of carbohydrate-like material reacting with unbound amino groups and resulting in destruction of amino acids.[26–28] However, under these conditions of amino N losses the amount of lysine recovered was unaltered.

As was pointed out by Melnick & Oser,[14] some valuable conclusions can be drawn by comparing unt eated proteins with processed products on the basis of *in vitro* susceptibility to enzymic digestion. They have also emphasised that the rate of enzymic liberation of amino acids, rather than the degree of amino acid availability is of critical importance in determining the efficiency with which the absorbed amino acids are retained for tissue protein synthesis. Indications of improved pancreatic enzymolysis *in vitro* owing to heat treatment are not necessarily reflected in feeding tests with higher animals, but if the application of heat to a product is severe enough to depress measurably the hydrolysis of its proteins by proteases *in vitro*, this change is often detectable by feeding trials.

In the present study, autoclaving decreased the rates of both pepsin and trypsin hydrolyses for mowra and pisa meals presumably because of destruction and inactivation. The large increase in the rates of pepsin and trypsin digestion of undi meal on heating is open to question, since the calculation of % hydrolysis is based upon the total amino nitrogen recovered by acid hydrolysis as 100, and this amount is reduced very much on autoclaving.

The carbohydrate-like materials in mowra meal could have been responsible for its poor degradation by trypsin in view of the reported retarding effect of mono- and di-saccharides on trypsin[29] and μ-amylase.[30] However, even isolated mowra protein was not rapidly digested by trypsin, and mowra meal extracts fail to inhibit trypsin *in vitro* (Venkatesan & Rege, unpublished work). These results indicate the presence of natural enzyme-resistant linkages in the native structure of the protein molecule analogous to those found in arachin[31] and several plant proteins.[32] Haurowitz *et al.*[33] observed that denaturation is required for efficient attack by enzymes of proteins such as egg albumin and serum globulins because the groups which the enzymes attack are relatively inaccessible. This arises from the positions of these groups inside the closely packed peptide chains of the protein molecule. In the present work, heat treatment is not effective in rendering the lysine groups of mowra meal accessible for trypsin attack. Disproportionately large amounts of the total lysine and histidine were found in that fraction of arachin that was resistant to digestion *in vitro*.[31] The possibility of such peculiarities of protein structure and of sequence of amino acids, resulting in poor digestibility of mowra meal cannot be precluded.

It is rather difficult to find any direct relation between available lysine content and susceptibility to trypsin cleavage. For example, FDNB-reactive ε-NH_2 lysine is 37% for mowra meal and 29% for pisa meal, but the former meal is resistant, and the latter highly susceptible to trypsin attack. On autoclaving, this figure increases to 49% for mowra meal, without concomitant improvement in the rate of digestion *in vitro*. These data point to the possibility that some ε-NH_2 groups do not react with FDNB but contribute to the proteolytic breakdown of protein.

The observations[2,3] of the fairly good correlation between content of essential amino acids in pisa and undi meals and growth response of rats also indicate that the 'available lysine' values may not necessarily reflect the protein quality.

These results suggest that while pisa and undi meals could be digested without difficulty by higher animals, mowra meal would be degraded very slowly. This, however, could be confirmed only by actual feeding tests. For example, some peptides from zein which resisted pepsin and trypsin action *in vitro* were found to be split in the intact rat.[34] In view of the considerable amount of destruction of amino acids, consequent upon heating the meals, it is logical to expect impaired nutritional value for autoclaved meals.

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SOIL SALINITY STUDIES

II.*—The relation of plant growth to salinity in soil and soil mixtures of differing physical properties

By D. M. MASSEY and G. W. WINSOR

Lettuce plants were grown at six levels of salinity in soil and in mixtures of soil with peat and sand. Soil salinity was determined in saturation pastes and paste extracts, and in 2·5 : 1 extracts prepared by weight and by volume, with and without added calcium sulphate. The correlations between plant growth (fresh weight) and salinity were calculated from two sets of data, each based on four lettuce crops. Sampling by volume rather than by weight for salinity measurements improved the correlations with growth, and gave a reasonably satisfactory measure of salinity over the range of growth media examined (bulk density 0·82–1·37 after air-drying and grinding). Still higher correlations resulted ($r = -0·95$ to $-0·98$) when corrections were applied for differences in bulk density. It is concluded that 2·5 : 1 extracts prepared by volume, saturated with calcium sulphate and corrected where necessary for bulk density, can provide a rapid and reliable estimate of high salinity levels in soils differing considerably in moisture-retaining properties.

Introduction

The merits of various procedures for the determination of soil salinity were discussed in a previous paper.[1] Although displaced soil solutions provide the most fundamental approach, the technique is too slow for routine use. Saturation extracts[2] provide a somewhat more rapid technique whilst retaining some of the advantages of displaced soil solutions, but the procedure is still somewhat time-consuming where large numbers of samples are involved and has not been adopted widely in this country. Extracts prepared at known water : soil ratios (e.g. 2·5 : 1, 5 : 1) are far more convenient for routine use. Errors due to sparingly soluble calcium sulphate in old glasshouse soils may be overcome by saturation with excess of this salt.[1] The question still arises, however, as to whether soil extracts can provide a satisfactory measure of salinity when applied to soils of differing water-holding capacity; saturation pastes, for example, automatically provide some compensation for this factor. The object of the present work was to study the relation between plant growth and soil salinity in media differing in water-retaining properties and bulk density.

Experimental

Glasshouse trials

Thirty six concrete troughs were constructed, each measuring 18 by 27 in. internally and filled to a depth of 8 in. with the various soil mixtures. The rooting media consisted of: field soil, a silt loam; a mixture of soil (two-thirds by volume) and sphagnum peat; and a mixture of soil (two-thirds by volume) and sand.

A mixture of potassium nitrate and sodium nitrate (K_2O/N ratio = 1·5) was added at rates of 0·2, 0·35, 0·5, 0·65, 0·8 and 0·95% (w/v) to give a range of high salinity values in the composts. Lime (carbonate) was added to maintain the pH in the range 6·6–6·8, and superphosphate was applied uniformly throughout.

Two replicates of the eighteen combinations of growing medium and salinity were provided.

The beds were watered by hand. Tensiometers (one or more per plot) were introduced for observation in Experiment 7, and were used as a guide to watering in Experiments 8–12; the tops of the porous pots were placed 3 in. (later 2 in.) below the soil surface. Each plot was brought to field capacity when the tension had reached 8 cm Hg, the leachate being returned to the bed.

The lettuce varieties planted were Southdown 5B or Cheshunt 5B (from which Southdown 5B was selected) in the autumn and winter, with other varieties (May Queen, May Princess, Cobham Green and Feltham King) in the spring or summer. In order to avoid managanese toxicity the first two lettuce crops were grown without steam sterilisation, but showed much variability in growth. Unsatisfactory results were also obtained with other test crops (tomatoes, peas and beans) in Experiments 3 and 4, and the beds were therefore steamed for further experiments (5–8) with lettuce. The beds were later re-soiled with steamed loam for a further four lettuce crops (Experiments 9–12). The test crops were not grown to maturity, the average period of growth being 40 days. Each plot contained twelve lettuce plants.

Soil sampling and salinity measurements

The soil samples consisted of at least eight vertical borings per plot, made with a 1¼ in. auger to the full depth of the beds. The soil was air-dried and ground to pass a 2 mm sieve.

Saturation pastes were made by the procedure of the United States Salinity Laboratory;[2] the pastes were either filtered under vacuum (Experiments 1–8) or centrifuged (Experiments 9–12) to give a clear extract. The electrical conductivity of the pastes was also measured directly, using a cell with annular carbon electrodes (Electronic Switchgear Ltd.). The 2·5 : 1 water/soil suspensions by weight were prepared by shaking 20 g air-dried ground soil with 50 ml distilled water for 30 minutes and standing for 30 minutes. A 20 ml brass cup (2·8 cm internal diameter), filled without tapping or com-

* Part I: *J. Sci. Fd Agric.*, 1963, **14**, 42

paction, was used for sampling on a volume basis; the weight of the contents of the calibrated container was used in the calculation of bulk densities. Both weight and volume tests (2·5 : 1) were repeated with the addition of an excess of calcium sulphate (0·3 g $CaSO_4 \cdot 2H_2O$).[1] For the data shown in Figs. 2, 3, 5, 6 and 8 the aqueous suspensions were centrifuged to give clear extracts. All conductivity measurements (other than on soil pastes) were made in a water bath at 20°, using an a.c. bridge.

Results

Correlations between growth (fresh weight) and salinity in Experiments 5–8 (combined)

The correlation coefficients of fresh weight with the various salinity tests are given in Table I.

All the salinity procedures tested were significantly correlated with plant growth, but in many cases were seriously affected by differences in soil texture. Thus the coefficients of determination ($r^2 \times 100$), giving a measure of the proportion of the total variation accounted for by linear regression, show a wide range from 27 to 91%.

As was expected, the saturation extracts gave a very satisfactory correlation with plant growth (Fig. 1). Among the four variants of the 2·5 : 1 water/soil extracts clarified by centrifuging, sampling by volume (Fig. 2a, 3a) was more satisfactory than sampling by weight (Fig. 2b, 3b). Saturation with calcium sulphate improved the correlation whether sampling was by weight or volume.

The correlations between fresh weight and the conductivity of the 2·5 : 1 soil suspensions (not centrifuged or filtered) were, somewhat surprisingly, higher than those for the corresponding clear extracts. This was apparently due to different degrees of settling of the suspensions according to texture. Centrifugation increased the conductivity more for soil than for soil–sand, and thus further separated the regression lines and slightly decreased the overall correlations.

The soil suspensions prepared (by weight) with 25% aqueous acetone gave a correlation coefficient (−0·70) intermediate between those for the corresponding aqueous suspensions with and without calcium sulphate (−0·84 and −0·60, respectively). The use of aqueous acetone was based on the decreased solubility of calcium sulphate in the presence of organic solvents such as acetone or ethanol,[3] thus providing an alternative method of minimising interference from this sparingly soluble salt.

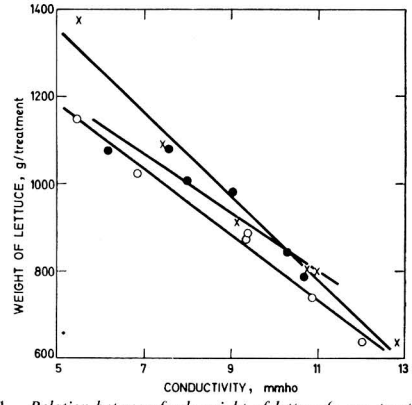

FIG. 1. *Relation between fresh weight of lettuce (g per treatment; 24 plants) and the conductivity (millimhos) of saturated soil extracts. Experiments 5–8 combined.*

● Soil × Soil + Peat ○ Soil + Sand

Correlations between growth and salinity in Experiments 9–12

In the earlier experiments (1–8) no further additions of potassium and sodium nitrates were made after the initial filling of the beds. The salinity levels thus decreased somewhat throughout the series. In Experiments 9–12, however, the salinity levels were maintained by small additions of salts before each successive crop. The salinity was therefore higher in the last four experiments (9–12), and plant growth was more severely restricted. The relation between plant weight and soil salinity was no longer linear, and the data were examined after logarithmic transformation. The correlation coefficients are given in Table II.

In every case the correlation coefficients were higher than the corresponding values in Table I, and all were significant at P = 0·001. The saturation extracts again gave the highest correlation of all (Fig. 4), but not significantly higher than the

TABLE I

Correlations between average fresh weight of lettuce plants and soil salinity measurements (Experiments 5–8 combined)

Salinity test		Correlation coefficient r	Significance P	Coefficient of determination %
Saturation extract		−0·954	0·001	91·0
Saturation paste		−0·685	0·01	46·9
2·5 : 1 water:soil extracts	by volume	−0·746	0·001	55·7
	" " + $CaSO_4$	−0·814	0·001	66·3
	by weight	−0·518	0·05	26·8
	" " + $CaSO_4$	−0·673	0·01	45·3
2·5 : 1 water:soil suspensions	by volume	−0·802	0·001	64·3
	" " + $CaSO_4$	−0·889	0·001	79·0
	by weight	−0·602	0·01	36·2
	" " + $CaSO_4$	−0·843	0·001	71·1
2·5 : 1 suspension (by weight) in aqueous acetone*		−0·701	0·01	49·1

* 1 part acetone + 3 parts water by volume, pre-saturated with calcium sulphate

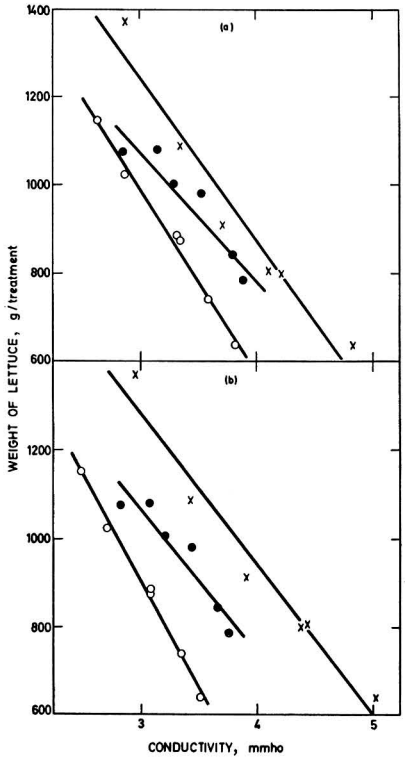

FIG. 2. *Relation between fresh weight (g) of lettuce and the conductivity (millimhos) of centrifuged 2·5 : 1 water:soil extracts + $CaSO_4$ prepared (a) by volume and (b) by weight. Experiments 5–8 combined.*

● Soil × Soil + Peat ○ Soil + Sand

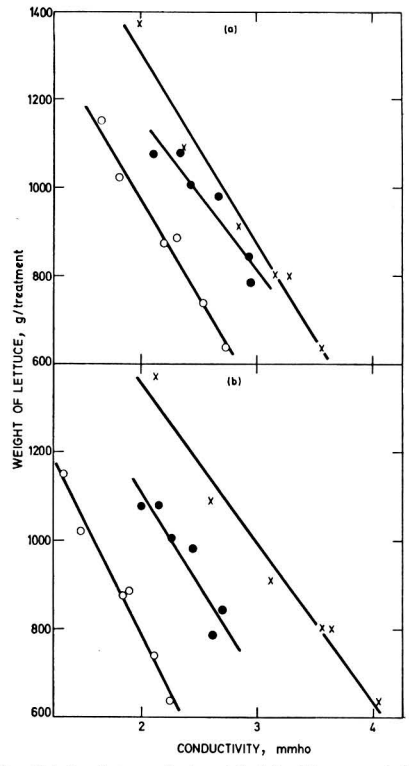

FIG. 3. *Relation between fresh weight (g) of lettuce and the conductivity (millimhos) of centrifuged 2·5 : 1 water:soil extracts prepared (a) by volume and (b) by weight. Experiments 5–8 combined.*

● Soil × Soil + Peat ○ Soil + Sand

TABLE II

Correlations between average fresh weight of lettuce plants and soil salinity measurements (Experiments 9–12; logarithmic transformation)

Salinity test			Correlation coefficient r	Significance P	Coefficient of determination %
Displaced soil solution			−0·956	0·001	91·4
Saturation extract			−0·982	0·001	96·4
Saturation paste			−0·888	0·001	78·9
2·5 : 1 water:soil extracts	by volume		−0·923	0·001	85·2
	,, ,,	+ $CaSO_4$	−0·953	0·001	90·8
	by weight		−0·716	0·001	51·3
	,, ,,	+ $CaSO_4$	−0·810	0·001	65·6
2·5 : 1 water:soil suspensions	by volume		−0·934	0·001	87·2
	,, ,,	+ $CaSO_4$	−0·971	0·001	94·3
	by weight		−0·776	0·001	60·2
	,, ,,	+ $CaSO_4$	−0·899	0·001	80·8
2·5 : 1 suspension (by weight) in aqueous acetone*			−0·816	0·001	66·6

*see footnote, Table I

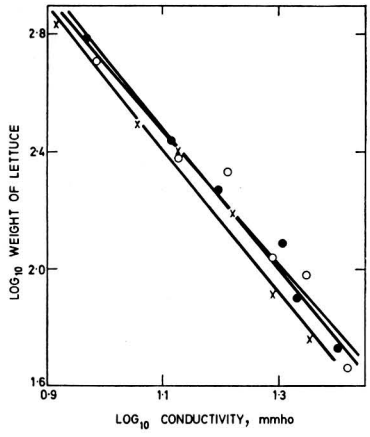

FIG. 4. *Relation between log.10 fresh weight (g) of lettuce and log.10 conductivity (millimhos) of the saturated soil extracts. Experiments 9–12 combined.*

● Soil × Soil + Peat ○ Soil + Sand

2·5 : 1 aqueous extracts by volume + $CaSO_4$, whether centrifuged or not. Comparison of Figs 5 and 6 shows the advantages both of sampling by volume and of including calcium sulphate. As noted previously, removal of suspended particles by centrifugation slightly impaired the correlation between plant growth and salinity.

Bulk density and moisture-holding characteristics of the growth media

Negative relationships were found between the bulk densities of the air-dry ground media and their saturation percentages, field capacities and moisture contents within the range 0–8 cm tension. Thus peat increased the saturation percentages and decreased the bulk densities, whereas sand had the opposite effect.

The amounts of water added per 100 g and per 100 ml of air-dry soil for saturation extracts, and for the 2·5 : 1 suspensions, are shown in Table III. Expressed on a weight basis the saturation pastes required 47–57% more water for soil–peat, and 28–35% less water for soil–sand, than for soil alone. The suspensions prepared by volume gave partial compensation for the moisture characteristics of the growth media because of differences in bulk density.

Alternatively, if expressed on a volume basis, the amounts of water required in the preparation of the pastes varied less (20–22% higher for soil–peat and 17–20% lower for soil–sand). On this basis the suspensions prepared by volume provided no compensation for soil characteristics, but were preferable to those prepared by weight since the latter showed differences in water/soil ratio (expressed on a volume basis) in the opposite direction to those for saturation pastes.

The data for moisture content at field capacity and during cropping (Table IV) show somewhat greater percentage differences between the soil mixtures than were found in the saturation pastes. In the peat–soil mixtures this could be due to incomplete hydration of the peat when preparing

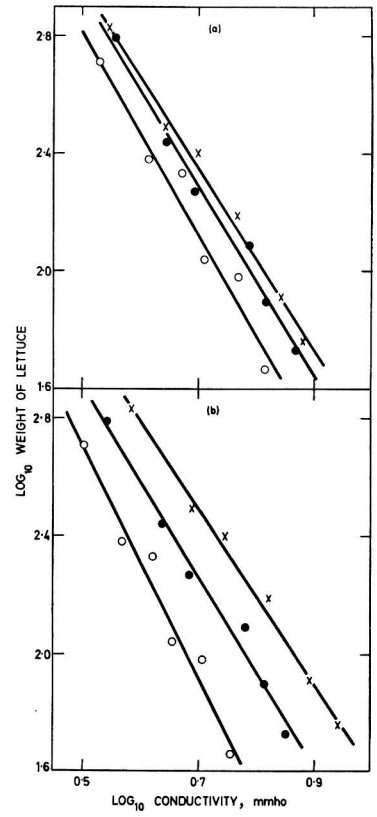

FIG. 5. *Relation between log.10 fresh weight (g) of lettuce and log.10 conductivity (millimhos) of centrifuged 2·5 : 1 water:soil extracts + $CaSO_4$, prepared (a) by volume and (b) by weight. Experiments 9–12 combined*

● Soil × Soil + Peat ○ Soil + Sand

saturation pastes from the air-dried material. Richards[2] previously noted that the saturation paste procedure underestimated salinity in sandy soils; the large pores which were filled with water in the pastes did not correspondingly retain water under field conditions.

Conductivity of soil saturation pastes

Direct measurements of the electrical conductivity of soil pastes have, for convenience, been used widely for soil survey. When interpreting the results it is however necessary to take account of the soil class (sand, loam or clay).[4]

The relation between the fresh weight of lettuce plants and the electrical conductivity of the saturation pastes is shown for Experiments 9–12 in Fig. 7a. Despite the significant correlation (Table II), the regression lines show considerable separation, particularly between the soil and soil–sand pastes; still wider separation was found in Experiments 5–8.

The conductivity of a soil paste depends both on the soluble salts present and on the conducting path between the elec-

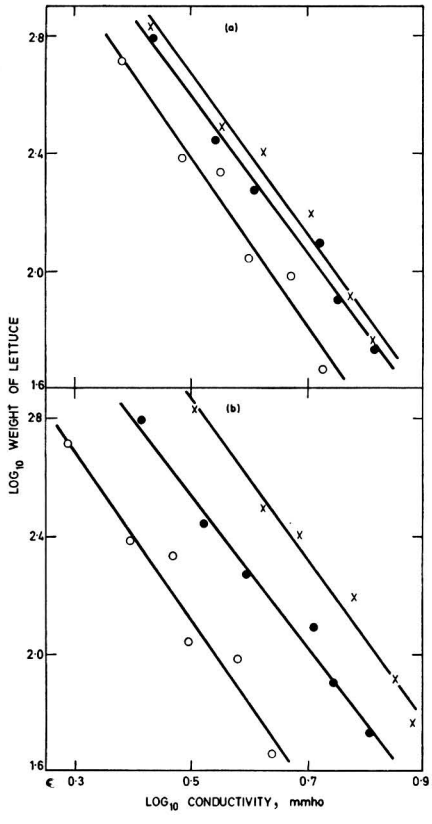

FIG. 6. *Relation between log.10 fresh weight (g) of lettuce and log.10 conductivity (millimhos) of centrifuged 2·5 : 1 water:soil extracts, prepared (a) by volume and (b) by weight. Experiments 9–12 combined.*

● Soil × Soil + Peat ○ Soil + Sand

trodes. To compensate for differences in the amounts of solution and solid phase between the electrodes the water in excess of the air-dry state was calculated per unit volume of paste. When the conductivities of the soil–peat and soil–sand pastes were adjusted on this basis to match the moisture content of the soil paste the effects of texture were greatly reduced in Experiments 5–8, and virtually eliminated in Experiments 9–12 (Fig. 7b). The overall correlations with plant growth were increased from −0·69 to −0·91 in Experiments 5–8, and from −0·89 to −0·98 in Experiments 9–12. As stated by Richards,[2] however, 'No method has been found for improving the reliability of the paste resistance method that does not destroy its simplicity'.

Multiple regression of plant growth on salinity and bulk density

Although the use of 2·5 : 1 soil suspensions prepared by volume rather than by weight improves the correlation of salinity with plant growth, the degree of compensation for physical differences between growth media is still insufficient. The regression of plant weight on soil salinity and bulk density was therefore calculated for experiments 5–8: $y = 2990 - 366x_1 - 662x_2$ where y = fresh weight (g) of lettuce (24 plants), x_1 = electrical conductivity (millimhos) of the centrifuged extracts prepared at 2·5 : 1 ratio by volume and saturated with calcium sulphate, and x_2 = bulk density (g per ml) of the air-dry ground soil. Despite sampling by volume, the term for bulk density in this equation was significant at P = 0·001. When a correction of 1·81 (x_2–1·18) was added to the conductivity, where 1·81 is the ratio of the regression coefficients $b_{yx2} \cdot x_1 / b_{yx1} \cdot x_2$ and 1·18 is the average bulk density of the three rooting media, the correlation with plant growth was raised from −0·81 to −0·97 (Fig. 8). Similar treatment of the data for 2·5 : 1 extracts prepared by weight (+$CaSO_4$) indicated a correction of 2·80 (x_2–1·18), and the amended conductivities were again highly correlated with plant growth (r = −0·97); the term for bulk density in the multiple regression equation was significant at P = 0·001.

When applied to the data from Experiments 9–12 the same corrections, based on Experiments 5–8, again improved the relation between plant weight and salinity after transformation to logarithms. Thus for 2·5 : 1 extracts (+$CaSO_4$) by volume the correlation coefficient was raised to −0·98. For extracts prepared by weight the correlation also improved from −0·81 to −0·96.

TABLE III

Water (g) added per 100 g and per 100 ml of air-dry soil for saturation pastes and for 2·5 : 1 suspensions prepared by weight and by volume

Experiments	Growing medium	per 100 g of soil			per 100 ml of soil		
		Saturation paste	2·5 : 1 *suspension* by wt.	2·5 : 1 *suspension* by vol.	Saturation paste	2·5 : 1 *suspension* by wt.	2·5 : 1 *suspension* by vol.
5–8	Soil	40·1	250	210	47·7	298	250
	Soil/peat	58·8	250	258	57·0	243	250
	Soil/sand	29·0	250	182	39·7	343	250
9–12	Soil	50·5	250	236	53·5	265	250
	Soil/peat	79·3	250	305	65·1	205	250
	Soil/sand	32·8	250	192	42·6	325	250

TABLE IV

Physical data for the soil and soil mixtures

Experiments	Growth medium	Bulk density (g/ml)	*Water per 100 g oven-dried material*		
			Field capacity	During cropping	Moisture equivalent
5–8	Soil	1·19	30·2	—	21·8
	Soil/peat	0·97	48·3	—	26·8
	Soil/sand	1·37	20·3	—	14·6
9–12	Soil	1·06	—	31·6	23·7
	Soil/peat	0·82	—	50·6	29·1
	Soil/sand	1·30	—	17·8	13·7

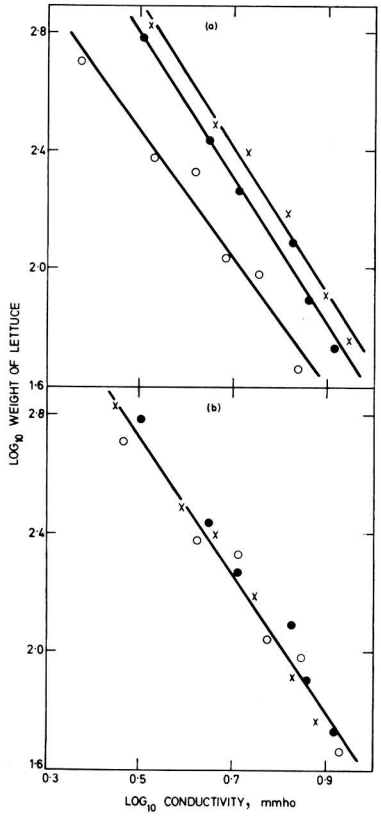

FIG. 7. *Relation between log.10 fresh weight (g) of lettuce and log.10 conductivity (millimhos) of the saturation pastes: (a) direct readings (b) adjusted for moisture content. Experiments 9–12 combined*

● Soil × Soil + Peat ○ Soil + Sand

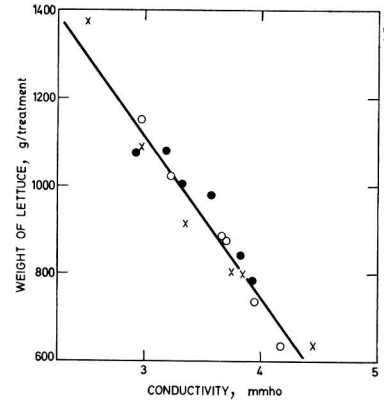

FIG. 8. *Relation between fresh weight (g) of lettuce and the conductivity (millimhos) of centrifuged 2·5 : 1 soil extracts (by volume, + $CaSO_4$) after correction for bulk density. Experiments 5–8 combined*

● Soil × Soil + Peat ○ Soil + Sand

Discussion

The results show that 2·5 : 1 soil suspensions prepared by volume are preferable to those prepared by weight for the assessment of salinity in soils differing in water-retaining properties and bulk density. The data support those of Bunt & Adams,[5] working with composts for pot plants, who concluded that valid comparison of analytical results for such media required expression on a volume basis rather than a weight basis. Somewhat different results were obtained by Drews,[6] who studied the growth of tomato plants over a range of salinity levels in three soils having humus contents of 1·5, 5·0 and 11·0%. His results showed only a slight improvement in the correlation between the conductivity of the soil solutions and of 5 : 1 extracts when prepared by volume rather than by weight. Sampling by volume did not, however, fully compensate for physical differences between the various growth media. Thus in the first series of experiments (5–8) the saturation extracts proved superior to those prepared at a constant ratio (2·5 : 1).

Multiple regression technique provided corrections to the salinity data according to the bulk densities of the media, and greatly improved the correlations with plant growth. Whilst the correction formulae used proved very satisfactory over the range of high salinities studied, it cannot be assumed that the same formulae would necessarily apply at lower salinities. Since the corrections apparently depend on the relation between bulk density and moisture retention, however, it should be possible to develop correction formulae applicable to all salinity levels. Such corrections would prove particularly useful for potting composts containing a high proportion of peat; many of the potting composts now in use have very low bulk densities, and difficulties arise in the interpretation of salinity data.

Saturation of the 2·5 : 1 soil extracts with calcium sulphate improved the correlation of soil salinity with plant growth, although differences in the calcium sulphate content of the plots had not been created deliberately. The benefit from including calcium sulphate was greater for extracts prepared by weight than by volume (Tables I and II).

The variability encountered among lettuce plants under saline conditions necessitated extensive repetition of the trials. It appears that lettuce grown under somewhat adverse conditions may reveal variability which would not show up under the more favourable conditions of a normal breeding programme.

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FUNGICIDAL ACTIVITY AND CHEMICAL CONSTITUTION

XIV.*—Preparation of 4-substituted 2,6-dinitrophenols

By DIANA M. FIELDGATE and D. WOODCOCK

Six 4-(1-phenylalkyl)-, eight 4-(1-cyclohexylalkyl)- and seven 4-(1-cyclopentylalkyl)-2,6-dinitrophenols were prepared for testing against powdery mildew of apple (caused by *Podosphaera leucotricha* (Ell. & Everh.) Salm.) and other fungi.

Introduction

Work by Kirby and his associates[1] and in this laboratory[2,3] showed that, in general, 4-substituted-2,6-dinitrophenols are more effective against apple mildew than the isomeric 2,4-dinitro compounds. The former are also much less phytotoxic, so much so that many could be used with safety as free phenols.[2,4,5] The high activity shown by 4-(1-ethylhexyl)- and 4-(1-propylpentyl)-2,6-dinitrophenols prompted the synthesis of analogues containing an α-substituent, other than an alkyl group, in the n-alkyl chain.[6]

* Part XIII: *Ann. appl. Biol.*, 1966, **57**, 223

Experimental and Results

Infra-red spectra were determined for liquid films or Nujol mulls on a Perkin-Elmer Infracord Spectrophotometer, Model 237, and only those absorption bands which are significant for structural assignments or identification are noted.

Thin-layer chromatography

The systems used were Kieselgel G (light petroleum (b.p. 40–60°)–ether–formic acid 80 : 20 : 2) and Kieselgel G (benzene).

Plates were sprayed either with a solution of 4% ceric sulphate in 10 wt.–% sulphuric acid or with a saturated

solution of chromic acid in 50 wt.-% sulphuric acid, and were baked at 140°.

Grignard reaction

This was carried out by refluxing the appropriate carbonyl compound in benzene solution for 3 hours with the required alkyl or cycloalkyl magnesium halide (2–3 equivalents) prepared in anhydrous ether. The mixture was cooled, and decomposed by means of saturated aqueous ammonium chloride, the ethereal layer was washed with water, dried over anhydrous sodium sulphate, and the solvent was distilled off.

Dehydration

The crude carbinol from the Grignard reaction was heated at 120–125° with an equal weight of powdered fused potassium hydrogen sulphate for 1 hour. The mixture was cooled and the product was extracted with ether, the ethereal solution was washed with aqueous sodium hydrogen carbonate and dried, and the solvent was removed. If necessary the product was purified by elution from a Grade I alumina column with light petroleum (b.p. 60–80°) alone and then with the addition of 10% benzene.

Hydrogenation

A solution of the olefin in ethyl alcohol or tetrahydrofuran was shaken with 10% palladised charcoal in hydrogen at laboratory temperature and pressure until there was no further uptake of gas. Filtration of the solution and removal of the solvent left the product, purified if necessary by elution from a Grade I alumina column as above.

De-methylation

In early de-methylation experiments, the use of hydrobromic acid (*d*, 1·48) or hydriodic acid (*d*, 1·9) at reflux temperatures either alone or with the addition of acetic acid or sulpholane was found to cause some fission of the 4-substituent with the formation of phenol. The method of Prey,[7] in which the methyl ether was refluxed for 3 h with an excess of pyridine hydrochloride was satisfactory, but preparation and manipulation of this hygroscopic reagent was tedious. More recently the use of pyridine and hydrochloric acid[8] has proved satisfactory. In all cases the cooled mixture was diluted with water and extracted with ether, the extract was washed free of pyridine with dilute hydrochloric acid, then with water and dried (Na_2SO_4). Removal of the solvent left the phenol, which if solid was crystallised to constant m.p. before nitration, but completeness of de-methylation was always checked using t.l.c. (System 1). For higher members of the series which tended to give a two-phase mixture, refluxing was necessary for a much longer period to ensure complete de-methylation.

Di-nitration

Method (a) (Two-stage)

Mono-nitration was carried out by the method of Jones.[9] A solution of the phenol (1 g) in chloroform (5 ml) was cooled and treated below 0° with a two-fold excess of nitric acid (*d*, 1·42) added dropwise with stirring. After 1·5 h, at laboratory temperature water was added, the chloroform layer separated, washed with water and dried ($CaCl_2$). Removal of the solvent gave the mononitrophenol which was examined by t.l.c. (System 1: yellow spot enhanced by ammonia vapour) and if not pure it was eluted from a Grade II alumina column using benzene, followed by benzene to which 1% ethanol had been added to promote 'banding'. Di-nitration was carried out by stirring a solution of the mononitro compound (1·5 g) in chloroform (6 ml) with a two-fold excess of nitric acid (*d*, 1·42) at 45° for 2 hours. The mixture was poured on to ice and the product isolated as before. It was examined by t.l.c. (System 1: orange spot enhanced by ammonia vapour) and if necessary purified as before, the addition of 0·1–0·2% acetic acid to the benzene–ethanol mixture being necessary in this case to promote 'banding' and to make removal of the dinitrophenol possible.

Method (b) (Single stage)

In both 4-(1-cycloalkylalkyl)-phenol series, nitration was carried out by dropwise addition of a solution of the phenol in glacial acetic acid (3 parts) to a stirred mixture of nitric acid (*d*, 1·5, 2 mol) and glacial acetic acid (2 parts), the temperature being kept below 30°. After standing overnight at laboratory temperature, the mixture was poured on to ice and extracted with ether, the extract was washed with water, then sodium hydrogen carbonate solution and then dried (Na_2SO_4). Removal of the solvent gave the dinitro compound which was examined and purified as described above, solid products being recrystallised to constant m.p.

2,6-Dinitro-4-(1-phenylalkyl)phenols

1-(p-*Methoxyphenyl*)-1-*phenylprop*-1-*ene*

4-Methoxybenzophenone (17 g) in dry benzene (50 ml) was added dropwise to a cooled solution of ethyl magnesium iodide, prepared from magnesium (5·5 g) and ethyl iodide (13·7 g) and then refluxed for 1·5 h. The red-brown product (21 g) isolated as described above showed no sign of the original ketone (t.l.c. System 2; no C = 0 peak at 1648 cm^{-1}), and absence of any absorption in the 3100–3600 cm^{-1} region indicated that spontaneous dehydration of the carbinol had occurred. After several crystallisations from ethyl alcohol it had m.p. 53–54°. (Found: C, 85·3; H, 7·1; $C_{16}H_{16}O$ requires C, 85·7; H, 7·1%).

1-(p-*Methoxyphenyl*)-1-*phenylpropane*

A solution of the above solid (7 g) in tetrahydrofuran (80 ml) was shaken with palladised charcoal (0·7 g) in hydrogen at laboratory temperature and pressure until there was no further uptake of gas (~2 h). Filtration of the solution and removal of the solvent gave an oil (8·1 g), purified by elution from a short Grade I alumina column.

4-(1-*Phenylpropyl*) *phenol*

The above methoxy compound (7 g), de-methylated using pyridine hydrochloride as already described, gave a pale yellow oil (6 g) which showed only a single spot of lower R_f than the corresponding methyl ether (t.l.c., System 2). ν_{max} 3350, 1240 cm^{-1} (OH bands) replacing peaks at 2840, 1250 cm^{-1} (OCH_3.)

2-*Nitro*-4-(1-*phenylpropyl*) *phenol*

The above phenol (6 g) was mono-nitrated by method (a). The product was a yellow oil which, crystallised from light petroleum (b.p. 40–60°), had m.p. 41·5–43° (Found: C, 69·6; H, 5·5; N, 5·5. $C_{15}H_{14}NO_3$ requires C, 70·0; H, 5·8; N, 5·45%). ν_{max} (cm^{-1}) 3245 (bonded OH), 1538 (NO_2).

2,6-*Dinitro*-4-(1-*phenylpropyl*) *phenol*

A solution of the above mononitrophenol (1·5 g) on further nitration (Method a) gave an orange-coloured oil (0·5 g), purified by elution from Grade II alumina. (Found: C, 60·4; H, 4·6; N, 9·0. $C_{15}H_{14}N_2O_5$ requires C, 59·6; H, 4·6; N, 9·3%). V_{max} (cm^{-1}) 3200 (strongly bonded OH), 1538 (NO_2).

Four other new 2,6-dinitro-4-(1-phenylalkyl) phenols, which were all yellow oils, were similarly prepared by way of the corresponding mononitro-compounds. The product at each stage was examined by t.l.c. and by infra-red spectroscopy for purity and identity. The following were prepared:

2,6-*Dinitro*-4-(1-*phenylethyl*) *phenol*, 60% yield (based on original ketone). (Found: N, 9·9%. $C_{14}H_{12}N_2O_5$ requires N, 9·7%).

2,6-*Dinitro*-4-(1-*phenyl-n-butyl*) *phenol**, 10% yield. (Found: N, 9·0. $C_{16}H_{16}N_2O_5$ requires N, 8·9%).

2,6-*Dinitro*-4-(1-*phenyl-n-pentyl*) *phenol*, 55% yield. (Found: N, 8·6. $C_{17}H_{18}N_2O_5$ requires N, 8·5%).

2,6-*Dinitro*-4-(1-*phenyl-n-hexyl*) *phenol*, 50% yield. (Found: N, 8·1. $C_{18}H_{20}N_2O_5$ requires N, 8·1%).

The isolation and identification (mixed m.p. with an authentic specimen) of 4-benzyl-2,6-dinitrophenol (m.p. 86°) in the final product, when earlier stages had not been rigorously purified by elution from an alumina column, showed that two-electron reduction of the 4-methoxybenzophenone by the Grignard reagent could take place to some extent.

2,6-Dinitro-4-(1-cyclohexylalkyl) phenols

1-(p-*Methoxyphenyl*)-1-*cyclohexylpent*-1-*ol*

A solution of cyclohexyl magnesium bromide prepared from magnesium (1 g) and cyclohexyl bromide (5·5 ml) was cooled to 0° and stirred during the dropwise addition of a solution of 4-methoxyphenyl butyl ketone (3·9 g) in dry benzene (14 ml), and then refluxed for 3 hours. The product, isolated as usual, was a pale yellow oil (9·2 g) showing a medium OH peak (V_{max} 3480 cm^{-1}).

1-(p-*Methoxyphenyl*)-1-*cyclohexylpent*-1-*ene*

The above oil (9·2 g) was dehydrated as previously described and the brown oily product (6·7 g) was purified using an alumina column (Grade I). Dicyclohexyl (0·42 g) was eluted first by means of light petroleum (b.p. 60–80°) (V_{max} 2920, 2850, 2450, 1000, 995 cm^{-1}),[10,11] Subsequent elution with light petroleum (b.p. 60–80°) containing 10% benzene gave the olefin (3·8 g) which showed a single spot (t.l.c., System 2) and complete disappearance of the band at 3480 cm^{-1}.

1-(p-*Methoxyphenyl*)-1-*cyclohexyl-n-pentane*

The above olefin (3·8 g) reduced catalytically gave a colourless oil (3·8 g) which showed only a single spot of higher R_f than the olefin (t.l.c., System 2).

4-(1-*Cyclohexyl-n-pentyl*) *phenol*

The above methoxy pentane (3·8 g), de-methylated using pyridine hydrochloride, gave a solid (3·6 g) which after several crystallisations from light petroleum (b.p. 60–80°) had m.p. 112–113°. (Found: C, 82·7; H, 11·0. $C_{17}H_{26}O$ requires C, 82·9; H, 10·6%) V_{max} 3300 cm^{-1} (OH broad band), 1320 (OH peak, replacing band at 1250).

4-(1-*Cyclohexylpentyl*)-2,6-*dinitrophenol*

A solution of the above phenol (2·9 g) was nitrated in glacial acetic acid as previously described (Method b). The product was a yellow oil (3·9 g, 75% yield based on original ketone) which was purified by elution from a Grade II alumina column. The i.r. spectrum was typical of a 2,6-dinitro-4-substituted phenol (V_{max} 3180, 1550 cm^{-1}—strongly bonded OH and NO_2 respectively). (Found: N, 8·1. $C_{17}H_{24}N_2O_5$ requires N, 8·3%). Other members of this series, which were yellow oils where no m.p. is given, were prepared similarly, the intermediates involved being checked for purity and identity by t.l.c. and infra-red spectroscopy before proceeding to the next stage. The following were prepared:

4-(1-*Cyclohexylmethyl*)-2,6-*dinitrophenol** was crystallised from aqueous methanol, m.p. 72–73° in 75% yield. (Found: N, 10·0. $C_{13}H_{16}N_2O_5$ requires N, 10·0%). 4-(1-*Cyclohexylethyl*)-2,6-*dinitrophenol* was crystallised from light petroleum (b.p. 40–60°) m.p. 51–52° in 70% yield. (Found: N, 10·0. $C_{14}H_{18}N_2O_5$ requires N, 9·5%). 4-(1-*Cyclohexyl-n-propyl*)-2,6-*dinitrophenol* was crystallised from light petroleum (b.p. 40–60°), m.p. 62–63° in 50% yield. (Found: N, 9·5. $C_{15}H_{20}N_2O_5$ requires N, 9·1%). 4-(1-*Cyclohexyl-n-butyl*)-2,6-*dinitrophenol* gave 50% yield. (Found: N, 8·2. $C_{16}H_{22}N_2O_5$ requires N, 8·7%), and 4-(1-*Cyclohexyl-n-hexyl*)-2,6-*dinitrophenol*, 80% yield. (Found: N, 7·9. $C_{18}H_{26}N_2O_5$ requires N, 8·0%). 4-(1-*Cyclohexyl-n-heptyl*)-2,6-*dinitrophenol* gave 50% yield. (Found: N, 7·8 $C_{19}H_{28}N_2O_5$ requires N, 7·7%) and 4-(1-*Cyclohexyl-n-octyl*-2,6-*dinitrophenol*, 50% yield. (Found: N, 7·7 $C_{20}H_{30}N_2O_5$ requires N, 7·4%).

2,6-Dinitro-4-(1-cyclopentylalkyl) phenols

4-(*Cyclopentylmethyl*) *anisole*

An ethyl alcoholic solution of *p*-methoxyphenyl cyclopentyl ketone (1 g), prepared according to a method of Hey & Musgrave,[12] was shaken with 10% palladised charcoal (0·1 g) in hydrogen at laboratory temperature and pressure until there was no further uptake of gas. Removal of the solvent from the filtered solution left a colourless oil (1 g) which showed complete absence of a peak at 1670 cm^{-1} (C = O).

4-*Cyclopentylmethyl phenol*

De-methylation of the above anisole (1 g) using pyridine and hydrochloric acid[8] gave a light brown oil (0·8 g) showing only a single spot (t.l.c., System 2). (V_{max} 3320, 1255 cm^{-1}).

2,6-*Dinitro*-4-*cyclopentylmethyl phenol*

The above phenol (0·8 g) was di-nitrated and the product (1·1 g) was isolated by method (b). It was purified by graded elution from an alumina column (Grade II) using successively benzene, benzene + 1% ethanol, benzene + 2% ethanol + 0·2% acetic acid, to give a yellow oil produced in 75% yield. (Found: N, 10·5. $C_{12}H_{14}N_2O_5$ requires N, 10·5%). (V_{max} cm^{-1} 3190 (strongly bonded OH), 1530–1550 (NO_2))

* Prepared by Mr. D. R. Clifford

* Prepared by Mr. E. D. Evens

Reaction of cyclopentylmagnesium bromide with 4-methoxyphenylpropyl ketone

A solution of cyclopentyl magnesium bromide, prepared from magnesium (2·2 g) and cyclopentyl bromide (11 ml) in anhydrous ether, was cooled to 0° and stirred during the dropwise addition of 4-methoxyphenylpropyl ketone (8 g) dissolved in dry benzene (28 ml). After reflux for 3 h the mixture was processed as described earlier. The product was a brown oil (10·7 g) which showed two major low R_f spots when examined by t.l.c. (System 2). This oil was dehydrated using potassium hydrogen sulphate (10·7 g) as already described, and distillation of the product gave two fractions (A) 5·8 g, b.p. 145–150°/20$_{mm}$ and (B) 1·1 g, b.p. ~200°/20$_{mm}$. Each fraction was catalytically hydrogenated and the products were de-methylated as already described. The phenol from fraction (A) had an i.r. spectrum almost identical with that of 4-n-butylphenol. Nitration (Method b) gave a yellow dinitro derivative which showed an orange-coloured spot of the same R_f value as 4-n-butyl-2,6-dinitrophenol (t.l.c. System 1). After several recrystallisations from light petroleum (b.p. 40–60°) it had m.p. 43–45°, which was not depressed by admixture with an authentic specimen. A study of the C-H str. (2800–3000 cm^{-1}) and C-H def. (1310–1370, 1440–1470 cm^{-1}) regions of the i.r. spectrum of the phenol from fraction (B), indicated the presence of the cyclopentyl group. Nitration (Method b) gave an orange-coloured oil which showed two orange spots using t.l.c. (System 1), the R_f of the smaller of these being the same as that of 4-n-butyl-2,6-dinitrophenol. The mixture was purified by column chromatography using Kieselgel (0·05–0·2 mm, activated at 120°) and elution with light petroleum (b.p. 60–80°) followed by that solvent mixed with an increasing proportion of benzene. Crystallised from light petroleum (b.p. 40–60°) it had m.p. 53–54.° (Found: N, 9·3. $C_{15}H_{20}N_2O_5$ requires N, 9·1%).

1-(p-*Methoxyphenyl*)-1-*cyclopentylbut*-1-*ene*

A solution of propylmagnesium bromide prepared from magnesium (4·8 g) and n-propyl bromide (20 ml) in anhydrous ether was cooled to 0° and stirred during the dropwise addition of a solution of *p*-methoxyphenyl-cyclopentyl ketone (20·4 g) in dry benzene (70 ml). After reflux for 3 h the mixture was processed as described earlier and the resultant oil (28·9 g) was dehydrated as usual. The product (26·1 g) was a light brown oil showing no absorption at 1675 cm^{-1} (C = O) and 3100–3600 cm^{-1} (OH) in the i.r. spectrum, and a single spot of different R_f from the original ketone (t.l.c. System 2).

4-(1-*Cyclopentylbutyl*) *phenol*

The above olefin (25 g) was catalytically hydrogenated, and the resultant 4-cyclopentylbutyl anisole was de-methylated using pyridine and hydrochloric acid as previously described. The product (18·6 g) showed only a single spot (t.l.c. System 2) and after several recrystallisations from light petroleum (b.p. 60–80°) had m.p. 77–78°. (Found: C, 82·4; H, 10·4. $C_{15}H_{22}O$ requires C, 82·5; H, 10·1%).

2,6-*Dinitro*-4-(1-*cyclopentylbutyl*) *phenol*

The above phenol (11·9 g) was nitrated (Method b) and the product (15·8 g) was isolated as previously described. It crystallised from light petroleum (b.p. 40–60°) (charcoal) and had m.p. 54–55°, yield 60% (overall). (Found: N, 9·3. $C_{15}H_{20}N_2O_5$ requires N, 9·1%). The following analogues were prepared in a similar way:

2,6-*Dinitro*-4-(1-*cyclopentylethyl*) *phenol* was crystallised from light petroleum (b.p. 40–60°) (charcoal) and had m.p. 45–46° (Found: N, 9·6. $C_{13}H_{16}N_2O_5$ requires N, 10·0%) 50% yield, based on *p*-methoxyphenyl cyclopentyl ketone.

2,6-*Dinitro*-4-(1-*cyclopentylpropyl*) *phenol* was crystallised from light petroleum (b.p. 40–60°) and had m.p. 76–77° (64% yield overall). (Found: N, 9·5. $C_{14}H_{18}N_2O_5$ requires N, 9·5%).

2,6-*Dinitro*-4-(1-*cyclopentylpentyl*) *phenol* was an orange-coloured oil prepared in 50% yield and purified by elution from an alumina column (Grade II). (Found: N, 9·0. $C_{16}H_{22}N_2O_5$ requires N, 8·7%).

2,6-*Dinitro*-4-(1-*cyclopentylheptyl*) *phenol* was an orange-coloured oil, purified as for the pentyl analogue (50% yield). (Found: N, 8·3. $C_{18}H_{26}N_2O_5$ requires N, 8·0%).

2,6-*Dinitro*-4-(1-*cyclopentylhexyl*) *phenol* was only prepared by reaction of *p*-methoxyphenyl n-pentyl ketone and cyclopentyl magnesium bromide, successive dehydration and hydrogenation of the product yielding a mixture of 4-n-hexylanisole and 4-(1-cyclopentylhexyl) anisole. These were separated on an Autoprep A 700 gas chromatograph, the 10 ft × $\frac{3}{8}$ in. o.d. copper column being packed with acid-washed Chromosorb G coated with LAC-2R-446 (2·5%) and orthophosphoric acid (0·2%) and run at 215° during the passage of helium at 150 ml min^{-1}. The cyclopentyl compound, which had the longest retention time, was collected, de-methylated and nitrated (Method b). The dinitro derivative produced in 10% overall yield was an orange coloured oil. (Found: N, 7·9. $C_{17}H_{24}N_2O_5$ requires N, 8·3%.)

Discussion

Members of the 2,6-dinitro-4-(1-phenylalkyl) phenol series were conveniently prepared from the commercially available *p*-hydroxybenzophenone. After methylation, introduction of the alkyl group was effected by the normal 1,2- addition of the appropriate alkyl magnesium halide, followed by dehydration, reduction and de-methylation. In the last stage, hydrobromic or hydriodic acids could not be used either in acetic acid or in sulpholane because of breakdown leading to phenol formation, and hydrochloric acid was preferred. One-stage di-nitration of the de-methylated product also lead to a certain amount of fission, with the formation of 2,4-dinitrophenol and picric acid, but this difficulty was resolved by a preliminary mono-nitration in chloroform solution.

p-Hydroxyphenylalkyl ketones, prepared by Fries reaction from the corresponding phenyl esters, were the starting materials for the synthesis of the 4-(1-cyclohexylalkyl) phenols. Reaction with cyclohexylmagnesium bromide was essentially by a normal 1,2- addition, though a small amount of dicyclohexyl was formed—presumably as a result of one-electron reduction of the carbonyl group leading to pinacol formation.[13] Often the resultant carbinols underwent dehydration during processing, but the subsequent reaction sequence was the same as in the phenylalkyl series, except that single-stage di-nitration was possible in this case. An analogous use of cyclopentylmagnesium bromide for the preparation of 4-(1-cyclopentylalkyl) phenols, however, could not be made since with this Grignard reagent, two-

electron reduction of the *p*-methoxyphenylalkyl ketone takes precedence over the normal 1,2- addition process (cf. Hey & Musgrave,[12] Kharasch & Weinhouse.[14]). Separation of the cyclopentylalkyl from the n-alkyl derivative at any subsequent stage of the reaction sequence proved very tedious, although 4-(1-cyclopentylhexyl) anisole has been separated relatively easily from 4-n-hexyl anisole by preparative gas–liquid chromatography. This series was therefore more conveniently prepared starting with *p*-methoxyphenylcyclopentyl ketone, reaction with various alkyl magnesium halides being followed by a procedure analogous to that used on the phenylalkyl series.

Tested at various concentrations for the ability to protect apple seedlings against mildew caused by *Podosphaera leucotricha* many of the compounds, particularly some members of the two cycloalkyl series, proved highly active. Results of the tests with this and other fungi have still to be published.

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SENSORY AND OBJECTIVE MEASUREMENTS OF THE QUALITY OF FROZEN STORED COD OF DIFFERENT INITIAL FRESHNESSES

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The eating quality of cod kept for different periods in melting ice before being frozen and stored at three different temperatures has been evaluated by a taste panel using a new score sheet. Objective measurements of both initial freshness before freezing and deterioration during frozen storage were carried out on the same samples. Correlations were obtained between the objective measurements and various aspects of eating quality. The relative contributions of the various aspects of eating quality to the final overall acceptability were obtained, and the value of the objective measurements in predicting overall acceptability assessed.

Introduction

Between the time a fish is caught and it is eaten after being frozen, it will have undergone a complex and often variable series of temperature changes each occurring over a different time interval. In general, the temperature history of the fish will include the following phases: unfrozen storage, freezing, frozen storage, thawing, storage in the thawed state. In some types of product the freezing–thawing phase may occur more than once. During each of these phases the fish may change in ways which will adversely affect its acceptability as an article of food. These changes are of three kinds, spoilage due to microbiological action, changes due to the action of endogenous enzymes, and chemical or physical changes such as oxidation or loss of flavour components through leaching or 'weeping'. All three kinds of change can occur during the unfrozen storage phases, whilst

during the frozen storage phase only the last two kinds occur, if it is assumed that the temperature is below about $-7°$. If the freezing or thawing phases are unduly prolonged, significant microbiological deterioration may also occur in them. The rates, and in some cases, the exact nature of each kind of deteriorative change will depend on the temperature experienced by the fish, whilst their extent will depend on the length of the phase. Therefore a method, or group of methods, which attempts to evaluate comprehensively the final eating quality of any samples of frozen fish needs to take into account a wide variety of processing histories and deteriorative changes.

However, it is practicable to select for investigation a small number of processing histories which are related to the main commercial freezing operations. One such important operation, around which is centred the investigation reported in this paper, is the freezing of fish fillets in the form either of blocks weighing 7 or 14 lb for catering use or of consumer packs weighing about 8 or 12 oz. In the manufacture of this type of product, fillets are cut from whole fish which have been kept, usually in the gutted state, for several hours or days after capture, and the fillets are then frozen rapidly and stored under conditions which minimise desiccation. In some cases, the fillets are cut from fish which have been previously frozen whole and subsequently thawed, but this type of double-freezing treatment is not specifically covered here. Normally the product will be thawed rapidly and used immediately, so that the phases in the entire history of the fish which are of importance so far as deterioration in eating quality are concerned, are the period before freezing and the period of frozen storage. This paper describes the results of a study confined to the effects on final eating quality of changes in the fish during both these periods. The species examined is cod; it is hoped to examine further species in similar detail in future work.

In the period before freezing, the temperature of, and therefore amount of deterioration in, the fish under commercial conditions may vary considerably, but if this period is of any appreciable length, attempts are usually made to chill the fish so that its temperature is effectively that of melting ice. It has therefore been thought appropriate to adopt for pre-freezing conditions, whole gutted cod held in melting ice for various periods. As far as frozen storage is concerned there are two stages of commerical importance, firstly, storage in a holding store or warehouse at a temperature of usually $-20°$ to $-30°$ but sometimes higher, and secondly, storage in a retail cabinet or deep freezer in a catering establishment at a temperature of about $-10°$ to $-20°$. A range of constant storage temperatures has therefore been selected which will cover most of the variations likely to be met with under commercial conditions of production, distribution and sale; the three temperatures used are $-14°$, $-22°$ and $-29°$.

In this study, both sensory and objective methods of evaluating the eating quality of the cod have been used. The sensory method adopted was a special taste panel technique developed for this purpose and described in a previous publication.[1] It seems highly unlikely that a single objective method would be capable of assessing the eating quality of frozen cod of different initial qualities, because the fish will have undergone spoilage during storage in melting ice, and during frozen storage; it is known that the underlying chemistry of these two types of deterioration is quite different. However, tests are available, or have been proposed, which aim to monitor both of these types of deterioration separately, and the approach has therefore been to apply several such tests simultaneously. In this way it was hoped to find out which tests or which combination of tests were most useful in predicting the various aspects of eating quality separately, and also the overall acceptability.

A number of the main changes in the characteristics of frozen cod measured in this investigation have been described in a previous publication.[2]

Experimental

Material

The storage tests described in this paper were carried out on North Sea cod (*Gadus callarias* L.) which was caught by trawling throughout the period June, 1965 to August, 1966. The fish were gutted immediately after they had been caught and were packed in melting ice. After periods of 2, 5, 8, 14 and 17 days in ice the fish were filleted, and the fillets were frozen in an air blast at $-34°$. The frozen fillets were closely wrapped in aluminium foil in bundles of three to prevent desiccation, and stored at temperatures of $-14°$, $-22°$ and $-29°$. Each bundle contained fish of only one icing treatment. After being stored for periods ranging from a few days to a year, the fillets were thawed overnight in still air at $1·5°$.

A number of tests were also carried out on fish frozen and thawed without intermediate storage. For these tests cod caught at various times between October, 1966 and April, 1967 were used. In these tests the paired fillet technique was used, one fillet from a fish being frozen and thawed, and then compared in the same tasting session with the other unfrozen fillet which was kept at $0°$ until tasted. In one series of tests fillets from two or three fish were frozen, partly thawed for a few hours and tasted at the same session on the same day as the paired unfrozen fillets. In a second series of tests the frozen fillets from the same number of fish were thawed overnight as described above and tasted at the same session as the paired unfrozen fillets which had been kept overnight at $0°$.

Sensory methods

For tasting, samples weighing 4–6 oz were cut from the centre portion of the thawed fillets, and cooked in a closed casserole over boiling water for 30 min. The samples, kept at $60°$, were tasted usually by 7 trained tasters immediately after cooking. Either one or two samples for each processing treatment were tasted.

The scoring system developed by Baines *et al.*[1] was used throughout. In this system, deterioration in the period before freezing is assessed in terms of the 11-point scales for freshness odour and freshness flavour proposed by Shewan *et al.*[3] in which scores of 10 and 0 represent absolutely fresh and putrid, respectively. During frozen storage, quality deteriorates in several ways which are all covered by the scoring system. There is a gradual increase in amount of unpleasant odour and flavour called, for convenience, 'cold storage odour' and 'cold storage flavour', respectively. At the same time the fish becomes 'firmer' and 'drier' until it is unacceptably tough and dry. These four attributes are scored on separate numerical scales; in the scales for cold storage odour and cold storage flavour the extreme scores of 0 and 5 denote 'absent' and 'very strong' respectively; in the scale for firmness, 0 and 6 denote 'very soft' and

'extremely tough', respectively, a score of 2 representing unfrozen fish of normal firmness; in the scale for dryness, 0 and 4 denote 'sloppy, watery' and 'extremely dry' respectively a score of 1 representing unfrozen fish of normal moistness. In addition, an assessment of the overall acceptability is obtained using a 9-point hedonic scale[4] in which scores of 9 and 1 denote 'like extremely' and 'dislike extremely', respectively. In all cases the reported scores are the mean panel scores.

In all, 280 frozen and stored samples were tasted in 25 sessions; 14 unfrozen samples were randomly distributed among the frozen samples. The numbers of samples which were tasted after being stored at temperatures of $-14°$, $-22°$ and $-29°$ were 138, 70 and 72, respectively. Each of these lots contained an approximately equal number of samples of each icing history. The 294 samples were presented to the panel in random order.

Objective methods

The criteria for selecting the objective methods used were: relative simplicity, and attainment of a reasonable degree of development. For evaluating the freshness of cod stored in melting ice a number of fairly good and well tried methods are available,[5,6] but two were finally chosen: the determination of the concentrations of trimethylamine and hypoxanthine in the fish. Rather less work has been done on developing tests for frozen-storage deterioration of cod, and, in fact, only three methods, all of which are employed in this study, have received even moderate attention. These are: the determination of the amount of protein extractable from the frozen stored cod by means of neutral approximately 0·5 M salt solutions;[7] the cell fragility method;[8] and the colour ratio method.[9] Only in the case of the extractable protein and colour ratio methods have previous attempts been made to correlate directly the results with sensory assessments of quality.

Recently it has been shown[10,11] that the firmness or 'toughness' of cod is appreciably affected by the ultimate pH of the fish. An approximately linear relationship exists between firmness and pH, firmer fish being associated with low pH. Thus the firmness of any sample of frozen cod may be affected by two factors: pH and the amount of textural deterioration undergone during storage. In order to distinguish the effect of storage alone it is therefore necessary to measure the pH of each sample and apply a correction according to the manner in which firmness depends upon pH; this procedure has been adopted in this work.

Experimental details of the objective methods used have been given.[2]

Extractable protein and colour ratio determinations were carried out on 205 and 246 of the samples, respectively; the other objective determinations were carried out on all 294 samples.

Statistical methods

The variables included in this study with their designations are as follows:

Month caught (June, 1965 = 0)	M
Days in ice	D
Weeks of frozen storage	W
Freshness odour	FO
Freshness flavour	FF
Cold storage odour	CSO
Cold storage flavour	CSF
Firmness	F
Dryness	Dr
pH	pH
Colour ratio	CR
Cell fragility	CF
Trimethylamine (mg N/100 g fish)	TMA
Protein extractability (%)	PE
Hypoxanthine (μM/g fish)	Hy
Overall acceptability	OA

The interrelationships between the variables were examined by means of their correlation coefficients and multiple regression analysis in a manner similar to that employed by Kramer.[12,13] Preliminary selection of the dependent variables used in the analyses was made on the basis of *a priori* judgement about their possible usefulness. Best fitting exponential regressions were obtained by an iterative method in which different values of the exponent were substituted until the maximum correlation between independent and dependent variables was found. This method does not provide a measure of the error in the exponent so that differences between exponents cannot be tested for significance. Nearly all the statistical computations were carried out on an Elliott 803 digital computer using a standard library programme. Throughout this paper the symbols **, *, and n.s. represent values which are significant at $p < 0{\cdot}01$, $p < 0{\cdot}05$ and not significant, respectively. The abbreviation d.f. means degree of freedom.

Results and Discussion

Changes caused by freezing and thawing alone

Extrapolation of the results of frozen storage experiments to zero time of storage would be expected to give information about the effect of freezing and thawing isolated from the effect of storage. However, in some cases, the experimental scatter is so large that from extrapolation alone it is not possible to say with any degree of accuracy what the effect of freezing and thawing is. Therefore, a number of direct comparisons of unfrozen and frozen cod have been made, using sensory measurements. In addition, a number of indirect comparisons of objective measurements have been made.

Sensory measurements

Two series of observations were made: Series 1—the comparison between unfrozen cod and cod frozen, partly thawed before cooking and then tasted on the same day, was made with fish iced for 2, 6, 7, 10 and 14 days; Series 2—the comparison between unfrozen cod and cod frozen, thawed overnight and then tasted next day was made with fish iced for 1 and 2 days.

The results of these two comparisons are shown in Table I. The changes in Series 1 and Series 2 scores are not significantly different for the fish of different freshnesses, and therefore the mean values for all icings are shown. It can be seen that for Series 1 tests freshness flavour (FF) is unaffected by the freezing and thawing treatment, whilst firmness (F) and dryness (Dr) increase significantly. In the case of Series 2 tests, FF falls significantly by 0·59 units whilst F and Dr increase significantly by larger amounts than for Series 1 tests. Thus, freezing and thawing, if carried out rapidly enough, are without effect on the freshness characteristics of cod but definitely increase firmness and dryness, whereas if

TABLE I

Effect of freezing and thawing without intermediate storage on the flavour and texture of cod of different initial freshnesses

	Mean score differences*		
	Freshness Flavour	Firmness	Dryness
Series 1 (frozen and thawed immediately)	0·04$^{n.s.}$	0·23*	0·21*
Series 2 (frozen and thawed overnight)	−0·59*	0·72**	0·36*

*difference calculated as follows:
Score of fillets frozen and thawed—score of corresponding paired fillets which are left unfrozen

thawing is carried out relatively slowly overnight then some freshness is lost from the freshest fish, firmness and dryness again increasing. The conditions used in Series 2 tests are similar to those applied in the frozen storage experiments, and it can be expected, therefore, that all the scores recorded in these experiments will include on average an amount of change similar to that shown for Series 2 tests. Although the effect of freezing and thawing under Series 2 conditions on fish older than 2 days was not examined, it is known that the mean FF of cod iced for 8, 14 and 17 days and, frozen-stored under all conditions, are not significantly different from that of unfrozen fish of the same age in ice.[3] Therefore, it is concluded that for fish iced for 8, 14 and 17 days, freezing and thawing under Series 2 conditions is without effect on FF.

These results show that a single freezing and thawing process is sufficient to produce textural changes in cod which are easily detectable by a trained taste panel tasting only 2 or 3 samples. It has been suggested in early work[15,17,22] that the effects on texture of a single freezing could not be detected by means of tasting. It should be emphasised that the changes in texture and flavour resulting from the kind of freezing and thawing examined in this paper are relatively small and still within the range of normal acceptance.

Objective measurements

Although no direct examinations were made of the effect of freezing and thawing on TMA and Hy concentrations, the mean TMA and Hy concentrations of frozen cod[2] did not differ from the concentrations occurring in unfrozen cod having the same icing history and which had been caught in the same locality. It is concluded, therefore, that freezing and thawing of themselves have little, if any, effect on the concentrations of TMA and Hy in cod of this kind stored in melting ice for the periods examined.

It appears from previous work that freezing and thawing of fresh cod cause both a slight reduction in cell fragility values[14] and a decrease in extractable protein.[15] These observations would indicate that freezing and thawing might at least, cause an increase in the firmness of cod of this kind; such a result was demonstrated.

From previous work[9] it is known that freezing and thawing without intermediate storage has no effect on the colour ratio value of cod iced for 2–3 days.

Changes occurring during frozen storage

Changes in sensory measurements

Freshness scores.—As was reported previously,[2] under only three conditions (fish iced for 2 and 5 days and stored at −22°, or for 17 days and stored at −29°) were slight significant changes in freshness flavours observed over 1 year, the scores of fish of all other icings and storage temperatures remaining constant. As stated above, the FF of fish iced for 8, 14 and 17 days averaged over all periods of frozen storage did not differ from those of the corresponding unfrozen fish. However, as shown in Table II significant differences usually existed between the average scores of unfrozen and frozen stored fish iced for 2 and 5 days. These differences were similar in magnitude to the fall of 0·59 reported for the freshest fish which had been frozen and thawed overnight.

Freshness odour scores were highly correlated with FF:

$$FO = 0{\cdot}88\ FF + 0{\cdot}70\ (r = 0{\cdot}910 \text{ with } 262 \text{ d.f.})$$

Cold storage deterioration scores.—The change of cold storage flavour (CSF) with storage time at −14° is shown in Fig. 1. In the case of the initially fresher fish the scores increase fairly rapidly at first and then more slowly afterwards. Single term exponential, reciprocal or squared relationships between score and storage time do not entirely account for this behaviour. For this reason the curves shown are smoothly drawn freehand through the experimental points. It appears from these curves that CSF increases in fresher fish more rapidly than in staler fish. This behaviour can be accounted for perhaps on the grounds that bacterial spoilage or off-flavours associated with stale cod are physiologically masking to a certain extent CSF, the result being that relatively lower scores are given to the stale samples. However, this cannot be the entire explanation because the phenomenon is observed even with fish iced for 2, 5 and 8 days which all possess bland intrinsic flavours unlikely to interfere with the perception of CSF. It would seem rather that the actual development at −14° of the compounds responsible for CSF is more rapid in fresh fish than in stale fish.

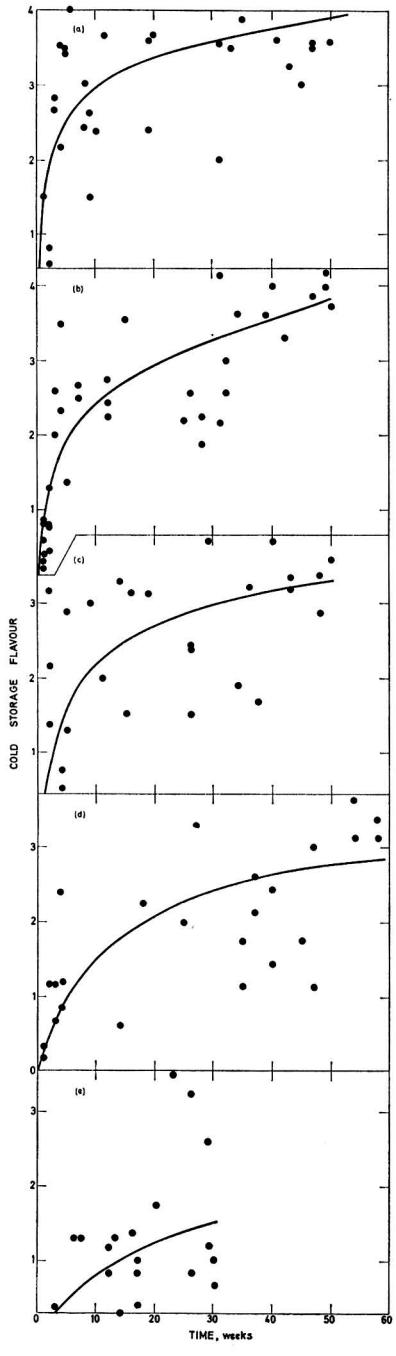
At −22° and −29° the change of CSF with time for any icing can be adequately represented linearly, and the results are presented in Table III. At −22°, CSF of 2 and 5 days iced cod increases at a moderate rate until at 50 weeks a mean score of about 2·5 units is given, whilst there is no significant increase with cod iced for 8 and 14 days, and actually a significant decrease of score with cod iced for 17 days. The difference in the rates of increase between the 2 or 5 and the 8 day samples is significant ($p < 0{\cdot}05$). At −29°, the only

TABLE II

Differences between average freshness scores of frozen stored and unfrozen cod of the same initial freshness

Days in ice	Temperature of storage° −14	−22	−29
2	−1·26**	−0·61**	−0·74**
5	−0·69**	−0·22$^{n.s.}$	−0·18$^{n.s.}$

The differences are calculated as follows:
(average FF of samples stored for up to 1 year)—
(average FF of unfrozen samples)



samples to show significant increases in CSF are the 2 day ones; in this case a score of about 1 unit is reached after 50 weeks' storage. Thus in all cases, the rate of development of CSF is greatest in the freshest samples. Although the differences between the rates of development are small they could be important because CSF considerably affects OA. Whereas an initial high degree of freshness would be expected to be associated with high OA in unfrozen fish, this situation is likely to be reversed in some kinds of frozen stored cod.

Multiple regression analyses of the data at $-22°$ and $-29°$ for all icings showed that CSF was significantly dependent upon pH. pH increased with the number of days' storage in ice, particularly for 14 and 17 day samples. Therefore it seemed possible that the effect of pH on CSF was merely a reflection of the effect of initial freshness on CSF as just described. However, separate analysis of the data for samples of different initial freshnesses showed that the dependence of CSF on pH almost reached significance $0{\cdot}05 < p < 0{\cdot}10$. As there were only 6 to 15 samples at each icing, the data for 2, 5 and 8 days in ice were pooled, this being considered justifiable because the mean pH of these samples were very similar, and the following regressions were calculated:

at $-22°$: CSF = 13·28 + 0·0312* W—1·88** pH
at $-29°$: CSF = 8·92 + 0·0087$^{n.s}$ W—1·22** pH

The inclusion of the pH data in the regression of CSF on W increased the percentage of variance accounted for from 38 to 50 for the $-22°$ samples and from 12 to 28 for the $-29°$ samples. On the other hand, the inclusion of terms for days in ice did not increase the proportion of variance accounted for, which justifies the pooling of the data for 2, 5 and 8 day samples. Thus it is concluded that the development of CSF is, in fact, dependent upon the pH of the sample, high pH being associated with low CSF.

The linear regression shown above for the effect of pH on CSF at $-22°$ is not the best way to represent the data because it indicates erroneously that CSF at zero time of storage is dependent upon pH. A better way of representing the data which leads to the same CSF at zero time is:

CSF = 0·739 + 0·431** W—0·0599** W pH.

Cold storage odour scores (CSO) were highly correlated with CSF:

CSO = 0·729 CSF—0·071 (r = 0·945 with 262 d.f.).

As firmness (F) is influenced by the pH of the sample, in order to determine the effect of frozen storage alone on the development of F, the scores obtained in this study were corrected to the same notional pH using a relationship between F and pH obtained both from multiple regression analyses on samples stored at $-22°$ and $-29°$, and from unfrozen samples. The reason for using samples stored only at $-22°$ and $-29°$ was that under these conditions linear regressions could be obtained from them as follows:

for all samples at $-22°$, F = 15·14 + 0·0117** W—1·85** pH
for all samples at $-29°$, F = 9·80 + 0·0029$^{n.s.}$ W—1·08** pH

The mean pH's of the samples at $-22°$ and $-29°$ were 6·81 and 6·85, respectively.

FIG. 1 (left). *Change of cold storage flavour scores during storage at $-14°$*
Cod stored initially for the following days in ice: (a) 2, (b) 5, (c) 8, (d) 14, (e) 17

TABLE III

Change of cold storage flavour score during storage at −22° and −29°
Linear regression: CSF = a + bW

Temperature of storage°	Days in ice	b	Standard error of b	Degrees of freedom	Correlation coefficient	a
−22	2	0·0377*	0·0150	10	0·62*	0·65
	5	0·0386*	0·0134	15	0·60**	0·37
	8	0·0126$^{n.s.}$	0·0104	14	0·31$^{n.s.}$	0·90
	14	0·0070$^{n.s.}$	0·0103	14	0·18$^{n.s.}$	0·91
	17	−0·0098**	0·0027	7	0·81**	0·70
−29	2	0·0113	0·0038	12	0·66**	0·14
	5			13		0·81
	8			16		0·70
	14			11		0·96
	17			6		0·44

For samples of unfrozen cod having a mean pH of 6·75:

F = 15·60—2·02** pH

If the assumption is made that the relationship between F and pH obtained from experiments on unfrozen samples is applicable to frozen samples, the coefficients 1·85, 1·08 and 2·02 can be pooled, allowing for the number of samples of each kind. When this is done it is found that changing the pH of a sample of frozen cod by 1·0 units results in a mean change in F of 1·8 score units. By using this mean relationship, firmness scores were corrected to the same mean pH of 6·85, which is the mean pH of all the frozen samples analysed.

The change of corrected F at −14° is shown in Fig. 2. As with CSF there appears to be no simple relationship between score and storage time, and the curves shown for the separate samples of different initial freshnesses have been drawn freehand. There is an indication that at this temperature of storage F increases in the fresher fish more rapidly, particularly in the early stages of storage, than in the staler fish. However, a multiple regression analysis of F against the dependent variables of pH, W and D showed that the last of these had no significant effect at the lower temperatures of −22° and −29°. At these temperatures the change in F is linear with storage time; at −22° there is a significant increase in F of 0·0117 score units per week of storage whilst at −29° there is no significant change.

It should be noted that the corrected F values of the frozen stored samples at zero time of storage are 2·3 to 2·5, which are, as expected, similar to the scores recorded for cod frozen and thawed without intermediate storage.

Dryness scores (Dr) were highly correlated with F and pH:

Dr = 0·541**F + 0·309**pH—1·718 (r = 0·844 with 262 d.f.)

The changes in Dr at −14°, being similar to those for F cannot be simply related to time, but attain a value of about 2·8 after 50 weeks storage. At the lower storage temperatures, Dr changes can be represented linearly:

at −22°: Dr = 5·59 + 0·00853**W + 0·0149$^{n.s.}$ D—0·586**pH

at −29°: Dr = 5·92 + 0·00247$^{n.s.}$W + 0·0219** D—0·649**pH

Thus at −22°, Dr increases significantly during storage but does not depend upon initial freshness, whilst at −29° Dr does not change during storage but does depend upon initial freshness. At all three temperatures of storage Dr at zero time of storage is about 1·5 which is significantly higher than the mean value of 1·0 for unfrozen fish, and probably reflects partly the effects of freezing and thawing alone.

The results in this section cannot be compared directly with the few previously published results because the methods of sensory assessment differ. For example, the term 'taste' has been used to cover all flavour attributes including those associated with initial freshness. Possibly as a result of this, it has been found,[18,19] in contrast to the present results, that inital freshness had little or no effect on the rate of deterioration of cod stored at −9°, −12°, −23° or −26°. A number of other results bearing on the question of whether initial freshness affects keeping quality in the frozen state have been reviewed by Lane,[20] but these refer to species other than cod.

Overall acceptability scores (OA).—Changes in OA at the three temperatures of storage are shown in Fig. 3. The scores at −14° for the samples iced for 2, 5 and 8 days follow an approximately exponential decline with time, the best fitting lines being as follows:

2 days iced: OA = 4·34 + 2·48exp (−0·33 W)
5 days iced: OA = 4·11 + 1·86exp (−0·10 W)
8 days iced: OA = 3·76 + 2·71exp (−0·07 W)

When fitted to a linear regression the scores for samples iced for 14 and 17 days and stored at −14° show, respectively no significant change and a significant ($p < 0·01$) positive change with time of storage as shown in Fig. 3. The mean score of the 14 day samples stored at −14° is 3·43. At the two lower temperatures of storage, all changes of scores with time can be represented linearly. Where there is no significant change with time this is shown by means of a line drawn parallel to the x axis, at a distance from it equal to the mean score for all periods of frozen storage.

In the case of fish iced for 2, 5 and 8 days and stored at −14°, the rates of decline in OA are all different, though the significance of the differences has not been obtained. At −22° the corresponding rates for 2 and 5 days are not significantly different but both of them are significantly different ($p < 0·01$) from the rate for the fish iced for 8 days. Thus it appears that on storage at both −14° and −22° OA declines more rapidly in the freshest fish. It has already been noted that the rates of development of CSF and F are more rapid in the freshest fish and it would therefore seem likely that these two attributes, either separately or in combination, exert a considerable influence on the preferences of the panel.

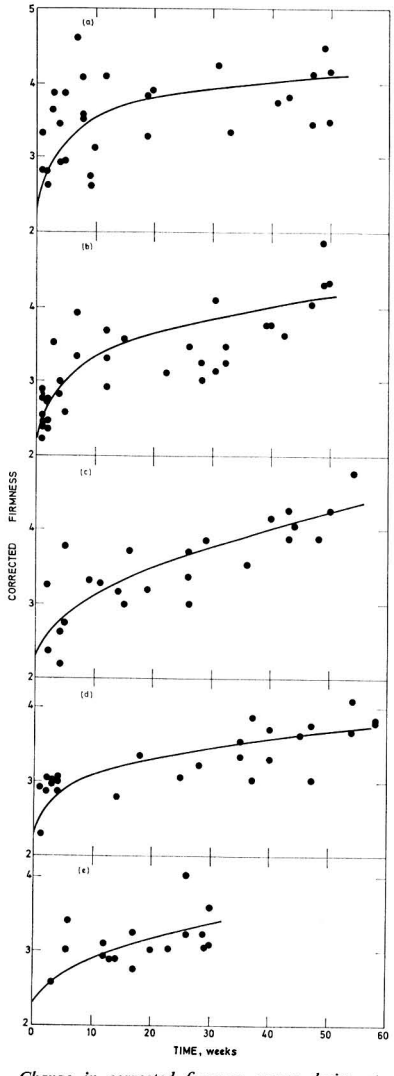


FIG. 2. *Change in corrected firmness scores during storage at −14°*

Cod stored initially for the following days in ice: (a) 2, (b) 5, (c) 8, (d) 14, (e) 17

The results on 17 days iced fish at −14° and −22° show that the acceptability of very stale fish may actually increase during frozen storage at some temperatures. Since the samples are scored as unacceptable at any stage this point is of academic importance, but it points to a gradual loss of objectionable bacterial spoilage odour and flavour constituents during frozen storage at high temperature, a process which outweighs the opposite effect of the accumulation of objectionable characteristics resulting from frozen storage.

The data shown in Fig. 3 enable a prediction to be made of the period of storage at a given temperature and initial freshness which leads to a particular value of OA. However, one level of acceptability is probably of most importance and this is at a score of 5 where an attribution of acceptability changes to one of unacceptability. Scores of 5 and 4 are listed as 'neither like nor dislike' and 'dislike slightly', respectively. When the score falls below 5 it can be reasonably concluded that the point has been reached where the sample would not normally be eaten. The period leading up to this point might be defined as the ultimate storage life (USL) of the sample treated in a given way. The USL is not, of course, the same as the period after which the first detectable difference between the unstored and stored samples is observed; it will in general be longer than this period.

Only the samples iced 2, 5 and 8 days are relevant as far as predictions of USL are concerned because the scores for 14 and 17 day samples are all below 5.

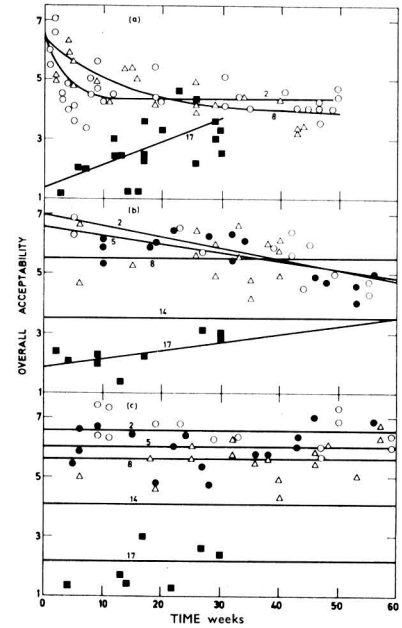


FIG. 3. *Change in overall acceptability scores during frozen storage*

Cod stored at the following temperatures: (a) −14°, (b) −22°, (c) −29°, and initially for the following days in ice: ○ 2, ● 5, △ 8, ■ 17. Some experimental points have been omitted for the sake of clarity

For the data at $-14°$ the procedure adopted was to transform OA into a logarithmic form so allowing a linear regression of log OA versus W to be calculated. From this regression and the corresponding regression using untransformed data at $-22°$, the predicted times (and the error in these times) at which OA assumes a value of 5 could be obtained. Thus, at $-14°$ and $-22°$ the USL values for the 2, 5 and 8 day samples taken as a group are 7·7 (±3·8) and 56·0 (±25) weeks, respectively, the value in brackets being the 95% confidence limits of the estimate. Although for this series of tests USL is not dependent upon initial freshness up to a period of 8 days in ice, it is clear that for fish iced for 8 to 14 days, the USL will be less than for 2, 5 and 8 days.

It should be emphasised that these conclusions about preferences are strictly applicable only to the particular group of tasters involved and for fish treated and cooked in the way described in this paper.

Fish-to-fish variation.—Examination of the data shows that there is some fish-to-fish variation as far as changes in sensory measurements are concerned. For example, Table IV shows that for CSF and F of samples stored at $-22°$, the variances unaccounted for after allowing for weeks stored and pH are usually significantly greater than the variances of the sample mean score. The latter variances were obtained by first calculating for each session the within-sample variances which were then pooled over all the sessions and divided by the number of tasters (that is, 7).

It is reasonable to conclude that the variation not accounted for by either storage or pH arises as a result of differences in the storage behaviour between individual fish.

Changes in objective measurements

Trimethylamine and hypoxanthine concentrations.—As shown in a previous publication,[2] the concentrations of these two substances are unaffected by frozen storage at any temperature up to about 50 weeks, and can therefore be used as indices of the initial freshness of a sample before it was frozen.

pH.—This increased in an approximately quadratic manner with days in ice (D), the mean increment in pH over the value for fish iced for 2 days being 0·020, 0·051, 0·155 and 0·229 for fish iced for 5, 8, 14 and 17 days, respectively. In addition, the pH changed in an approximately cubic manner with month when caught (M):

$$pH = 6{\cdot}44 + 0{\cdot}150^{**}M - 0{\cdot}0174^{**}M^2 + 0{\cdot}00059^{**}M^3 + 0{\cdot}000791^{**}D^2$$

high pH being associated with the mid-winter months and low pH with the mid-summer months. This behaviour, which is presumably cyclical with season, is clearly the result of variations in the biochemical composition of the cod in this area; in summer the fish are feeding actively and have high glycogen reserves, whilst in the winter the opposite is true. After the death of summer-caught fish larger amounts of lactic acid are formed than in winter-caught fish with the result that the ultimate *post-mortem* pH is lower in the summer-caught fish.

No obvious change of pH with frozen storage occurred, though any such change may have been obscured by the seasonal changes.

Cell fragility (CF).—The changes in CF for all periods of icing and at the three temperatures of storage are shown in Fig. 4. The curve shown through the data at $-14°$ is the best fitting exponential regression, accounting for 54% of the variance:

$$CF = 0{\cdot}207 + 0{\cdot}459\exp(-0{\cdot}200\ W)$$

At this temperature the pH of the sample does not significantly affect the values, but there was an effect of days in ice which was of borderline significance but increased the percentage of variance accounted for from 54 to 59. This relationship is different from that found by Love[15] for a uniform batch of fresh cod, namely:

$$CF = 0{\cdot}052 + 0{\cdot}862\exp(-0{\cdot}150\ W)$$

It has been found recently that the readings obtained by this method are to some extent dependent upon the pH of the homogenisation medium, and this discrepancy is believed to have arisen from the use of formaldehyde of unusually high pH. At $-22°$ and $-29°$ the values are not significantly dependent upon initial freshness but are significantly dependent upon pH:

at $-22°$: $CF = 2{\cdot}02 - 0{\cdot}199^{*}pH - 0{\cdot}00632^{**}\ W$

at $-29°$: $CF = 2{\cdot}94 - 0{\cdot}349^{**}pH$

Because of the dependence of CF on pH the lines shown in Fig. 4 are for a pH of 6·85 corresponding to the mean value of the samples.

The sample-to-sample variation with this method was high, the standard deviations round the lines shown in Fig. 4 being 0·163 and 0·187 for $-22°$ and $-29°$, respectively.

TABLE IV

Comparison of variation in cold storage flavour and firmness scores of samples stored at $-22°$

Initial freshness of sample (days in ice)	Number of samples	Residual variance after accounting for time in the frozen state and pH	
		Cold storage flavour scores	Firmness scores
2	12	0·669	0·054
5	17	0·615	0·094
8	16	0·260	0·059
14	16	0·279	0·065
17	9	0·335	0·121
Variance of mean score*	280	0·137	0·054

* Calculated as described in the text

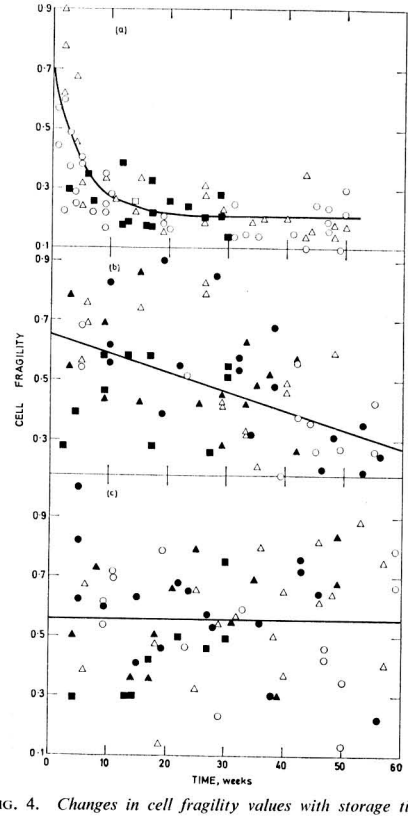


FIG. 4. *Changes in cell fragility values with storage time*

Cod stored at the following temperatures: (a) −14°, (b) −22°, (c) −29°, and stored initially for the following days in ice: ○ 2, ● 5, △ 8, ▲ 14, ■ 17. Some experimental points have been omitted for the sake of clarity

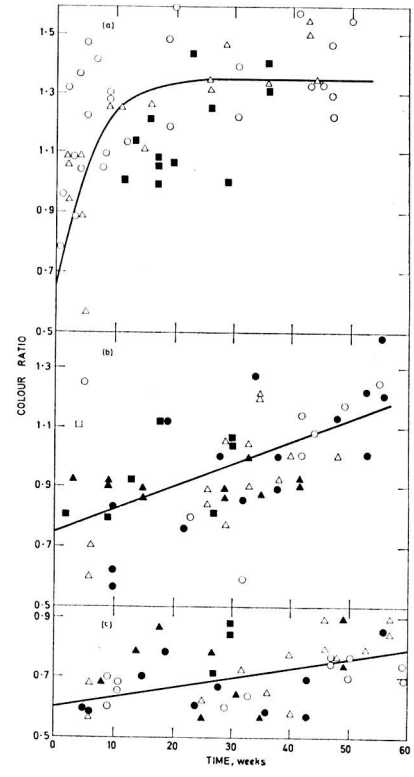


FIG. 5. *Changes in colour ratio values with storage time*

Cod stored at the following temperatures: (a) −14°, (b) −22°, (c) −29°, and stored initially for the following days in ice: ○ 2, ● 5, △ 8, ▲ 14, ■ 17. Some experimental points have been omitted for the sake of clarity

Colour ratio (*CR*).—The changes in CR for all periods of icing and at the three temperatures of storage are shown in Fig. 5. The curve through the data at −14° is the best fitting exponential regression, accounting for 74% of the variance:

$$CR = 1{\cdot}353 - 0{\cdot}707\exp(-0{\cdot}180\ W)$$

which agrees closely with a previous result.[9] For the two lower temperatures the lines are:

at −22°: $CR = 0{\cdot}752 - 0{\cdot}00743^{**}\ W$

at −29°: $CR = 0{\cdot}594 + 0{\cdot}00317^{**}\ W$

At none of the three temperatures of storage were the results dependent upon pH or days in ice.

The sample-to-sample variation is high though relatively less than with CF, the standard deviations round the lines shown in Fig. 5 being 0·147 and 0·103 at −22° and −29°, respectively.

Protein extractability (*PE*).—The changes in PE for all periods of icing and at the three temperatures of storage are shown in Fig. 6. The curve through the data at −14° is the best fitting exponential regression, accounting for 78% of the variance:

$$PE = 25{\cdot}0 + 75{\cdot}3\exp(-0{\cdot}0752\ W)$$

This regression is different to one published previously[15] namely:

$$PE = 26{\cdot}5 + 58{\cdot}90\ (-0{\cdot}130\ W)$$

For the two lower temperatures the lines are:

at −22°: $PE = 93{\cdot}3 - 0{\cdot}637^{**}\ W$

at −29°: $PE = 89{\cdot}9 - 0{\cdot}225^{**}\ W$

At none of the three temperatures of storage were the results dependent upon pH or days in ice.

The sample-to-sample variation with this method was similar to that of CR, the standard deviations round the lines shown in Fig. 6 being 11·5 and 9·8 at −22° and −29°, respectively.

Correlations between variables

A number of correlations between the variables in the experiment were examined with the main purpose of finding how the individual objective tests were correlated with the individual subjective assessments. The usual purpose of an objective test is to predict quality in terms of one particular attribute; therefore, in the present analysis the dependence

of the various sensory attributes as independent variables on the objective test as dependent variable are presented.

Freshness flavour (FF) and trimethylamine or hypoxanthine

As shown above, TMA and Hy do not change with period of frozen storage, but FF is somewhat dependent both upon freezing and upon frozen storage. Therefore the chief value of TMA and Hy determinations on frozen cod is for the prediction of the initial freshness before freezing and frozen storage. The relative value of TMA and Hy as predictors of initial freshness will depend therefore upon their relative value as predictors of freshness in unfrozen cod. In this regard Hy is superior to TMA over the whole range of freshnesses because intrinsic sample variability tends to be less with Hy than with TMA, and perhaps because Hy concentrations increase from the commencement of icing whereas TMA concentrations tend to remain relatively constant in the early stages of icing.[2,16] Therefore, if the aim is to predict with moderate accuracy the initial FF of a sample of cod before freezing, the determination of Hy is the method of choice.

FF is one of the main determinants of overall eating quality in frozen fish, and the question therefore arises whether for any given frozen sample Hy is a better predictor of FF than TMA. The correlation coefficients between FF and Hy, log Hy, TMA and log TMA for 262 samples of all icing and frozen storage histories, have the highly significant values of −0·843, −0·800, −0·823 and −0·842, respectively. The corresponding values for the three separate temperatures of frozen storage are very similar. Therefore, neither test offers an advantage over the other as far as the accuracy of prediction of actual FF in the frozen material is concerned. However, taking all factors into account including the amount of information provided by the test, the method of choice is the determination of Hy concentration. For all conditions of icing and of frozen storage the following relationship accounts for the highest percentage of the variance of FF:

$$FF = 7{\cdot}505 - 0{\cdot}832\ Hy$$

Cold storage flavour (CSF), firmness (F) and cell fragility (CF)

The relationships between CSF and CF, and corrected F and CF are shown in Figs 7 and 8, respectively. In these figures only the data on fish iced for 2, 5 and 8 days are included on the grounds that the assessments of CSF and F are considerably different with 14 and 17 days iced cod, compared with the fresher samples. In addition, it was felt that attempts to predict these cold storage attributes in very stale fish is of somewhat academic interest as the fish would be rejected on grounds of bacterial spoilage anyway. The lines shown in Figs 7 and 8 are obtained from the following best fitting relationships:

$$CSF = 17{\cdot}13 - 3{\cdot}50^{**}\ CF - 2{\cdot}03^{**}\ pH$$

$$F = 17{\cdot}01 - 1{\cdot}72^{**}\ CF - 1{\cdot}93^{**}\ pH$$

for pH values of, respectively, 6·76, which is the mean value of the samples analysed, and 6·85 which is the mean pH used, to correct F as described above. Strictly speaking the cell fragility data shown in Fig. 7 at −22° and −29° should be corrected for pH according to the finding in a previous section, but this small correction has been omitted. The

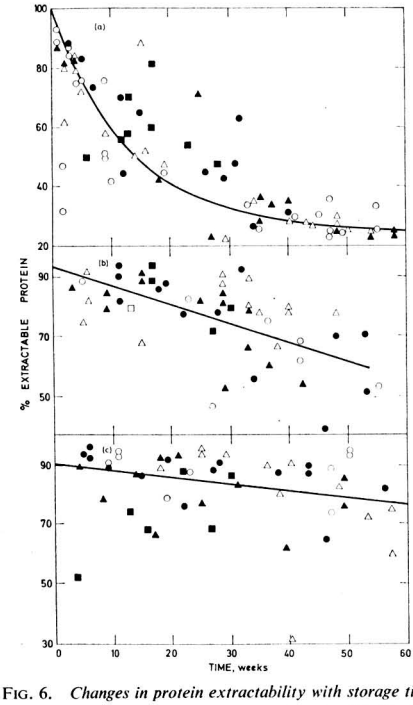


FIG. 6. *Changes in protein extractability with storage time*

Cod stored at the following temperatures: (a) −14°, (b) −22°, (c) −29°, and stored initially for the following days in ice: ○ 2, ● 5, △ 8, ▲ 14, ■ 17

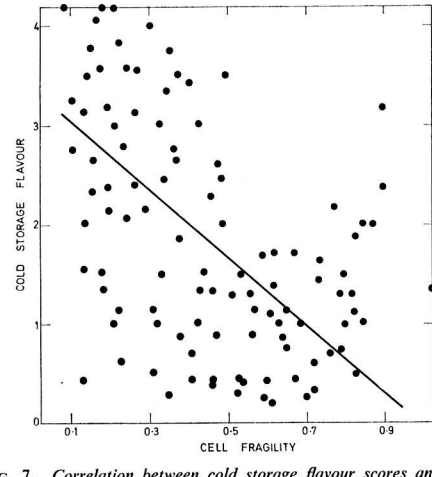


FIG. 7. *Correlation between cold storage flavour scores and cell fragility values for samples stored initially for* 2, 5 *and* 8 *days in ice, and held under all conditions of frozen storage*

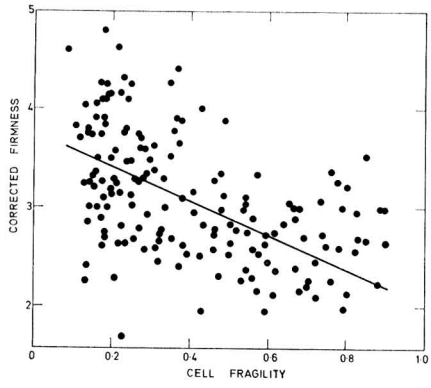


FIG. 8. *Correlation between corrected firmness scores and cell fragility values for samples stored as in Fig. 7*

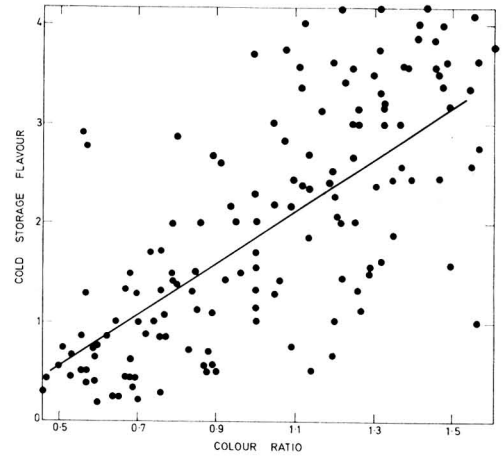


FIG. 9. *Correlation between cold storage flavour scores and colour ratio values for samples stored as in Fig. 7*

percentage variance, in CSF and F accounted for by the above regressions are 46·9 and 47·6, respectively.

It is likely that a linear regression is not the best fit to the data shown in Figs 7 and 8 because in the samples frozen-stored for the longest period CF is relatively constant whilst CSF and F continue to increase.

Cold storage flavour (CSF), firmness (F) and colour ratio (CR)

The relationships between CSF and CR, and corrected F and CR are shown in Figs 9 and 10, respectively. The selection of data is as described in the previous section. The lines shown are obtained from the following best fitting relationships:

$$CSF = 8{\cdot}39 + 2{\cdot}57^{**}\,CR - 1{\cdot}35^{**}\,pH$$
$$F = 12{\cdot}86 + 1{\cdot}26^{**}\,CR - 1{\cdot}62^{**}\,pH$$

using the same pH values as described in the previous section.

The percentage variances in CSF and F accounted for by the above regressions are 50·4 and 50·0, respectively. These are slightly higher values than for the data on CF and to this extent CR can be said to be a better predictor of either CSF or F. A similar conclusion was previously reached from an examination of the correlations between a wider selection of samples.[2] As with the CF, that for CR is probably not best represented by a linear regression.

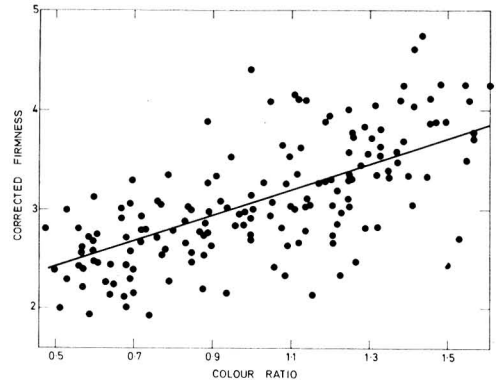


FIG. 10. *Correlation between corrected firmness scores and colour ratio values for samples stored as in Fig. 7*

Cold storage flavour (CSF), firmness (F) and protein extractability (PE)

The relationships between CSF and PE and between corrected F and PE are shown in Figs 11 and 12, respectively, only data for fish iced for 2, 5 and 8 days being included as before. The lines shown are obtained from the following best fitting relationships:

$$CSF = 9{\cdot}96 - 0{\cdot}0370^{**}\,PE - 0{\cdot}835^{**}\,pH$$
$$F = 14{\cdot}16 - 0{\cdot}0148^{**}\,PE - 1{\cdot}50^{**}\,pH$$

using the same pH as described before.

The percentage variances in CSF and F accounted for by the above regressions are 56·3 and 55·2, respectively. These values are higher than those for the data on either CF or CR thus showing that of the three objective measurements PE is the most precise predictor of the two sensory attributes.

Selection and use of objective methods for the quality assessment of frozen cod

The complete evaluation by objective means of eating quality in any sample of frozen stored cod must involve at least three tests. One test is required to give an accurate assessment in terms of changes resulting from 'wet' spoilage, and a second test in terms of changes resulting from frozen storage. A third test is required to describe the differences in texture resulting from differences in initial pH. If texture is not considered to be an important contributor to the eating quality of a particular sample then a measurement of pH can be dispensed with.

As discussed above, the best objective test for evaluating quality changes in the 'wet' state is Hy. The FF score predicted from Hy will then provide one facet of the overall

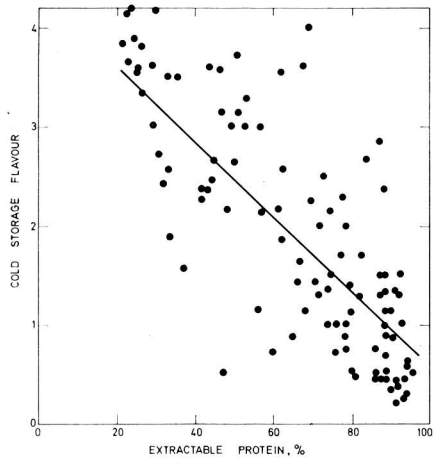


FIG. 11. *Correlation between cold storage flavour scores and protein extractability for samples stored as in Fig. 7*

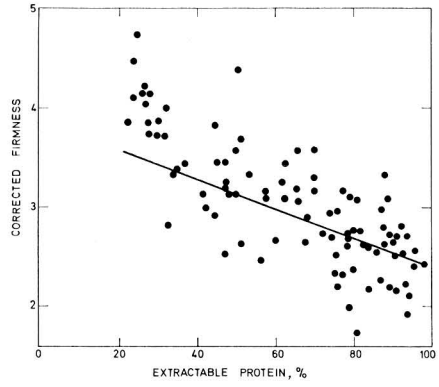


FIG. 12. *Correlation between corrected firmness scores and protein extractability for samples stored as in Fig. 7*

eating quality of the sample. Special considerations apply to limiting FF which correspond to the stage at which the fish become inedible. Normally this stage is reached with cod when a freshness score of 4·5 or less is given, corresponding to the freshness of fish held in melting ice for about 14 days or longer. In the case of the type of cod used in this work, this stage is reached when the Hy concentration in fish exceeds 3·00 (standard error ±0·14) μM/g flesh.[2] If the Hy concentration is greater than this the fish is likely to be too spoiled to be edible regardless of the amount of accompanying frozen storage deterioration.

The selection of an objective test of frozen storage deterioration depends, in the present stage of development of such tests, largely upon the ability of the test to predict the degree of quality loss as measured by the accumulation of CSF and the development of F. It is concluded that of the three tests, PE is the best predictor of cold storage deterioration in cod when equivalent numbers of determinations are carried out. Previous results[2] on a wider range of samples showed that PE was correlated much better with CSF and F than were the other two tests. PE determinations are somewhat tedious and time-consuming; CF or CR determinations are considerably less precise but are rapid and fairly easily carried out. It might be advantageous therefore to carry out more replicate determinations using these last two methods to achieve the same precision as with the PE method.

A PE determination on a given sample will allow a prediction to be made of either CSF or F scores using one of the equations given above. In the case of F, the value needs to be corrected using a separate determination of pH and the relationship between F and pH. As with FF, there will be limiting scores for CSP and F above which the sample will be judged to be inedible regardless of its FF, and in such circumstances a knowledge of the FF is irrelevant. At the moment there is very little information on the extent of cold storage deterioration in cod which on this ground alone will lead to it being classed as inedible, but preliminary experience among personnel at this Station indicates that this point is reached when CSF and F exceed about 3·0 and about 3·5, respectively.

If all the predicted freshness flavour and cold storage deterioration scores lie above the absolute rejection values then the overall eating quality will depend upon some combination of these scores. The exact way in which these predicted scores or the results of objective methods should be combined and the weighting to be given to each cannot, in general, be decided *a priori*, and may vary with the consumer, or batch of consumers. It is probably necessary, in the first place, to find out empirically which is the best way of combining the results of objective tests in order to obtain an assessment of overall acceptability. Some idea of the way in which combinations of the results of objective methods can be used to predict overall eating quality is illustrated for the OA scores of the tasters used in this experiment. Using only the Hy and PE data for fish iced for 2, 5 and 8 days and for all conditions of frozen storage, the following linear regression, accounting for 35·6% of the variance, was obtained:

$$OA = 4{\cdot}10 - 0{\cdot}493^{**}\ Hy + 0{\cdot}0266^{**}\ PE$$

It is possible that some non-linear combination of the data would provide a better prediction of OA but of all the possible linear combinations of TMA, Hy, CF, CR and PE, the regression quoted gives the best prediction.

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Torry Research Station,
Ministry of Technology,
Aberdeen

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INFLUENCE OF WATER LOSS ON THE LOSS OF VOLATILES BY APPLES

By R. B. H. WILLS

A study was made of the effect of different rates of water loss on the loss of volatiles from Jonathan apples stored at 30°F. The rates of loss of n-butanol, iso-amyl alcohol and n-hexanol decreased with increasing rates of water loss while the corresponding acetate esters increased. The water loss was considered to be enhancing the production of acetate esters as well as providing a 'carrier' for their removal from the fruit.

Introduction

Low-temperature breakdown is a physiological disorder, common to many varieties of apples, which may limit the storage life of susceptible varieties. In 1923, Overholser, Winkler & Jacob[1] suggested that breakdown was caused by the accumulation of deleterious substances in the fruit and that the reduction in breakdown they achieved by increasing the rate of air circulation over the fruit was due to evaporation of these substances from the fruit. Since then, no critical study has been published to confirm the validity of this hypothesis. Chromatographic techniques provide more sensitive methods whereby this problem can now be better examined.

Recent storage work[2–6] has shown that treatments which promote water loss from the fruit markedly reduce the incidence of breakdown and that where other treatments reduce breakdown,[7,8] their effect can also be explained in terms of water loss (Scott, K. J., & Roberts, E. A, Unpublished results). However no explanation was offered for the rôle of the water loss beyond the suggestion that some toxic compound could be removed with the water.[5,6]

If the loss of water provides a 'carrier' in the evaporation of toxic volatile compounds, the amounts of these volatiles given off, and hence the reduction in breakdown, would increase as the rate of water loss increased. However, a preliminary study with Delicious apples suggested that the effect of water loss on the loss of volatiles could not be fully explained on this physical basis alone, as some compounds had a lower rate of evolution at the higher rate of water loss. This paper reports an experiment which attempted to determine the influence of water loss on the production of volatiles from Jonathan apples.

Experimental

Fruit samples

Mature Jonathan apples were harvested from commercial orchards at Batlow and Bilpin, N.S.W. The fruit were systematically distributed into 10 units of 15 for Batlow fruit and 20 for Bilpin fruit to provide two replicates of five treatments from each area. The weight of fruit in each unit was approximately two kg. Each unit was placed in a ventilated glass jar and stored at 30° F, a temperature at which breakdown readily occurs with Jonathan apples.

Treatments

A variation in water loss between the units, was obtained by ventilating the jars with air at five different humidities. The air flow was 10 l/h. Air, previously dried over calcium chloride and sodium hydroxide and purified by passage through activated charcoal, was passed over saturated solutions of various salts to obtain a range of relative humidities from near zero to approximately 100%.[9] The fruit units were weighed each week to determine the weight lost during storage. As the weight lost by apples is mainly water,[10] the measured weight loss was taken to be the water loss.

Analysis of volatiles

The volatiles given off by the fruit were collected over a 24 hour period once a week by inserting a cold trap (ethanol–dry ice mixture) in the air lines leaving the jars. Collections were made on the Batlow fruit for 12 weeks and on the Bilpin fruit for 13 weeks.

The trapped material was melted, decanted into a tared sample vial, weighed and then re-frozen until analysed. A 10 μl sample of the aqueous material was injected into an Aerograph gas chromatograph (Model Hy-Fi 600D) equipped with a flame ionisation detector. A 20 ft column of FFAP ($3\frac{1}{2}$%) on Chromosorb G (60–80 mesh) was used at 100° c with nitrogen as the carrier gas (20 ml/min). The hydrogen flow rate was 20 ml/min, and air 300 ml/min.

Identification of compounds

The volatiles were identified by comparing their retention times on the FFAP column with those of known compounds chromatographed under the same conditions. The known compounds were injected together with 10 μl of water to obtain times comparable with the apple volatiles.[11]

Comparisons were also made of retention times obtained on a 6 ft glycerine (25%) on Celite (45–60 mesh) column at 50° c. The gas flows were: nitrogen 40 ml/min, hydrogen 20 ml/min, and air 300 ml/min.

Treatment of data

Standard curves were used to convert the measured heights of peaks to the weights of volatiles (μg) collected for the 24 hr period.

The converted data were treated by the analysis of covariance applicable when treatments are applied to produce differences in covariate values. The responses were concentration of alcohol, ester and the ratio of alcohol/ester. The covariate was the rate of water loss.

Results

Identification of the volatiles

An example of the chromatograms obtained with the FFAP column is shown in Fig. 1. The principal peaks found were ethanol; n-butanol, n-butyl acetate; iso-amyl alcohol, iso-amyl acetate; n-hexanol, and n-hexyl acetate. Fractionations on the glycerine column confirmed these identifications. These compounds have been reported previously as being present in apples.[12] As the other peaks showed no apparent variation with water loss, no attempt was made to identify them.

Effect of water loss on loss of volatiles

The effects of the rate of water loss on the amounts of n-butanol, iso-amyl alcohol, n-hexanol, n-butyl acetate, iso-amyl acetate and n-hexyl acetate, given off by the fruit are shown in Fig. 2.

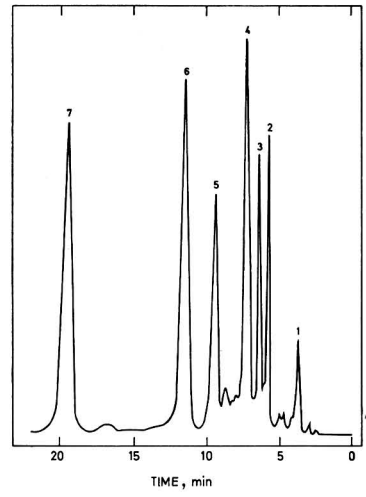

FIG. 1. *Chromatogram of apple volatiles run on FFAP*

1 ethanol
2 n-butyl acetate
3 *iso*-amyl acetate
4 n-butanol
5 *iso*-amyl alcohol
6 n-hexyl acetate
7 n-hexanol

An examination was made of the relationship between the ratio of each alcohol to its corresponding acetate ester and the water loss. The regression analysis showed that each ratio decreased significantly with increasing rate of water loss (Fig. 3). The levels of significance were $0{\cdot}01 < P < 0{\cdot}02$ for the ratios n-butanol/n-butyl acetate and iso-amyl alcohol/iso-amyl acetate and $0{\cdot}10 < P < 0{\cdot}20$ for the n-hexanol/n-hexyl acetate ratio. The analysis was carried out on the data pooled for all the storage times as there was no interaction between the time in store and treatments.

Effect of water loss on the incidence of breakdown

Other workers in this laboratory have examined the effect of water loss on breakdown production using fruit from the same trees as those used in this study. They found that an increase in water loss decreased the incidence of the disorder.

Discussion

The results show that an increase in water loss, even though only a small fraction of the water in the fruit, increased the rate of evaporation of some volatiles and decreased that of others. The increase in the rate of ester removal could be attributed to a 'carrier' effect of the water lost but the decreased evolution of alcohols cannot be explained in this way. Since the most significant effect was the decrease in the ratios of alcohol/acetate ester with increasing water loss, this loss apparently enhanced the production of esters from their components. Increased ester formation would lower the concentrations of alcohol and acid in the fruit. A decrease in the levels of alcohols in the fruit would explain why their rates of evolution decreased at higher rates of water loss.

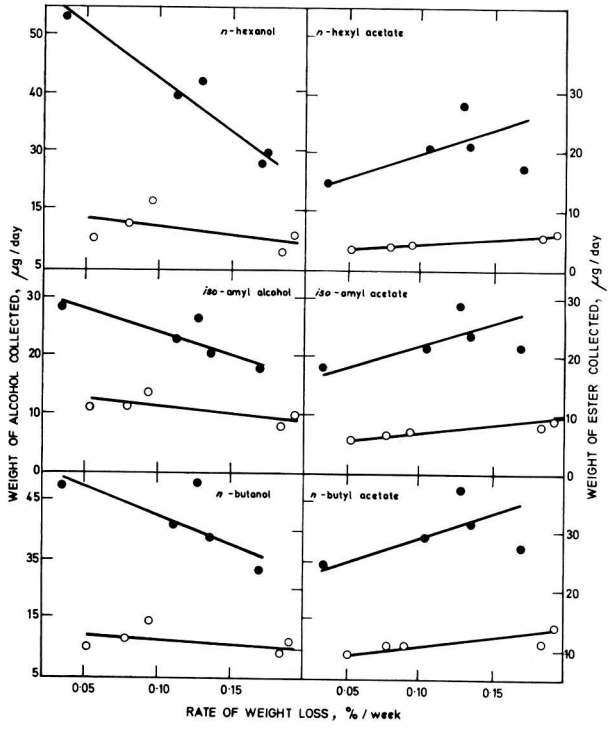

FIG. 2. *Effect of the rate of water loss on the amounts of alcohols and esters given off during storage*
Each point is the mean of all the weekly readings of both replicates.
● Batlow ○ Bilpin

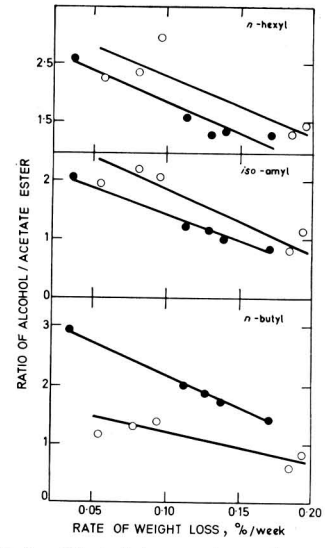

FIG. 3. *Effect of the rate of water loss on the ratio of alcohol/ acetate ester*
Each point is the mean of the ratios obtained from all the weekly readings of both replicates.
● Batlow ○ Bilpin

It appears that accumulation in the fruit of the constituent parts of the acetate esters is associated with breakdown, and that removal of water reduces breakdown because it reduces the concentrations of these chemicals in the fruit. Since acetate has a central rôle in many biochemical reactions, the effect of water loss on acetate metabolism in apples merits further investigation.

Acknowledgments

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JUNE, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

The 'Bolböden' of the Eifel. G. Jaritz and J. Schalich (*Z. PflErnähr. Bodenk.*, 1967, **117,** 89–96).—The soils described are transported *terra fusca* overlain by a shallow layer of humic colluvium. Chemical, physical and mineralogical characteristics are recorded. The soils are rich in illite, show obvious shrinkage properties, are deficient in nutrients, notably associated with temporary fixation of K, and produce irregular crop yields.
A. G. POLLARD.

Classification of hydromorphic soils. D. Schroeder (*Z. Pfl-Ernähr. Bodenk.*, 1967, **116,** 199–207).—A review. The basis of the classification is explained and discussed. Variations in classification adopted in different countries are noted, together with the grading of arable soils. A. G. POLLARD.

Occurrence of imogolite in pumice tuff soils of Western Germany. G. Jaritz (*Z. PflErnähr. Bodenk.*, 1967, **117,** 65–77).—The presence of imogolite (**I**) in a brown-earth of fine-grained pumice tuff was demonstrated by electron micrographs, *X*-ray diffraction, D.T.A. and u.v. spectroscopy. Data for six soils are presented. The max. concn. of **I** (~10% of the clay fraction) occurred in the B_{v1} horizon; the concn. in B_{v2} was lower, the difference corresponding with an increased proportion of halloysite–metahalloysite in B_{v2}. Other profiles examined were developed under similar climatic conditions but were derived from different parent material, or were derived from the same material under different climatic conditions.
A. G. POLLARD.

Framework for man-made soil changes: an outline of metapedogenesis. D. H. Yaalon and B. Yaron (*Soil Sci.*, 1966, **102,** 272–277).—Man-induced changes in soil processes resulting from normal land utilisation practices and involving profile changes, are discussed. It is suggested that a systematic study should be made of such changes, as being of value in the prediction of further anticipated changes. A. G. POLLARD.

Thermal properties of a pumice soil. P. H. Cochran, L. Boersma, and C. T. Youngberg (*Proc. Soil Sci. Soc. Am.*, 1967, **31,** 454–459).—Calculated thermal conductivities of dacite pumice materials agreed closely with measured values. The values were very low, which may account for the frequent occurrence of night frost in this region. The results are discussed in relation to the establishment of different pine species. A. H. CORNFIELD.

Effect of water content on axial strain in a loam soil under tension and compression. D. A. Farrell, E. L. Greacen, and W. E. Larson (*Proc. Soil Sci. Soc. Am.*, 1967, **31,** 445–450).—A method of measuring the tensile strain energy of soil at varying moisture contents is described, and results are presented for a loam.
A. H. CORNFIELD.

Hydraulic properties of disturbed soil materials affected by porosity. G. E. Laliberte and R. H. Brooks (*Proc. Soil Sci. Soc. Am.*, 1967, **31,** 451–454).—The effects of changes in soil porosity on saturated permeability, bubbling pressure and pore-size distribution were determined. A. H. CORNFIELD.

Comparison of water-potential measurements made with two types of thermocouple psychrometer. W. D. Zollinger, G. S. Campbell, and S. A. Taylor (*Soil Sci.*, 1966, **102,** 231–239).—Comparison is made of the wet-loop (*W*) and the Peltier (*P*) type of thermocouple psychrometer, both units being mounted in the same holder for measurement of the relative v.p. Some changes occur during the measurement by both instruments. With the *W* method water vapour is added to the system from the inserted water droplet whereas in the *P* method a small amount of water is condensed and is removed from the system. Thus, the former method records the higher water potential, the difference being more pronounced in dried soils. The small amount of water condensed in the *P* apparatus is probably negligible in most cases. Measurements made with plant material did not indicate significant differences between the two systems. A. G. POLLARD.

Evaporation of water from soils as influenced by drying with wind or radiation. R. J. Hanks, H. R. Gardner, and M. L. Fairbourn (*Proc. Soil Sci. Soc. Am.*, 1967, **31,** 593–598).—Evaporation in response to wind or radiative drying caused different magnitudes and direction of temp. gradients. For two of three soils cumulative evaporation was greatest for wind drying. Water contents within about 10 cm of the surface were lower for radiative drying than for wind drying. A. H. CORNFIELD.

Relations between air and water permeability in soil samples. K. H. Hartge (*Z. PflErnähr. Bodenk.*, 1967, **117,** 97–107).—Permeabilities were measured in undisturbed core samples. Permeability to air was determined at tension ≡200 cm of water. Values for air and for water were equal only if saturation with water was effected under vacuum conditions. With all other saturation methods tested permeability to water was less than that to air. This is attributed to the inclusion of air in the soil used for the water measurements; a nomogram is presented to correct for this. Water percolation measurements made without previous saturation under vacuum, may be compared among themselves provided the 'saturation' is carried out under constant conditions.
A. G. POLLARD.

Theory of suction drain from the saturated ideal soil: analysis of capillary moisture-distribution curve. T. Tabuchi (*Soil Sci.*, 1966, **102,** 161–166).—The mathematical theory associated with a new model system of soil pores is presented. Its influence on capillary moisture distribution and suction drainage is discussed.
A. G. POLLARD.

Soil water and chloride redistribution under various evaporation potentials. D. D. Fritton, D. Kirkham, and R. H. Shaw (*Proc. Soil Sci. Soc. Am.*, 1967, **31,** 599–603).—The depth zone in which vaporisation of soil water occurs for different evaporation potentials was determined for laboratory cylinders 38 cm long, using $CaCl_2$ as a tracer. Most soil water evaporated at depths shallower than 7 cm. Water and chloride profiles and cumulative evaporation for four evaporation potentials are presented. A. H. CORNFIELD.

Determination of p_F curves [of soils] from texture using multiple regressions. G. Husz (*Z. PflErnähr. Bodenk.*, 1967, **116,** 115–125).—In comparable soils p_F curves may be determined from their clay and silt contents with sufficient accuracy for practical purposes. The relationship between clay content alone and vol.-% of water at different tensions is subject to large errors; no consistent relation is apparent between silt content alone and vol.-% water at different tensions. Regression equations determined from experimental data are not linear, but over the ranges examined can approximate to linearality when a clay and silt are used. A. G. POLLARD.

Transient state oxygen diffusion in soil. II. A case in which oxygen consumption varies with time. R. I. Papendick and J. R. Runkles (*Soil Sci.*, 1966, **102,** 223–230, cf. *ibid*, **100,** 251).—Apparatus is described by which the rate of O_2 consumption by a remoistened air-dry soil is shown to be max. at or very shortly after moistening; subsequently the rate gradually declines and approaches a constant value after about a week. A mathematical treatment of the data is presented. The period of storage of the air-dry soil influenced the rate of O_2 consumption after moistening. Measurements of O_2 concn. at different depths up to 135 cm in a silty clay loam one week after moistening showed a decreasing $[O_2]$ with depth, reaching a min. at each depth soon after wetting, followed by a slow increase with a considerable time lag at the lower depths. Consideration of $[O_2]$ at 15 cm depth in the soil indicates that low $[O_2]$ could occur temporarily in an otherwise well-aerated soil.
A. G. POLLARD.

Influence of moisture on erodibility of soil by wind. F. Bisal and J. Hsieh (*Soil Sci.*, 1966, **102,** 143–146).—A method is described for

determining the effects of soil moisture and texture on the initiation of movement of erodible particles of the soil by measured wind velocity. Differences in the % of soil moisture necessary to prevent movement are determined in a sandy loam (*a*), a loam (*b*) and a clay soil (*c*). The erosive fraction of a soil (particles < 0·84 mm) is separated by mechanical sieving of an air-dried sample. This is placed on trays and after addition of measured amounts of water the trays are placed in a wind-tunnel. Data for the wind velocity–soil moisture relationship are recorded for the three soils. With (*a*) and (*b*) at 3·5% and (*c*) at 11·0% moisture the wind velocity needed to initiate movement was similar. These values approximate to the moisture content of the soils in the air-dry condition. A. G. POLLARD.

Relations between signs of poor drainage, hydraulic conductivity and continuity of pores of loess-derived Gray-Brown podzolic soils in Niedersachsen. K. H. Hartge and F. Bailly (*Z. PflErnähr. Bodenk.*, 1967, **116**, 10–25).—Eleven profiles of para-brown earths and pseudo-gley soils formed on loess in Lower Saxony and showing evidence of poor drainage were examined. Hydraulic conductivity was measured and the continuity of pores was calculated; values were compared with morphological characteristics of the profiles. Hydraulic conductivity (*k*) values ranged from $>10^{-1}$ to $<10^{-6}$ cm. sec.$^{-1}$ Frequency of high conductivity values diminished with increasing depth of soil. Pores showing very great-to-medium continuity influenced conductivity to the greatest extent. Pores of small continuity occur less frequently and only in A_1 and B_1 horizons. In well drained soils pores of great conductivity occur largely in upper horizons. Pores of medium conductivity ($\sim 5 \times 10^{-5}$ cm. sec^{-1}) were found in deep horizons having morphological evidence of poor drainage. A decrease in hydraulic conductivity and pore continuity was always indicated by small black Mn stains and concretions. These afford the first signs of restricted drainage. A. G. POLLARD.

Formation of micro-aggregates from silt–clay mixtures. C. Ehrhardt (*Z. PflErnähr. Bodenk.*, 1967, **117**, 118–129).—The influence of hydrated Fe oxide and humic acid on the aggregation of various mixtures of silt and clay is examined by means of vertical and horizontal thin sections. With Fe oxides large spheroidal aggregates with loosely-packed components are formed. Harder aggregates are obtained with silt–Ca-clay mixtures then with silt–H-clay mixtures. Fe gels are unevenly flocculated on the mineral particles. Addition of humic acid (**I**) to the silt–clay mixtures yields laminated and encrusted aggregates in which the constituents are densely packed, **I** being homogenously flocculated on the clay. Mixtures of silt and H-clay are highly encrusted and silt–Ca-clay mixtures are greatly aggregated. When Fe oxide and **I** are deposited alternately they are mutually flocculated and encrusted. Fe gels play a part in the formation of spheroidal aggregates. **I** yields laminar structures with sedimentation layers forming the greater part of the aggregate. A. G. POLLARD.

Changes in exchangeable potassium on drying soils after treatment with organic compounds. II. Reversion. A. D. Scott and T. E. Bates (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 481–485).—A study was made of treatments for converting exchangeable K, which often increases when soils are air-dried, into non-exchangeable form so that the exchangeable K would be the same as in the original moist samples. Re-wetting the air-dried soil and maintaining it at room temp. or 90° reduced exchangeable K level, but the level attained was still twice as high as in the original moist sample. Re-wetting oven-dried soil with high-b.p. alcohols and water followed by oven-drying was more effective in reverting exchangeable K to non-exchangeable form. $C_8H_{17}OH$ was the most effective in this respect. A. H. CORNFIELD.

Negative adsorption by vermiculite: salt exclusion from interlayer volumes. S. El-Swaify, N. T. Coleman, G. Bredell, and M. Arca (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 464–466).—The negative sorption of Cl^- (the exclusion of Cl^- by water) by vermiculite was unaffected by salt concn. (0·001–1·0 N-NaCl or -$CaCl_2$) or particle size of vermiculite (0·2–74 μ). The amount of water that excluded Cl^- was 0·2 g per g of vermiculite. Implications of these findings are discussed. A. H. CORNFIELD.

Cation exchange capacity variations with pH in soil clays. J. M. de Villiers and M. L. Jackson (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 473–476).—The pH-dependent cation exchange capacity of soil clays was shown to result from the presence of pedogenically formed aluminous chlorite, a complex consisting of positively charged hydrous alumina attached to negatively charged silicate surfaces. Synthetic aluminous chlorite showed properties almost identical with that in soil clays. A. H. CORNFIELD.

Isotopic exchange of calcium-45 in calcium carbonate, phosphate and sulphate. M. A. Abdel Salam and R. H. Abu Zahra (*Soil Sci.*, 1966, **102**, 255–257).—The extent of the exchange of ^{45}Ca in the three Ca salts is examined. The salts were collected from natural deposits; the solid samples were washed, dried and powdered, the 60 μ fractions being used for the tests in which aq. $CaCl_2$ labelled with ^{45}Ca, was stirred with the various Ca salts at 25° for different periods. The % exchange σ_t was greater in the case of $CaSO_4.2H_2O$ than in those of the two other salts. The exchange is regarded as due to recrystallisation as well as to surface effects. Curves showing the values of σ_t as a function of time, are presented. In determinations of exchangeable Ca by the equilibration method, correction for this exchange should be considered. A. G. POLLARD.

Exchange reaction between potassium and sodium in Braňany bentonite. E. Podlešáková, J. Kremer and K. Bičovský (*Z. PflErnähr. Bodenk.*, 1967, **116**, 1–10).—Solutions of NaCl and KCl in various concn. in the range 0·01–0·1 N and with Na : K ratios from 1 : 7 to 7 : 1 were shaken with Na bentonite for 2 h and after 24 h the suspension was centrifuged. In the clear (equilibrium) solution (*ES*) Na and K were determined by flame photometry and Cl^- by titration. Throughout the whole range of concn. and Na/K ratios K showed an affinity for bentonite exceeding that of Na. This effect increased with diminution in salt concn. and with decrease in K/Na ratio. The relationship between equilibrium constant and composition of the *ES* is described by the equation: $K = 1{\cdot}19 + 0{\cdot}12\,S^{-1}.\left(\frac{0{\cdot}02}{N + 0{\cdot}02}\right)$, in which N is the total normality and S is the ratio K/Na in the solution. The ion balance of the bentonite can thus be expressed in terms of the composition of the *ES* as a numerical basis for the amelioration of sandy soils by treatment with bentonite. A. G. POLLARD.

Negative-charge properties of synthetic aluminium silicates. A. Mehlich (*Z. PflErnähr. Bodenk.*, 1967, **117**, 193–204).—Aluminium silicates prepared from $AlCl_3$, $Na_2Si_4O_9$ and NaOH under various conditions showed a negative permanent charge, CEC_p and a negative variable charge CEC_v. The total charge CEC_t increased with the rise in Si/Al ratio and with increasing pH. CEC_t was highest with Si/Al ratio 1·8 when the synthetic prep. were made at pH 8·3 and highest with Si/Al 3·5 at pH 5·0. CEC_p increased and CEC_v fell with increase in Si/Al ratio. After ageing at pH 2 for 10 days the main charge was CEC_p which was independent of the Si/Al ratio. Coarse ppt. failed to form when the Si/Al ratio exceeded 1·8 at pH 5, 7 or 8·3 unless alkaline earth salts (Ba, Ca, or Mg chlorides) were added. By acidifying the $Na_2Si_4O_9$ initially or in presence of $AlCl_3$ in excess, coarse ppt. were obtained in absence of alkaline earth salts. Al silicates made by use of Na_2SiO_3 showed mainly variable charges, and ageing before titration affected the ratio CEC_p/CEC_v very little. Acidification and ageing of $AlCl_3$ and $Na_2Si_4O_9$ before titration resulted mainly in production of CEC_p. For the use of Al silicates as 'cracking' catalysts, prep. having high proportions of CEC_p and therefore highly acidic properties, are preferred. The formation of natural clays in soils is discussed in relation to factors involving CEC_p and CEC_v. A. G. POLLARD.

Rubidium-86 as a tracer for exchangeable potassium in soils. J. Deist and O. Talibudeen (*Soil Sci.*, 1967, **104**, 119–122).—^{86}Rb-labelled RbCl was utilised to determine the amounts of Rb fixed and adsorbed on cation-exchange sites not accessible to K. Values thus obtained were closely related to the clay content of the soils. The Rb–K exchange corrected for the fixed Rb gave values very similar to the amounts of K^+ exchangeable to NaOAc. A. G. POLLARD.

Apparent irreversibility of ion exchange reactions in clay suspensions. R. van Bladel and H. Laudelout (*Soil Sci.*, 1967, **104**, 134–137).—A method for examining ion-exchange in clay suspensions is described. By determining exchange isotherms at not less than two normalities and extrapolating to zero ion strength, the selectivity coeff. observed at various surface compositions suppress the apparent irreversibility of the exchange reaction and the calculation of salt activities in mixed electrolyte solution is avoided. A. G. POLLARD.

Factors affecting the net absorption of exchangeable potassium by the Neubauer rye seedling method. II. Disproportionate influences of nitrogen and phosphate. J. T. Gillingham (*Soil Sci.*, 1966, **102**, 147–150; cf *Idem.*, *ibid.*, 1965, **100**, 384).—Soils were examined by the Neubauer method following the addition of (*a*) N as NH_4NO_3, (*b*) P as superphosphate and (*c*) N + P. Exchangeable K may be only partially released to the plants in the Neubauer test as a result

of over-riding deficiences of soil-N and, especially, -P. Preliminary addition of P was followed by the uptake of all the exchangeable and some of the non-exchangeable K present in the soil. Neubauer tests for exchangeable K therefore cannot be used for predicting the K requirement of the soil for cropping. High contents of free Fe oxides in soil fixed considerable amounts of P and the soils showed acute P deficiency. A. G. POLLARD.

Infra-red studies of hydroxy-aluminium material. R. A. Weissmiller, J. L. Ahlrichs, and J. L. White (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 459–463).—Interlayer hydroxy-aluminium in Al-montmorillonite had OH groups vibrating at two distinct frequencies, 3695 cm^{-1} and 3570 cm^{-1}. An interlayer structure is proposed based on the frequencies, intensities, and degree of pleochroism exhibited by the i.r. absorption bands.
A. H. CORNFIELD.

Analysis of geophysical data at three locations. E. B. Penrod and O. W. Stewart (*Soil Sci.*, 1967, **104**, 86–98).—Geophysical data and the physical properties of soils at three stations are presented. Equations are derived for calculating soil temp. at any depth and time, the amount of solar energy received on horizontal, vertical and inclined surfaces at any time and air temp. for any day or month in the year. A. G. POLLARD.

Relative contribution of organic and clay fractions to the cation-exchange capacity of sandy soils from several soil groups. T. L. Yuan, N. Gammon, jun. and R. G. Leighty (*Soil Sci.*, 1967, **104**, 123–128).—Data obtained from 83 virgin soils classified as entisols (*E*), inceptisols (*I*), mollisols (*M*) and spodosols (*S*), were utilised to ascertain the proportions of the total cation-exchange capacity (*CEC*) attributable to the clay and to the org. components. It is calculated that the *CEC's* associated with 1 g of org. matter from *I*, *S*, *E* and *M* were 1·38, 1·50, 1·87 and 1·97 mequiv. respectively; the corresponding values per 1 g of clay were 0·90, 0·21, 0·58 and 1·28 mequiv. respectively. The overall contribution of the org. matter to the *CEC* of surface horizons ranged from 66·4% in *M* to 96·5% in *S*. A. G. POLLARD.

Nitrogen availability in California soils in relation to precipitation and parent material. J. O. Klemmedson and H. Jenny (*Soil Sci.*, 1966, **102**, 215–222).—In soils derived from acid igneous (*A*) and basic igneous (*B*) parent material the availability was studied in relation to annual pptn. Pot culture methods with barley as test plant demonstrated a significant relationship between soil-N and mean annual rainfall for both groups of soils at the site of formation; with increase in pptn. *B* soils became relatively richer in N than did *A* soils, due to their higher clay content. Availability of N was related linearly to soil-N level and, where similar types of vegetation occur the relationship was similar for *A* and *B* soils.
A. G. POLLARD.

Retention of anhydrous ammonia by soil. III. Dispensing apparatus and resulting ammonia distribution. R. I. Papendick and J. F. Parr (*Soil Sci.*, 1966, **102**, 193–201).—Apparatus is described for dispensing high-pressure NH_3 solutions into laboratory or greenhouse systems. The patterns of distribution of anhydrous NH_3 in greenhouse pots of a moist or dry sandy loam 36 h after injection were correlated with the apparent solubilisation of the org. matter and the resulting pH contour lines. A large proportion of the NH_3 from an injection of 680 mg was retained within a radius of 5 cm of the injection point (cf. *Idem.*, *ibid.*, 1966, **101**, 109). A. G. POLLARD.

Utilisation of nitrate, urea and ammonium nitrogen by *Chenopodium album*. E. A. Kirkby (*Z. PflErnähr. Bodenk.*, 1967, **117**, 204–209).—Water-cultured plants of *C. album* were supplied with NO_3^-, urea or NH_4^+ as sole source of N, the pH of the nutrient solution being 5·5. Dry matter production with NO_3^--N was much greater than with NH_4^+-N; urea gave very poor yields. The total N per unit dry matter in the plants was in the order NH_4^+ > urea > NO_3^-. Within the plant tissues inorg. N (NO_3^- + NH_4^+) contents were small; that of NO_3^- in NO_3^--fed plants was particularly small. Of the total N in the NO_3^--fed plants 72–77% was present as protein, in the urea-fed plants 60–65%, and in the NH_4^+-fed plants 42–58%. The sol. org. N concn. in plants given NH_4^+- or urea-N were 2–4-fold and 2-fold, respectively, that of the NO_3^--fed plants. A. G. POLLARD.

Inorganic nitrogen transformations through the oxidation and reduction of sulphur compounds. T. T. Chao (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 485–490).—Changes in free energy are shown for 154 reactions between inorg. N and S compounds of various oxidation states. On the basis of these changes, N transformations through oxidation or reduction of sulphur compounds which may occur in acidic or basic media are presented. Experimental evidence showed that under certain conditions N transformations could be carried out by chemical reactions with S compounds.
A. H. CORNFIELD.

Comparison and evaluation of methods of determining fixed ammonium in soils. J. M. Bremner, D. W. Nelson and J. A. Silva (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 466–472).—Nine published methods for determining fixed NH_4^+ in soils gave widely different results. The main defects were (*a*) pretreatments to eliminate interference by org. N were inefficient or led to changes in fixed NH_4^+ and (*b*) many of the procedures were not quant. in determining fixed NH_4^+ or led to the formation of NH_4^+ from org. N. The best of the published methods was that of Silva and Bremner (*ibid.*, 1966, **30**, 587). A. H. CORNFIELD.

Breakdown of cyanamide in arable soils. D. Ernst (*Z. PflErnähr. Bodenk.*, 1967, **116**, 34–44).—Neutral solutions of ^{15}N-labelled $CaCN_2$ were added to soils sterilised by steam at 120°, by liquid ethylene oxide or by γ-radiation (1 or 5 Mrad dosage). No NH_3 or urea was found in these soils. In unsterilised soil and in that irradiated with 1 Mrad breakdown is 2–4 times faster than in the soil irradiated with 5 Mrad. The non-bacterial function in the breakdown is due to a thermo-labile agent, probably an enzyme. Inorg. catalysts play only a small part (<8%) in the breakdown process. Irradiation at 5 Mrad causes slight damage to the urease in soil; however the enzyme is still capable of converting urea into NH_3. Decomposition of soil org. matter diminishes in presence of $CaCN_2$. A. G. POLLARD.

Fractionation of soil phosphates and the isotopically exchangeable phosphate in the different phosphate fractions. P. K. Khanna and B. Ulrich (*Z. PflErnähr. Bodenk.*, 1967, **117**, 53–65).—The method of Chang and Jackson (*Soil Sci.*, 1957, **84**, 133) is modified to make possible the prediction of the isotopically exchangeable portion of the fractions and thus the surface area and degree of crystallisation of various forms of P. Results of P fractionation are thus shown to depend on the degree of dispersion of the soil but not on its water content. Because fixation or re-pptn. of P may occur during the extraction, changes between P fractions may take place and mask the re-exchange of ^{32}P. In acid soils, the H_2SO_4-sol. P cannot be regarded as Ca phosphates but includes actually acid-sol. portions of occluded phosphates. The coeff. of variation (*v*) among different methods of analysis are within the range 2·9–6·3% except that for org. P which is calculated as total P less inorg. P. Estimation of ^{31}P in the equilibrium solution after labelling with ^{32}P showed a *v* value ~30%. A. G. POLLARD.

Relationship between uptake of phosphorus by plants and the phosphorus potential and buffering capacity of the soil. An attempt to test Schofield's hypothesis. N. J. Barrow (*Soil Sci.*, 1967, **104**, 99–106).—Under glasshouse conditions soft brome grass was grown in 42 different soils with two levels of P applied to each soil as aq. NaH_2PO_4 and Na_2HPO_4. Five clippings of grass were taken over a period of 183 days. Data obtained accorded with the Schofield theory and demonstrated that the chemical potential of phosphate expressed as $-(\frac{1}{2}\, p\,Ca + p\,H_2PO_4)$ and a measure of the rate of change of that potential can account for the progressive intake of P. An equation is developed expressing the intake in terms of the buffer capacity and time (days) since sowing, viz., uptake = 0·0375 $(t - 21)$ $(1 - b)$ $(8·92 + 0·0043 +$ potential$)^2$ where $(1-b)$ is an index of buffer capacity, *t* is time, in days, since sowing and uptake is in mg. (24 references.) A. G. POLLARD.

Enhanced solubility of dicalcium phosphate in presence of magnesium and sulphate ions and its edaphic significance in calcareous soils. S. C. Srivastava and M. P. Agrawal (*Soil Sci.*, 1967, **104**, 77–80).—The $[PO_4^{3-}]$ in suspensions of $CaHPO_4$ (**I**) was increased by addition of $MgSO_4$ to extents related to the diminution in the ionic ratio Ca^{2+}/Mg^{2+}. In presence of excess $CaCO_3$, $MgSO_4$ increased the stability of the system, making possible the formation of more Ca phosphate and increasing the $[PO_4^{3-}]$ of the system. The action of $MgSO_4$ appeared to be specific and could not be explained by a 'salt effect' alone. In pot cultures of sugar-cane the incorporation of $MgSO_4$ narrowed the Ca/Mg ratio in the exchange complex, increased the solubility of P and also the uptake of P by the plants. A. G. POLLARD.

Potash exchange in soils. II. Potash selectivity and fractionation of exchangeable potash. W. Ehlers, B. Meyer and F. Scheffer. **III. Potash exchange and crystallographic behaviour of three-layer clay minerals.** W. Ehlers, H. Gebhardt and B. Meyer (*Z. Pfl-Ernähr. Bodenk.*, 1967, **117**, 1–29, 29–53).—II. The technique of Schouwenburg and Schuffelen (*Neth. J. agric. Sci.*, 1963, **11**, 13) is

applied to differentiation of the K sorption sites in an illite, according to the positions planar (*p*), edge (*e*), interlayer (*i*) on the basis of the Gapon coeff. The method of Matthews and Beckett (*J. agric. Sci.*, 1962, **58,** 59) which avoids extraction of K by neutral salts was used for differentiation of the K fractions. In several Central European soils examined the portion of the total exchange capacity (*EC*) of the clay attributable to the *e*- and *i*-positions was related to the expandibility of the three-layer clay minerals present.

III. The clay fraction (< 6 μ) of an unweathered loess was used to examine the relationship between the K·Ca charge and the state of expansion of the minerals. Intermediate stages between max. expansion and max. contraction were distinguished by *X*-ray observation. Comparison is made of the data with that obtained by graphic methods with reference to the K-exchange positions. The minerals expand or contract when the *p*- and *e*-positions, and to a smaller extent the *i*-position are filled with or exhausted of K. Ease of expansion or contraction is largely influenced by e.g., Ca concn., physical strain. Tests under conditions of increased tendency towards contraction (lower [Ca]) show blockage of the *e*-position when taking up K by a diminution in the *EC* of the *e*-position. The partial alternate conversion of exchange positions in three-layer clay minerals is possible when the state of absorption of K is altered. K fixation does not involve alteration of lattice spacings but may depend on processes of diffusion within the interlayer spaces. A. G. POLLARD.

Ionic equilibria in a ferrallitic clay: specific adsorption sites for potassium. M. E. Sumner and J. M. Marques (*Soil Sci.*, 1966, **102,** 187–192).—Differences in the ability of different crops to take up K from this soil are examined. The exchangeable K in a K-Ca ferrallitic (ultisol) clay is divided on the basis of a Gapon equation. The specific adsorption of K is assumed due to the presence of a small amount of montmorillonite (**I**). Specific sites probably occur in the hexagonal holes of the tetrahedral layer of **I**; this provides an explanation of K deficiency in these soils. A. G. POLLARD.

Submergence and liming effects on soil. I. Changes in pH, E_h and manganese uptake by rice plants. A. Mukhopadhyay, T. R. Fisher and G. E. Smith (*Soil Sci.*, 1967, **104,** 107–112).—Air-dried soil of low available P content was placed in large, glazed earthenware pots having no drainage. $CaCO_3$ was added at rates of 0, 5200 or 7625 ppm, together with an NPK fertiliser and ^{32}P-labelled Na phosphates. Rice seedlings were planted and the soils were submerged. Samples of soil and plants were examined periodically. After 62 days submergence the pH of the limed soils was stabilised at pH ~7·0. E_h values fell rapidly in the first 2–3 weeks and then remained relatively stable. The Mn content of the plants after 26 and 51 days and of the harvested grain and straw was much higher in plants from the unlimed soil. Changes of E_h and pH within the acid soil system were possibly related to Fe transformations. Differences in uptake of Mn by rice on limed and unlimed soils were related to the theoretical stabilities of several Mn minerals. A. G. POLLARD.

Mechanisms for the movement of manganese from the soil to the plant root. E. H. Halstead (*Diss. Abstr.* B., 1967, **27,** 2225).—Growth experiments with five crops in nine soils with two rates of transpiration, are described. With high transpiration rates the uptake of Mn was largely by a mass-flow mechanism in five of the soils, while in the remaining four soils root interception and/or diffusion were mainly concerned. The apparent self-diffusion coeff. D*a* of ^{54}Mn for six soils ranged from $0{\cdot}36 \times 10^{-7}$ cm^2.sec.$^{-1}$ to $2{\cdot}20 \times 10^{-7}$ cm^2.sec^{-1}. The D*a* increased with increase in exchangeable Mn in the soil and usually decreased as *CEC* increased. Plant uptake of Mn was highly correlated with D*a*, exchangeable Mn and with saturation extract-Mn. Experimental assessments of the Mn supplied to maize roots by diffusion indicate that the level of Mn at the soil–root interface was reduced by only a small amount. Mn uptake due to root interception and/or diffusion showed a straight line relation to root area. A. G. POLLARD.

Fractionation of copper from soils. H. Grimme (*Z. PflErnähr. Bodenk.*, 1967, **116,** 207–222).—A modified system for the fractionation of soil-Cu is described. Fraction 1 is removed by 0·5 N-NaOH–0·01 M-EDTA and classed as organically bound Cu (Cu_{org}). Fraction 2 is extracted with 0·2 M-NH_4 oxalate at pH 3·0. It is incompletely defined but contains mostly org. Cu, and is designated Cu_{ox}. Fraction 3, represents Cu occluded in Fe oxide (Cu_{okk}) and is obtained by extraction of the Fe oxide with 0·4 M-NH_4 oxalate in presence of Zn. The amount of Cu extracted is proportional to that of Fe, increasing with the quantity of Zn used in the range 20 mg for the A-horizon to 40 mg in the B-horizon. Addition of larger amounts of Zn causes deposition of Cu (metal) in the Zn. The Fe and Cu concn. in the extracts are closely related. Fraction 4 (Cu_{si}) is obtained from the residue of soil after removal of Cu_{okk} by dissolution in conc. $HClO_4$. Satisfactory reproducibility of results is obtained by this means, the sum of the four Cu fractions representing 94–114% of the total Cu determined. A. G. POLLARD.

Distribution of copper in Gray-Brown podsolic soils developed from loess. H. Grimme (*Z. PflErnähr. Bodenk.*, 1967, **116,** 125–136).—The soils are examined by the method described in the previous abstract. Data are recorded for a series of para-brown earths. Cu_{org} and Cu_{ox} decreased and Cu_{okk} increased with depth below the soil surface through the A- and B-horizons. The Cu_{si} values were consistently highest in the B_f horizons. Of the total Cu present, 55–90% was in the Cu_{org} and Cu_{ox} fractions. Only on the A_h horizons of forest soils did Cu_{org} and Cu_{ox} reach as high as 10–50%; these two values are somewhat less than in arable soils. The forest soils also differ from arable soils in that the Cu_{org} and Cu_{ox} values diminish from the A_h to the A_l horizons and increase again towards the B_f horizon. The Cu_{okk} and Cu_{si} values were similar in forested and in arable soils. A. G. POLLARD.

Residual effect of a single borate application on Western Washington soils. A. S. Baker and W. P. Martensen (*Soil Sci.*, 1966, **102,** 173–179).—The residual effects are examined with regard to rate and timing of the application and to liming. Lucerne was sown in the year following soil treatments. Colwell-type tests (*ibid.*, 1943, **56,** 71) were used to assay available B in the soil. Values thus obtained correlated well with the level of sol. B; these levels showed changes over the whole 5 years of the experiment. These changes were directly related to the original sol. B levels in the soil. Retention of the applied B was greater on limed than on unlimed soils. The movement of B from the surface (0–6 in.) soil to the subsoil (10–16 in.) was depressed by lime. B-deficiency symptoms in lucerne occurred only during prolonged periods of low rainfall and when the sol. B level in the subsoil was < 0·05 ppm. Samples of the apical parts of lucerne grown in B-deficient soils contained < 20 ppm of B. A. G. POLLARD.

Fluorimetric determination of magnesium in soil extracts. R. A. Swanson, D. Hovland and L. O. Fine (*Soil Sci.*, 1966, **102,** 244–247).—The method is based on the fluorescence produced by the interaction of Mg salts with *o,o'*-dihydroxyazobenzene. Details are given of the prep. and use of reagents. Interference due to Al^{3+}, PO_4^{3-}, Mn^{2+}, Na^+, Cu^{2+} and SO_4^{2-} was insignificant. Ca^{2+} caused slightly high values, but if fairly high concn. of Ca are added to the standards and to the test solutions the discrepancy is very small. The method is suitable for determining Mg in N-NH_4 acetate extracts of soil. Results obtained have a level of accuracy similar to that of atomic absorption methods and superior to those by the thiazol yellow method. A. G. POLLARD.

Fractionation of humic substances of a podsol by dialysis. F. Martin-Martinez (*Z. PflErnähr. Bodenk.*, 1967, **116,** 89–96).—Five fractions from the fulvic and humic acids of a podsol were separated by dialysis and the process of humification was examined by i.r. spectra and neutralisation curves. In the fulvic fractions the acidity is attributed to ·CO_2H which diminish in number as humification proceeds. In the humic acid fractions there was also a decrease in ·CO_2H but the capacity for neutralisation increased as the process continued. The acidity of humic acid probably results from phenolic-OH groups. A. G. POLLARD.

Humic substances as radicals. F. Scheffer and W. Ziechmann (*Z. PflErnähr. Bodenk.*, 1967, **116,** 106–115).—A review (cf. Scheffer *et al. ibid.*, 1958, **80,** 126; 1959, **85,** 50) tracing the development of the concept of the structure of humic acid in terms of radical groupings. Further, it is considered that the reactive behaviour of the constituent radicals leads to a state of stable equilibrium such that humic material can be regarded as a system capable of experimental characterisation. At an early stage in the process of humification aromatic substances of reasonable stability are necessary. The stability of radicals increases as the rate of humification diminishes. A. G. POLLARD.

Factors affecting the accuracy of the carbon-dating method in soil humus studies. C. A. Campbell, E. A. Paul, D. A. Rennie and K. J. McCallum (*Soil Sci.*, 1967, **104,** 81–85).—Data presented show the accuracy of the carbon-dating method with particular reference to the possible effects of isotopic fractionation, the production of ^{14}C from nuclear bombs and the accuracy of analytical methods used in the study of humus. ^{14}C fractionation was constant in several soils and humus fractions and necessitated the subtraction of ~115 years from the mean residential time. In-

corporation of ^{14}C from nuclear bombs produced no apparent error. Possible errors in the analytical method used in C-dating studies of soil humus were small. Micro-organisms did not differentiate between C-isotopes during the decomposition of org. matter. A. G. POLLARD.

Nature of the amino-acid compounds of soil. I. Isolation and fractionation. F. J. Sowden (*Soil Sci.*, 1966, **102**, 202–207).—Partial hydrolysis by a conc. HCl–acetic acid mixture, of a Brown Forest soil removed approx. half of the total N. Various methods of separation of the amino-acids are examined. Details of the NH_2-acids in the original soil and in the partially hydrolysed product are recorded. A. G. POLLARD.

Nature of the amino-acid compounds of soil. II. Amino-acids and peptides produced by partial hydrolysis. F. J. Sowden (*Soil Sci.*, 1966, **102**, 264–271; cf. p. 180).—Among products of partial hydrolysis of a Brown Forest soil (*Idem., Can. J. Soil. Sci*, 1958, **38**, 147) NH_2-acids and peptides are subjected to further examination. The NH_2-acids were fractionated first on a cation- and then on an anion-resin. Elution of the latter yielded most of the free NH_2-acids (except aspartic and glutamic acids) and some small peptides similar to those obtained from proteinaceous matter in soil. The results do not accord with the view that most of the N-containing materials in soil are products of reactions between NH_2-acids and polyphenols or sugars, but lend support to the hypothesis that peptides, polypeptides and, possibly, proteins and NH_2-acids are stabilised by reactions of this type. A. G. POLLARD.

Complexes of montmorillonite with primary, secondary and tertiary amides. I. Protonation of amides on the surface of montmorillonite. S. A. Tahoun and M. M. Mortland (*Soil Sci*, 1966, **102**, 248–254).—Interactions between highly purified montmorillonite (**I**), and acetamide (**II**), *N*-ethylacetamide (**III**), or *N*,*N*-diethylacetamide are examined. Data obtained indicate that **II** is protonated on the surface of H-**I** or Al-**I**. The pH of the surface of either form of **I** is $<0{\cdot}8$. Dehydration of the **I**–amide complex caused the amide to revert to the mol. form. On rehydration the cation form was again produced. An appropriate mechanism for these effects is indicated. It is also probable that proteins and peptides may protonate through the CO-group as well as through free amino-groups in acid clay systems. A. G. POLLARD.

Dynamics of soil organic acids. T. S. C. Wang, San-Yao Cheng and H. Tung (*Soil Sci.*, 1967, **104**, 138–144).—Samples (100 g) of soil were mixed with 5 g of chopped fresh leaves of *Crotalaria juncea*. One sample was kept under aerobic conditions and a second was waterlogged. In a similar trial powdered sugar-cane leaf was used. After incubation of the two samples at 30° for various periods the volatile, aliphatic acids (*VAA*) formed were extracted and determined by gas chromatography, the non-volatile aliphatic acids (*NAA*) were separated by high-voltage electrophoresis and the phenolic acids (*PA*) by paper chromatography. Waterlogging and addition of *C. juncea* resulted in the production of a larger no. of *VAA* and in greater variety than when sugar-cane leaf was used; this activity gradually diminished with passage of time. Production of *NAA* and of *PA* varied with different soils, with the nature of the added org. matter and with conditions of aeration. Production of these acids also diminished gradually, though more slowly than in the case of *VAA*. Growth of young sugar-cane was suppressed by monocarboxylic acids but accelerated by hydroxy- and dicarboxylic acids in small concn. The possible influence of the production of such acids on the conditions of the rhizosphere are considered. (24 references.) A. G. POLLARD.

Movement of phytic acid in soil cores. B. T. Bowman, R. L. Thomas and D. E. Elrick (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 477–481).—Phytic acid (**I**) applied to the top of a core of sandy loam (pH 7·35) and subjected to leaching by water was bound by the soil and moved downward in only limited amounts. **I** displaced PO_4^{3-} from the soil. The movement of **I** was greater and its rate of mineralisation less at 5° than at 20°. Sterilisation of the soil by irradiation (3 Mrad) reduced, but did not eliminate, mineralisation of **I**. A. H. CORNFIELD.

Retention of carbon dioxide in the glasshouse atmosphere. M. Sturm (*Z. PflErnähr. Bodenk.*, 1967, **116**, 177–190).—The release of CO_2 from cylinders in a glasshouse produces a uniform distribution of gas and precautions necessary to maintain this are discussed. Application of farmyard manure before planting tomatoes caused a considerable increase in $[CO_2]$ during the night. This could be maintained readily in rainy weather until ventilation was necessary. With light intensity of 2500 lux the $[CO_2]$ in a tomato house diminished slowly. At 1200 lux the concn. rose and at 2000 lux it remained unchanged; at 10,000 lux the gas concn. fell to a very low level and stomata remained closed. On sunny days ventilation is necessary to counteract the rapid rise in air-temp., but gassing may be possible when light intensity falls below 10,000 lux. A. G. POLLARD.

Course of reduction and elimination of reduction phenomena in artificially compressed soils. U. Hochmuth (*Z. PflErnähr. Bodenk.*, 1967, **117**, 130–139).—Artificially compacted soil from the brown coal area of the Lower Rhine, used in *in vitro* tests, showed a faster and more extensive reduction of Fe compounds than did soils of lower humus content. Subsequent re-oxidation occurs initially at a faster rate than did the original reduction process. Large proportions of humus in the experimental soil are associated with higher rates of reduction of pH. After removal of the reducing conditions, $Ca(NO_3)_2$, in contrast with $(NH_4)_2\,SO_4$, causes a new increase in pH. A. G. POLLARD.

[A] Action of rhizosphere products of white mustard on ferrous phosphate (vivianite). [B] Complex chemical action of organic compounds on ferrous phosphate (vivianite) in model experiments with hydroxy-acids. F. Scheffer, R. Kickuth and E. Schlimme (*Z. PflErnähr. Bodenk.*, 1967, **116**, 53–62, 62–70).—[A] Org. compounds from sterile and infected rhizospheres of *Sinapis alba* were fractionated into amino-acids, org. acids and neutral components. The mobilisation of vivianite by these substances was tested at pH 5·8. Chemical interaction between substances in the rhizosphere and sparingly sol. phosphates may explain the known effect of mustard on soil phosphates.

[B] Hydroxy-acids found in the rhizosphere of mustard plants begin to mobilise Fe^{2+} phosphate in concn. $5 \times 10^{-5{\cdot}5}$ mol/l at pH 5·8 and 22°. Chelating agents e.g. citric and isocitric acids may also occur in the rhizosphere in similar concn. A. G. POLLARD.

Influence of non-protein amino-acids on dry matter formation and salt uptake by *Helianthus annus* in sterile culture. F. Scheffer, R. Kickuth and R. Aldag (*Z. PflErnähr. Bodenk.*, 1967, **116**, 25–33).—The altered morphology of shoots and roots of *H. annus* in sterile culture caused by 'foreign' NH_2-acids (those not forming part of the natural protein of the plant) is described. 'Foreign' NH_2-acids exerting notable effects on the morphology of the plants include *d*-serine and *d*-leucine causing symptoms resembling chlorosis and *d*-glutamic, *d*-aspartic acids and also *d*-serine which inhibit root growth. Salt uptake and dry matter production are strongly inhibited by these substances. A. G. POLLARD.

Acidity-dependent reactions between organic acids and amines and montmorillonitic clay surfaces. R. D. Harter (*Diss. Abstr.* B, 1967, **27**, 2226).—The adsorption of org. pesticides by soil colloids is examined in relation to pH. Mixtures of aq. Na benzoate and benzoic acid of varying pH were dried to a film and the ratio between absorption of the carboxylate and carbonyl stretch modes in the i.r. spectral region could be related to the pH of the system. Introduction of montmorillonite into the system lowered the absorption ratio considerably. The $[H^+]$ near the colloidal surface was estimated at approx. 10 times that measured electrometrically at pH ~3 and about 100 times the measured value at pH 7. By similar means the effect of pH on the adsorption of 2,4-D, amiben, aniline, urea and the hydrochlorides of the two last-named was characterised. A theoretical basis for the experimental data is suggested. A. G. POLLARD.

Enzyme activity and water content of some Pannonic alluvial gravel soils. G. Malicky-Schlatte and H. Malicky (*Z. PflErnähr. Bodenk.*, 1967, **116**, 190–199).—The soils occurred on an alluvial gravel near Wiener Neustadt; details of enzyme activity, pH, water (%), org. matter and $CaCO_3$ contents are recorded. On pure carbonate-gravel the transition from garden or field to older fallow land and to undisturbed or regenerated grassland is associated with increase in all values studied except pH and $CaCO_3$ content. Among artificial forest-like vegetation, soils carrying *Syringa vulgaris* have higher values than that under *Pinus nigra* or *P. sylvestris*. Grassland soils are superior in general quality to those under *Pinus*. Enzyme activity increases in *Pinus* soils and in deforested soils which have carried older trees. With increase in silicate content of the gravel, its water supply increases but the pH, enzyme activity and $CaCO_3$ content diminish. On carbonate-gravel soils there are no natural stands of trees. A. G. POLLARD.

Responses of soil microflora to volatile components in plant residues. J. D. Menzies and R. G. Gilbert (*Proc. Soil Sci. Soc. Am.*, 1967, **31**, 495–496).—Exposure of soil to volatile substances from a number of plant species rapidly increased the respiration rate of the soil microflora. This was followed by an increase in

bacterial numbers and by vegetative growth of fungal mycelia over the soil surface. The volatiles from lucerne leaves were the most effective in this respect, but volatiles from maize, bluegrass, and tea leaves and barley straw were also active. A. H. CORNFIELD.

Changes occurring in soil samples during air-dry storage. Z. Nevo and J. Hagin (*Soil Sci.*, 1966, **102,** 157–160).—Microbial and physico-chemical changes occurring in stored air-dry soils are examined. During 3 months' storage the major change is in the physical condition of the org. matter fractions; this takes place independently of the micro-organisms present. The nitrification rate in the remoistened soils is correlated with the surface area (*A*) of the particles in an org. soil. By increasing the *A* of the soil an estimate of the available N in a fresh soil sample may be made. A. G. POLLARD.

Effect of γ-irradiation on soil microbial population in relation to soil moisture content. N. E. Jackson, J. C. Corey, L. R. Frederick and J. C. Picken, jun. (*Proc. Soil Sci. Soc. Am.*, 1967, **31,** 491–494).—In general microbial counts of soils containing 30% water were reduced to zero by lower γ-irradiation doses than were those in air-dried soil. One Mrad was sufficient to kill all fungi, but 2–3 Mrad were required to kill all bacteria. At a given water content, samples with the higher initial microbial population required greater radiation doses for sterilisation than did samples with lower initial populations. A. H. CORNFIELD.

Distribution of nitrogen-fixing micro-organisms in paddy soils of south-east Asia. Michiharu Kobayashi, Eiichi Takahashi and Keizaburo Kawaguchi (*Soil. Sci.*, 1967, **104,** 113–118).—Soils of Thailand, Malaya, Philippines and Taiwan are examined and counts of N-fixing organisms are recorded. Photosynthetic bacteria are widely distributed. *Athisrhodaceae* can co-exist with *Azotobacter*, there being a symbiotic relation between them. A. G. POLLARD.

Nitrate reduction by bacteria isolated from waterlogged Crowley soil. J. H. Jordan, jun., W. H. Patrick, jun. and W. H. Willis (*Soil Sci.*, 1967, **104,** 129–133).—Organisms which reduce NO_3^- to NO_2^- in waterlogged soil are examined. Of 59 organisms isolated under anaerobic conditions, 22 reduced NO_3^-; some were unable to reduce NO_2^-. Accumulations of NO_2^- amounting to 100–200 ppm temporarily, prevented NO_3^- reduction by cultures of *Bacillus polymyxa* and permanently inhibited cultures of *B. megatherium*. *Arthrobacter simplex* was not affected by NO_2^--N at 300 ppm. Several strains of *B. polymyxa* showed similar rates of NO_3^- and of NO_2^- accumulation. A. G. POLLARD.

Quantitative relations between some species of *Fusarium* and *Trichoderma* in a citrus grove in Israel. A. Z. Joffe (*Soil Sci.*, 1966, **102,** 240–243).—The occurrence of three species each of *Fusarium* and *Trichoderma* in a citrus soil indicated a specific negative correlation between *T. lignorum* and *F. solani*. No such relationship was apparent between *T. lignorum* and *F. oxysporum* or *F. equiseti* or between *T. glaucum* or *T. koningi* and either of the *Fusarium* species. A. G. POLLARD.

Plant Physiology, Nutrition and Biochemistry

New gravimetric method for estimating root surface area. H. E. Carley and R. D. Watson (*Soil Sci.*, 1966, **102,** 289–291).—A modification of the method of Wilde and Voigt (*J. For.*, 1949, **47,** 643) is described. Air-dry roots are dipped for 10 sec. in a beaker containing a conc. solution of $Ca(NO_3)_2$ (6 g in 1 ml of water with warming) and then allowed to drain back into the beaker for 30 sec. The beaker and solution are weighed before dipping and after draining. The difference is the wt. of solution adhering to the roots plus a small amount absorbed. Values are standardised against carefully measured roots. A. G. POLLARD.

Effect of external salt concentration on water relations in plants. II. Effect of osmotic differential between external medium and xylem on water relations in the whole plant. J. J. Oertli (*Soil Sci.*, 1966, **102,** 258–263; cf. *ibid.*, 1966, **102,** 186).—The passage of water through plants is discussed as one phase of a system, soil or solution → plant → free atm. Consideration of the effects of changes of water potential in the rooting medium e.g. of soil salinity, of water potential within the plant tissues, of transpiration rates, of turgidity within plant tissue, are examined and a new model of the plant–water system is suggested. A. G. POLLARD.

Ion exchange resin media: micronutrient levels and the response of tomatoes. E. O. Skogley (*Soil Sci.*, 1966, **102,** 167–172).—Use of the exchange resin previously described (Skogley and Dawson: *Nature*, 1963, **198,** 1328) to examine intake of Fe, Mn, Mo and B by tomato plants is reported. The micronutrients were supplied in the resin. When the P supply was insufficient, yields of plant tops increased at first and then decreased as the supply of Zn and Fe fell below the calculated optimum. Changes in Fe and Zn supply influenced plant growth more than tissue concn. Plant growth and rates of nutrient availability were closely related. Leaves of the plants had the highest Fe contents when the Fe supply was adequate but as the Fe supply was lowered, petioles showed the highest Fe content. With all levels of Zn supply leaves had the highest concn. of the element, stems showed intermediate levels and were the most sensitive organs to changes in Zn supply. High-level Zn supply was needed to ensure max. absorption of Fe. B was also required in the nutrient, but Mo in the seed was an adequate source of supply. A. G. POLLARD.

Effects of external salt concentrations on water relations in plants. I. Absence of osmotic adjustment in the root xylem. J. J. Oertli (*Soil Sci.*, 1966, **102,** 180–186).—Plant injury due to highly saline conditions in soils is discussed, distinction being drawn between (*a*) the 'osmotic effect', the effect of external water potential whereby availability of water to the plant is depressed, and (*b*) specific effects of salts causing injury after being taken up by the plant. A new model of the passage of water and salts into and through the plant system, is proposed. This involves a barrier in roots, mass flow in the root xylem and another barrier around leaf vacuoles, the barriers being crossed by water and by solutes at different rates. It follows that osmotic adjustment is impossible under saline conditions and when transpiration rates are high. With low external salt concn. and low transpiration rates the same kinetics predict the development of root pressure and its dependence on concn. A. G. POLLARD.

Enzymic catalysis and the action of mineral matter. E. Latzko (*Z. PflErnähr. Bodenk.*, 1967, **117,** 179–193).—A review of mineral–enzyme systems, mainly in plants and micro-organisms. A. G. POLLARD.

Influence of chlorocholinechloride (CCC) on some characteristics of the water dynamics of winter wheat. M. Zemánek (*Z. PflErnähr. Bodenk.*, 1967, **117,** 210–223).—Spraying winter wheat plants with CCC during the heading stage, increased the lightly-held water in the leaves and also the transpiration rate. The water saturation-deficit showed no important differences. Segments of sprayed leaves, after severe wilting (provided that this did not reach the critical value of ~60%), took up more water than did control unsprayed leaves. A. G. POLLARD.

Diurnal variations in transpiration and root metabolism in maize. R. C. Long (*Diss. Abstr.* B., 1967, **27,** 2256).—Possible relationships between transpiration and root respiration, both of which exhibit diurnal variations, are examined. It was suspected that the basis of both functions lies in nucleic acid metabolism. Malic dehydrogenase concn. is shown not to vary diurnally. The capacity to induce nitrate reductase in leaf tissue was greater at night than by day. Absorption of ^{32}P and its incorporation into various pools occurs mainly during the day. P-pool levels e.g. lipid, inorg., acid-sol. org. and protein-nucleic acid, and their turnover rates (except that of the inorg. P pool), do not vary diurnally. A. G. POLLARD.

Trace-element contents of plant ribonucleic acid. H. E. Haeder (*Z. PflErnähr. Bodenk.*, 1967, **116,** 223–231).—Possible methods for determining trace elements in ribonucleic acids (**I**) extracted from plants are examined using spinach having adequate or deficient supplies of Cu. Methods for extracting **I** proved unreliable. Other tests with yeast-**I** in aq. solutions of $MgSO_4$ or precipitated in aq. quaternary NH_4 compound solution confirm that metal content of **I** varies with solvent used; thus determination of trace elements in **I** seems inevitably inaccurate. A. G. POLLARD.

Influence of temperature on yield and lipid synthesis in oat grain. H. Beringer (*Z. PflErnähr. Bodenk.*, 1967, **116,** 45–53).—Two varieties of oats were grown in pots and at the flowering stage growth was continued with day temp. of either 12° or 30° until maturity. Others were grown at 12° or 30° day temp. for 23 days after flowering when the two temp. were reversed until maturity. Other environmental factors were kept constant, viz., light intensity 20,000 lux, photoperiod 16 h, night temp. 12°. Day temp. of 12° up to the 23rd day after flowering increased grain yields by 30–40% for both varieties as compared with the 30° treatment. At 12° the lipid contents of the grain were higher than after growth at 30°. 75–80% of the total quantity of lipid produced was synthesised after the 23 days following flowering. In plants grown at 12° for the first 23 days after flowering the mature grain showed a ratio of unsaturated : saturated fatty acids ~5·5 whereas the ratio was

~4·1 in grain from plants grown at 30° for the same period. In the latter plants the relative amount of linoleic acid, 23 days after flowering, exceeded that of oleic acid but when grown at 12° the reverse was the case. Regardless of the growth temp. more oleic than linoleic acid was present in the grain at maturity.
A. G. POLLARD.

Uptake of brucine from nutrient solutions by plant roots. R. Dörr (*Z. PflErnähr. Bodenk.*, 1967, **116**, 96–105).—The uptake of org. substances by roots of intact wheat seedlings in water-culture is examined, using brucine (**I**) as test substance. Max. uptake of **I** was 6·5% of the total present or ~0·2% of the total dry matter of the plant. Approx. 24% of the **I** used disappeared in the course of the experiment, probably by microbial decomposition.
A. G. POLLARD.

Efficient and inefficient use of iron by two soya-bean genotypes and their isoclines. J. C. Brown, C. R. Weber and B. E. Caldwell (*Agron. J.*, 1967, **59**, 459–462).—An isocline of a soya-bean genotype which was efficient in absorbing Fe from soils of high pH was somewhat less efficient than the original genotype. This was related to a lesser ability of the isocline to reduce Fe^{3+} to Fe^{2+} at the root. An inefficient genotype and its isocline both showed equally low ability to absorb Fe. The stem exudates of the efficient Fe absorbers were higher in citrate than were the exudates of the poor absorbers.
A. H. CORNFIELD.

Determination of organic nitrogen in plant material. H. K. Quashu (*Agron. J.*, 1967, **59**, 486–487).—The digestion of plant material with conc. H_2SO_4 in sealed tubes at 470° gave higher values for org. N in plant material than did two standard Kjeldhal digestion procedures. The presence of NO_3^- did not interfere with the determination of org. N in the sealed tube method, but gave high results for org. N in the Kjeldhal methods.
A. H. CORNFIELD.

Crops and Cropping

Relative salt tolerance of rice during germination and early seedling development. G. A. Pearson, A. D. Ayers and D. L. Eberhard (*Soil Sci.*, 1966, **102**, 151–156).—Different varieties of rice seeds were germinated in sand saturated with a dil. Hoagland solution to which NaCl and $CaCl_2$ were added to produce an appropriate electrical conductivity. Germination occurred in solutions of conductivity 40 mmho/cm. A 50% reduction in germination was associated with conductivities in the range 21·2–30·5 mmho/cm in the 14 varieties tested. Rice seedlings showed much higher sensitivity to salinity a week after germination, e.g., a 75% reduction of growth occurred at conductivity 5·1 mmho/cm.
A. G. POLLARD.

Selection of index leaf for studying the critical concentration of nitrogen in rice plants. E. F. Wallihan and J. C. Moomaw (*Agron. J.*, 1967, **59**, 473–474).—The total N content in the last four leaves on rice culms of three varieties growing in paddy was determined during the period from formation of flower primordia until after flower emergence. The next-to-last-leaf (second leaf below the panicle) sampled at the time of flower emergence was the most suitable for showing differences in N supply. A. H. CORNFIELD.

Efficiency of utilisation of soil- and foliar-applied nitrogen and phosphorus as revealed by tuber production and nutrient uptake of potatoes. S. K. Mukherjee, Rahat De and P. N. Saxena (*Soil Sci.*, 1966, **102**, 278–283).—On a sandy loam (pH 7·5; available N and P, 120 and 26 lb/acre, respectively), potato plants received three different levels each of N and P fertilisers, (*a*) applied to the soil, (*b*) half the fertiliser applied to soil at planting, and half as foliar spray or (*c*) one quarter of fertiliser to soil and a second quarter as foliar spray. Treatment (*b*) produced an 18% greater yield than did (*a*). Treatment (*a*) and (*c*) gave similar yields. At the lower levels of fertiliser application, the efficiencies of utilisation (in terms of yield) of foliar applications of N and P were 1·99 and 1·54% respectively of that with (*a*). With increase in level of application, the efficiency of foliar applications diminished. Foliar treatments increased the total nutrient uptake of the plants. With treatment (*b*) the N and P contents/plant were 29 and 37% higher than when the same gross amount of fertiliser was given as (*a*).
A. G. POLLARD.

Influence of limestone and nitrogen on Coastal Bermuda-grass yields and soil pH. W. E. Adams, R. W. Pearson, W. A. Jackson and R. A. McCreery (*Agron. J.*, 1967, **59**, 450–453).—Without application of $CaCO_3$ forage yields declined over 3 years where 448 kg or more of N were added annually. Where 896 or 1793 kg N was applied chlorosis was severe and stands were reduced to 10–25% by the second or third year. Where $CaCO_3$ (7846 or 40,349 kg per ha) was applied in the first year forage yields increased with rate of application of N (224–1793 kg per ha annually) in each of the 3 years, with little difference between years. Bermuda-grass roots penetrated to 120 cm in soil having pH of 4·0–4·5.
A. H. CORNFIELD.

Effect of cutting frequency and root segregation on the yield from perennial ryegrass–white clover associations. B. F. Bland (*J. agric. Sci., Camb.*, 1967, **69**, 391–397).—The average yearly output in the years 1963 to 1965 was 4000, 7000 and 6000 lb/acre and the N harvested 132,184 and 179 lb/acre respectively. The mean annual dry matter yields for the two, four and six defoliations were 5300, 6100 and 6000 lb/acre and corresponding N yields 112, 166 and 217 lb/acre. Both segregation of the roots of the species and increase in the defoliation frequency were responsible for the higher contributions from the clover component. In the spring of the third year the underground transference of 30–31 lb of N/acre from clover to ryegrass could be demonstrated.
M. LONG.

Long-term effects of fertilisers on herbage production. I. Yields and botanical composition. R. G. Heddle. **II. Chemical composition.** R. G. Heddle and P. Crooks (*J. agric. Sci., Camb.*, 1967, **69**, 425–431, 433–441).—I. N and K applied to a mixed grass/clover sward have a marked effect on yield with marked interaction. P has very little effect on yield. At low N levels KCl has a depressing effect, probably due to the Cl effect on clover. Clover disappears early on plots receiving N and no K, due to K deficiency. At higher levels of N the disappearance is probably due to competition for light, and root competition. Yorkshire fog and broad-leaved weeds are very evident on plots receiving no N or K. *Poa* spp. respond to N, whilst red fescue responds to heavy N applications and is almost eliminated from plots receiving K.

II. The results of trials lasting 14 years show that, without N but with adequate K, clover can yield up to 871 lb of N/acre annually, whilst with dressings of 348 lb N/acre and adequate K, recovery of N goes up to 237 lb/acre. P concn. with no P applications falls with time, but does not fall when P is applied, irrespective of N application. K always depresses P concn. With 348 lb of N/acre, 43 lb P and 280 lb of K/acre max. removal of P occurs. Herbage-K concn. is increased by K applications and is reduced by N when no K is applied. Herbage-Ca is increased by Nitro-chalk in the absence of K. Superphosphate slightly increases Ca concn. Herbage-Mg is increased by N and reduced by K treatments.
M. LONG.

Effect of pH on the growth and ion-balance of tomato and mustard plants. A. Jungk (*Z. PflErnähr. Bodenk.*, 1967, **117**, 108–117).—In pot experiments the plants were grown in peat to which lime and nutrient solutions were added. Increase in pH of the substrate caused increases in inorg. cations and in org. anions in the plant shoots; effects on inorg. anions were variable. Max. yields were obtained with org. anion contents of 150 mequiv./100 g dry matter in tomato and with 130 mequiv./100 g dry matter in mustard. Lowered yields were obtained with org. anion levels above or below the max. Data obtained accord with the view that the pH of the nutrient medium influences yields through its action on the org. anions within the plant.
A. G. POLLARD.

Castor-bean production as related to length of growing season. II. Date of planting tests. D. L. Kittock and J. H. Williams (*Agron. J.*, 1967, **59**, 456–458).—Yields of irrigated castor-beans were highest when planted during the first 2 weeks of May, particularly when leaf spot, due to *Alternaria ricini*, was not present. For non-irrigated castor-beans yields were similar irrespective of planting date in May, whilst April and June plantings gave lower yields. Lodging was inversely correlated with hypocotyl dia. within, but not between, varieties. Seed wt. was correlated with germination %. Germination % was highest with seeds from early plantings.
A. H. CORNFIELD.

Effect of irrigation and fertilisers on castor-bean yield and quality. D. L. Kittock, J. H. Williams and D. G. Hanway (*Agron. J.*, 1967, **59**, 463–467).—Irrigation of a silt loam had no significant effect on castor-bean yields in 2 of 3 years. Application of N (180 kg per ha) increased seed yields of only one of two varieties. Irrigation, particularly when applied early, increased the field loss of seed of one variety, whilst N had no influence on field loss. Seed wt. and oil content were inversely related to the amount of irrigation water applied. Added N significantly reduced the incidence of leaf spot due to *Alternaria ricini*.
A. H. CORNFIELD.

Iron-manganese relationship of chlorotic sugar-cane plants grown on a high-lime soil. S. C. Srivastava, M. P. Agrawal and S. M. H. Jafri (*Soil Sci.*, 1966, **102**, 208–211).—Chlorotic ratoon cane plants

on a high-CaO soil were sprayed with (*a*) chelated Mn, (*b*) 'ultra'-S or (*c*) water, and measurements were made of the time, no. of plants recovering normal leaf colour, juice quality and yield of cane. Although symptoms were characteristic of Fe deficiency best results were obtained with (*a*) which caused marked improvement in juice quality. The apparent inactivation of Fe may be caused by deficiency of Mn. A. G. POLLARD.

Influence of sulphate and chloride ions in fertilisers on the growth of azaleas. K. Rathsack and H. E. Pawlowski (*Z. PflErnähr. Bodenk.*, 1967, **117**, 165–178).—First-year plants of *Rhododendron simsii* were fed various mixtures of K_2SO_4 and KCl (0–100% of each), the total K supplied being 150 or 600 mg/l. The N supply was 300 mg/l, given as $Ca(NO_3)_2$, alone or with NH_4NO_3, or as urea. Wt. of various plant parts are recorded. The ratio SO_4^{2-} : Cl^- had no apparent effect on the dry wt. of flowers, leaves or stems or on no. of leaves produced. Yields were similar whether K_2SO_4 or KCl was given. The form of N supplied had no apparent effect on any of the plant characteristics measured. When grown in constantly moist plots azaleas were not specifically sensitive to Cl^- in practical concn. The no. and dry wt. of leaves were unaffected by constant use of $CaCl_2$, supplying up to 200 mg Cl^-/l in the nutrient solution. The S and Cl contents of the plants were not altered appreciably by the SO_4^{2-} : Cl^- ratio in the nutrient until the total K concn. was raised to 600 mg/l; at this level the leaf-dry matter rose from 0·5 to 0·9% with increase in the [Cl^-] of the nutrient. Simultaneously the S content fell from 0·4 to 0·3%. The dry-matter contents of stem and leaves were unaffected by the SO_4^{2-}/Cl^- ratio of the nutrient. A. G. POLLARD.

Air pollution damage to Austrian pine, *Pinus nigra* var. *austriaca*, in New Jersey. E. Brennan and S. H. Davis jun. (*Pl. Dis. Reptr*, 1967, **51**, 964–967).—Following a period of heavy air pollution extensive needle damage occurred on Austrian pine trees, whilst many other species of conifers on the same sites were unaffected. There was great variability in susceptibility of individual Austrian pine trees to air pollution damage. A. H. CORNFIELD.

Plant damage due to air pollutants in New Jersey. E. Brennan, I. A. Leone and R. H. Daines (*Pl. Dis. Reptr*, 1967, **51**, 850–854).—Symptoms of damage to plants caused by presence in air of SO_2, O_3, peroxyacetylnitrate, and aldehydes are described. A. H. CORNFIELD.

Pest Control

Toxicity of five insecticides to several insect predators. P. D. Lingren and R. L. Ridgway (*J. econ. Ent.*, 1967, **60**, 1639–1641).—Insect predators from six genera were treated topically or exposed to residues of trichlorfon, demeton, Bidrin, phosphamidon and methyl parathion. The toxicity of each compound to each species is given. All were very toxic to hemipterous predators. (23 references.) C. M. HARDWICK.

Uptake of DDT by the American cockroach central nervous system. J. L. Eaton and J. G. Sternburg (*J. econ. Ent.*, 1967, **60**, 1699–1703).—The DDT content of the central nervous system was not directly related to the levels of activity observed. Large topical dosages of ^{14}C-DDT applied to the cerci induced more trains than a small injected dose. When DDT was injected into ganglia increasing temp. increased the number of DDT-induced trains. C. M. HARDWICK.

Use of *Folsomia fimetaria* and *Drosophila melanogaster* as test insects for the detection of insecticide residues. N. E. A. Scopes and E. P. Lichtenstein (*J. econ. Ent.*, 1967, **60**, 1539–1541).—The rearing and bioassay method for *F. fimetaria* are described. The insect was more susceptible to org. P and carbamate insecticides whereas *D. melanogaster* was more susceptible to chlorinated hydrocarbons. Comparative trials with other insecticides are recorded. C. M. HARDWICK.

Metabolic and enzymic degradation of several aromatic carbamate insecticides. E. G. Gemrich, II (*Diss. Abstr.* B., 1967, **27**, 2245).—Carbaryl (1-naphthyl *N*-methyl carbamate) (**I**), and Banol (2-chloro-4,5-dimethylphenyl *N*-methyl carbamate) (**II**), were oxidatively metabolised by *Musca domestica* and by *Blaberis giganteus*. Microsomes obtained from the fat bodies of *B. giganteus* produced three oxidative products of **II**. A. G. POLLARD.

Fungitoxicity of captan. V. Electron microscopy of captan-treated *Neurospora crassa conidia*. D. V. Richmond, E. Somers and P. F. Millington (*Ann. appl. Biol.*, 1967, **59**, 233–237).—The only observable effect of an ED_{50} fungicidal dose of captan on dormant *conidia* was to produce a characteristic convoluted form of the nuclear membrane. This may be due to the reaction of captan with the sulphydryl groups of the nuclear protein leading to an inhibition of cell division. The cytoplasmic membrane was unaffected by captan. After incubation in Fries medium, captan-treated spores showed an almost complete loss of internal fine structure. A. H. CORNFIELD.

Distribution of Bidrin in the foliage of treated trees. D. E. Donley (*J. econ. Ent.*, 1967, **60**, 1583–1585).—Bioassay of leaves with *Daphnia pulex* was carried out over 4 years in 13 species of trees. The best distribution patterns were obtained by implanting in roots on both sides of the tree. Root implants gave more even distribution than trunk implants. C. M. HARDWICK.

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), an active agent in resistance of maize to European maize borer. J. A. Klun, C. L. Tipton and T. A. Brindley (*J. econ. Ent.*, 1967, **60**, 1529–1533).—When DIMBOA was added to the diet of *Ostrinia nubilalis* it inhibited larval development and caused 25% mortality. Its isolation and bioassay are described. It is probably associated with resistance of maize to the borer. MBOA (6-methoxy-benzoxazolinone) showed attenuated activity in presence of various vitamins, particularly niacin. C. M. HARDWICK.

Ultrasonic extraction of insecticides in soil. I. Comparison of extraction methods and solvent systems over three time intervals. R. E. Johnsen and R. I. Starr (*J. econ. Ent.*, 1967, **60**, 1679–1682).—Use of the ultrasonic method of extraction of heptachlor-epoxide and dieldrin from a sandy loam after 1 day and 1 or 2 months was compared with the blender and roller extraction methods using six solvent systems. No method was markedly superior to the others. In all cases benzene was a poor solvent. C. M. HARDWICK.

Bioassay of field-treated soils to determine bioactivity and movement of insecticides. C. C. Burkhardt and M. L. Fairchild (*J. econ. Ent.*, 1967, **60**, 1602–1610).—Granular aldrin, diazinon, heptachlor, parathion and phorate were applied at 1, 2, and 4 lb/acre in a band at planting time. The soil was assayed with *Acheta domesticus*. Activity varied with soil type and moisture. Aldrin and heptachlor caused 85–100% mortality 8 weeks after treatment. Diazinon was the most active org. phosphate followed by parathion and phorate. (14 references.) C. M. HARDWICK.

Effect of detergents and inorganic salts in water on the persistence and movement of insecticides in soils. E. P. Lichtenstein, T. W. Fuhremann, K. R. Schulz and R. F. Skrentny (*J. econ. Ent.*, 1967, **60**, 1714–1721).—Water percolated through loam soil, containing insecticide (1 ppm), contained no aldrin but did contain a small amount of parathion. This amount varied with the concn. in the soil. The addition of detergents did not increase this and salts reduced the amount of parathion. Aldrin moved in the absence of water more than did parathion. Thus contamination of water in deep soil strata appears unlikely. (18 references.) C. M. HARDWICK.

Comparisons of soil and foliar applications of acaricides for control of the two-spotted spider mite on strawberries in southern California. H. H. Shorey, R. L. Hale and V. Voth (*J. econ. Ent.*, 1967, **60**, 1722–1724).—Granular phorate applied in the furrow with Nov. plantings gave 6 months control of *Tetranychus urticae*; this did not protect Aug.-planted strawberries from the spring infestation. Disulfoton granules were not satisfactory. Foliar sprays of dicofol, dimethoate and tetradifon + TEPP gave good but short-term mite reduction. C. M. HARDWICK.

Factors influencing the population fluctuation of *Pratylenchus penetrans* in soils of high organic content. I. Effect of soil fumigants and different crop plants. J. M. Ferris (*J. econ. Ent.*, 1967, **60**, 1708–1714).—Without treatment there was a regular build-up of *P. penetrans* during the growing season of onion, maize, potatoes, peppermint or spearmint. The use of D-D, Telone or Vorlex (a mixture of dichloropropenes, dichloropropanes etc.) gave dramatic reductions and low populations for at least one season. Greenhouse tests gave similar results. (15 references.) C. M. HARDWICK.

Soil treatments with broadcast or band applications of organophosphorus or carbamate insecticides for prevention of wireworm damage to potatoes. R. H. Burrage, J. A. Menzies and E. Zirk (*J. econ. Ent.*, 1967, **60**, 1489–1492).—Of seven granular treatments applied before planting only N-2790 (*O*-ethyl-*S*-phenyl ethylphosphonodithioate) and Zinophos gave 90% protection of potatoes from wireworm damage in two of three trials. Yield was not increased in proportion to control of damage. Two of the compounds produced an off-flavour. C. M. HARDWICK.

Sex pheromones of noctuid moths. XIV. Feasibility of behavioral control by disrupting pheromone communication in cabbage loopers. H. H. Shorey, L. K. Gaston and C. A. Saario (*J. econ. Ent.*, 1967, **60,** 1541–1545).—Synthetic female sex pheromones of *Trichoplusia ni* were released and the effect on males observed. In the laboratory 2×10^{-14} g/l was the lowest concn. inhibiting mating. A max. no. of males responded within 1 min.; at higher concn. the response lasted longer. In 2 l jars higher concn. were required. In the field 1×10^{-20} g/l prevented male orientation. (25 references.)
C. M. HARDWICK.

Evaluation of systemic insecticides incorporated in soil for control of lepidopterous larvae on cole crops in southern California. H. H. Shorey and R. L. Hale (*J. econ. Ent.*, 1967, **60,** 1567–1570).—Eight systemic insecticides were compared as foliar or soil treatments on cabbage, cauliflower or broccoli over 3 years. While NIA-10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was most effective against *Pieris rapae* and *Plutella maculipennis* larvae, American Cyanamid 47031 [cyclic ethylene (diethoxyphosphinyl) dithioimidocarbonate] was more effective against larvae of *Trichoplusia ni*. C. M. HARDWICK.

Chemosterilisation of male European maize borers by feeding of Tepa and Apholate to larvae. J. A. Harding (*J. econ. Ent.*, 1967, **60,** 1631–1632).—Seven-day-old *Ostrinia nubilalis* larvae were fed media treated with 7·5 and 8·5% Tepa and Apholate. Untreated females mated to treated males laid a reduced no. of fertile eggs. There was some larval toxicity but no effect on adult longevity.
C. M. HARDWICK.

Aspects of the wheat bulb fly problem. F. Raw (*Ann. appl. Biol.*, 1967, **59,** 155–173).—An address. A. H. CORNFIELD.

Effect of variety and fumigation on nematode populations in oats, wheat, and rye. D. T. Sechler, W. B. Tappan and H. H. Luke (*Pl. Dis. Reptr*, 1967, **51,** 915–919).—Soil fumigation with CH_3Br (2 lb per 108 sq. ft) one week before seeding a sandy loam resulted in lower populations of nine genera of nematodes over 3 years, although populations of *Aphelenchoides* were not affected over 2 years. Both size and type of nematode populations tended to be similar between the three genera and the varieties of small grain tested. Grain yields were generally inversely related to nematode populations. A. H. CORNFIELD.

Control of leaf blight of wheat caused by *Alternaria triticina*. I. N. Tandon, S. C. Verma and H. S. Lal Srivastava (*Pl. Dis. Reptr*, 1967, **51,** 950–952).—The most effective control of leaf blight was obtained by spray application of ziram (2 lb) or Dithane S-31 ($NiSO_4$ + maneb, 3 lb per acre) 6 times at 14-day intervals starting immediately after the disease appeared. A. H. CORNFIELD.

Factors affecting control of western maize rootworm larvae with soil insecticides. G. J. Musick and M. L. Fairchild (*J. econ. Ent.*, 1967, **60,** 1522–1525).—Control of *Diabrotica virgifera* by phorate, disulfoton, diazinon and a stabilised parathion was affected by date of planting. Treatment at planting time was more effective when this was earlier. Later planting increased the effectiveness of basal treatment. C. M. HARDWICK.

Gamma irradiation of European maize borer larvae. E. S. Raun, L. C. Lewis, J. C. Picken, jun. and D. K. Hotchkiss (*J. econ. Ent.*, 1967, **60,** 1724–1730).—Irradiation of non-diapausing larvae with ^{60}Co caused extensive somatic damage, which did not occur in diapausing larvae. Field collected diapausing larvae were irradiated after 3 or 5½ months refrigeration. There was resistance to damage in the early stages of oogenesis. The longer the pupation, the higher was the dose required to produce sterility. Differences in egg hatchability from treated females was not so marked.
C. M. HARDWICK.

Insecticidal control of regulated populations of cutworm on maize. J. W. Apple (*J. econ. Ent.*, 1967, **60,** 1612–1615).—Plots were artificially infested with 4th instar larvae of *Agrostis ipsilon* at the rate of 1 larva per plant. Diazinon granules at planting time or as a spray were as effective as aldrin + parathion. Carbaryl was effective as a spray but not as granules. Granules of Furadan (2,3-dihydro-2,2-dimethyl 7-benzofuranyl methylcarbamate or CL-47470 [cyclic propylene (diethoxy-phosphinyl) dithioimido carbonate] + phorate gave good protection.
C. M. HARDWICK.

Chemical control of the stem nematode, *Ditylenchus dipsaci*, in lucerne. G. D. Griffin (*Pl. Dis. Reptr*, 1967, **51,** 973–974).—Liquid formulations of Cynem (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate, 0·5–2·0 lb per acre), dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate, 0·5–2·0 lb] and Bay 37289 (*O*-ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate, 2 lb) applied as sprays immediately after sowing lucerne gave excellent control of *Ditylenchus dipsaci* infestation of the plants. A. H. CORNFIELD.

Systemic insecticides as lygus bug controls compatible with bee pollination on lucerne. C. Johansen and J. Eves (*J. econ. Ent.*, 1967, **60,** 1690–1696).—Of three systemic insecticides applied as granules and irrigated, Temik gave up to 3 months control of *Lygus hesperus*. Trichlorfon sprays needed renewing after 10 days. Dimethoate applied through irrigation sprinklers at high dosages provided good control with low bee mortality. Abate gave 3 weeks' control but was highly toxic to bees. C. M. HARDWICK.

Control of storage decay of crown vetch, *Coronilla varia*, crowns. H. Cole and G. W. McKee (*Pl. Dis. Reptr*, 1967, **51,** 820–822).—Storage decay, due to *Fusarium roseum*, of crown vetch crowns was prevented by a 5-sec dip in captan (1 oz 50% wettable powder per gal). A. H. CORNFIELD.

Control of endo- and ecto-parasitic nematodes in turf by hot-water treatments. C. M. Heald and H. D. Wells (*Pl. Dis. Reptr*, 1967, **51,** 905–907).—Cores of Bermuda-grass turf were freed of endo- and ecto-parasitic nematodes when held in water at 50° for 45 min. or at 55° for 15–30 min. Germination of sprigs was reduced at 55° and completely inhibited at 60°. A. H. CORNFIELD.

Control of *Fusarium* blight of turfgrass. G. A. Bean, R. N. Cook and A. E. Rabbitt (*Pl. Dis. Reptr*, 1967, **51,** 839–841).—Of a number of chemicals tested for control of *Fusarium* blight of turfgrass, the most effective control was obtained by spray application, at weekly to monthly intervals of 45% thiram + 10% 2-chloro-4-(hydroxymercuri)phenol (4–6 oz in 5 gal water per 1000 sq. ft).
A. H. CORNFIELD.

Effect of sawdust on incidence of root knot and yields of okra and tomatoes in nematode-infested soil. R. S. Singh, B. Singh and S. P. S. Beniwal (*Pl. Dis. Reptr*, 1967, **51,** 861–863).—Mixing sawdust (2000 lb per acre) with a silt loam 3 weeks before planting tomatoes and okra reduced the severity of root knot galls due to *Meloidogyne javanica*. The treatment increased okra yields by 70% and tomato yields by 125%. A. H. CORNFIELD.

Control of pickleworm on cucurbits. T. D. Canerday (*J. econ. Ent.*, 1967, **60,** 1705–1708).—In field experiments, carbaryl, lindane, endosulfan and three proprietary insecticides applied weekly as foliar sprays gave effective control of *Diaphania nitidalis*. There was no adverse effect on yields. C. M. HARDWICK.

Control of *Cytospora* canker disease of peach trees with systemic chemicals. R. E. Williams and A. W. Helton (*Pl. Dis. Reptr*, 1967, **51,** 834–838).—Spraying 3-year-old peach trees with 500–5000 ppm 8-quinolinol phosphate before infection reduced the extent of spread of infection. The treatment was not effective when applied after infection. Cycloheximide thiosemicarbazone and cycloheximide acetate sprays were most effective when applied before infection, but were also fairly effective when applied after infection; they were particularly effective in causing rapid healing of infection wounds. All compounds were translocated into the root system.
A. H. CORNFIELD.

Glasshouse experiments on apple scab. III. Fungicide mixtures, curative translocation, and the influence of mildew. M. H. Moore (*Ann. appl. Biol.*, 1967, **59,** 239–244).—Experiments with fungicide mixtures used protectively and curatively against apple scab showed no advantage from adding PhHgCl (*PMC*) to certain protective fungicides. S fungicides greatly impaired the curative activity of *PMC*, especially in mixtures applied 24 h after infection, but captan showed little and dodine acetate no such effect. *PMC*, however, contributed appreciably to the curative effectiveness of mixtures with captan or dodine acetate. Dodine acetate, Dichlofluanid, CaO–S, and iso-butyl-*o*-coumarate showed little translocated activity against scab when applied after infection. *PMC* was much more effective when applied in summer before infection than when applied in spring 24 h after infection. Uncontrolled powdery mildew early in the scab-incubation period greatly reduced the establishment of scab infection on test plants.
A. H. CORNFIELD.

Responses of pineapple orange trees to selected petroleum oil fractions. H. A. Dean and C. E. Hoelscher (*J. econ. Ent.*, 1967, **60,** 1668–1672).—Four oil fractions and one non-oil treatment were applied to trees in July for 3 years. Juice quality was analysed and differences were small. The pattern of leaf drop was analysed; it was 37–65% greater after various oil applications than after non-oil treatment. (17 references.) C. M. HARDWICK.

Control of the iris borer with systemic insecticides. J. E. Appleby (*J. econ. Ent.*, 1967, **60,** 1610–1612).—Phorate and disulfoton

granules applied to the soil surface did not control *Macronoctua onusta*. Dimethoate granules or spray gave good control. If applied while flower buds were within the sheath, flower stems were weakened. C. M. HARDWICK.

Bark *vs.* foliage applications of insecticides for control of *Psylla uncatoides* on *Acacia*. C. S. Koehler and S. S. Rosenthal (*J. econ. Ent.*, 1967, **60**, 1554–1558).—The effects of diazinon, dimethoate (**I**) carbaryl (**II**) and oil on various stages as spray and bark treatments are shown. Applied to bark, **I** gave control of nymphs for 4 months, whereas **I** and **II** afforded similar control for about 5 weeks only, when applied as foliage sprays. C. M. HARDWICK.

Bulb treatments for the control of the root-lesion nematode, *Pratylenchus penetrans*, in Easter lily. W. H. Hart, A. R. Maggenti and J. V. Lenz (*Pl. Dis. Reptr*, 1967, **51**, 978–980).—Dipping Easter lily bulbs in 0·5% aq. phorate for 15 min. was the most effective treatment for reducing the numbers of root-lesion nematodes in the roots during subsequent growth in infested soil. A. H. CORNFIELD.

Comparison of three rates of application of ultra-low-volume azinphosmethyl in a reproduction-diapause control programme against the boll weevil. E. P. Lloyd, F. C. Tingle, M. E. Merkl, E. C. Burt, D. B. Smith and T. B. Davich (*J. econ. Ent.*, 1967, **60**, 1696–1699).—Azinphosmethyl at 0·2 and 0·25 lb/acre gave as good reduction of *Anthonomus grandis* as did a water-emulsion spray of methyl parathion (0·5 lb/acre). The number of hibernating weevils found in the rubbish round treated fields was reduced by ~95%. C. M. HARDWICK.

Low-volume dusts for control of cotton bollworm. C. Lincoln and G. Dean (*J. econ. Ent.*, 1967, **60**, 1744–1745).—In all except one case, low-vol. dusts gave better control of a primary infestation of *Heliothis zea* than conventional sprays and dusts and equal control of re-infestation. C. M. HARDWICK.

Gamma irradiation of pupae of the tobacco budworm. H. M. Flint and E. L. Kressin (*J. econ. Ent.*, 1967, **60**, 1655–1659).—When pupae of *Heliothis virescens* were irradiated with 45 krad or more, there was reduced emergence in both sexes; males were completely sterile and the number of eggs laid was reduced. Life-spans were also reduced. The effect of radiation on the pattern of oviposition is discussed. The dosage response curve for males was sigmoidal. C. M. HARDWICK.

Lindane in diesel oil prevents western pine beetle attacks for at least one year. R. H. Smith (*J. econ. Ent.*, 1967, **60**, 1746–1747).—Application of lindane in diesel oil to a tree trunk prevented attack by *Dendroctonus brevicomis* for 10 ft above and 6 ft below the treated area. Application of an aq. solution to freshly attacked trees prevented additional attacks for at least 2 months. Untreated trees died. C. M. HARDWICK.

Late-season control of European elm scale with Bidrin trunk injections. H. E. Thompson (*J. econ. Ent.*, 1967, **60**, 1745–1746).—Injections (2 ml) of Bidrin at 3 in. spacings gave an 88% reduction of *Gossyparia spuria*, and 3 ml at 6 in. spacings a 98% reduction when carried out from mid-June to mid-Sept. C. M. HARDWICK.

Toxicity of insecticides to house crickets and bioassay of treated soils in the laboratory. C. C. Burkhardt and M. L. Fairchild (*J. econ. Ent.*, 1967, **60**, 1496–1503).—The effect of topical application of 21 insecticides on *Acheta domesticus* is given. Of these, aldrin, heptachlor, Niran, diazinon and phorate were added to four soil types at two moisture levels. Big differences in cricket mortality were found among soil types for each insecticide. Within most soil types, increased moisture content increased insecticidal activity. C. M. HARDWICK.

Screening of insecticides against the changa and southern mole cricket attacking seedling millet. E. W. Beck and J. L. Skinner (*J. econ. Ent.*, 1967, **60**, 1517–1519).—Of 10 insecticides tested N-2790 bait (*O*-ethyl *S*-phenyl ethylphosphonodithioate), Kepone bait and trichlorfon granules applied to the soil were most effective against *Scapteriscus acletus*. Increased stands and forage yields resulted. C. M. HARDWICK.

Herbicide-insecticide formulations for control of American dog tick. J. A. McKiel, D. A. Dever, J. R. Proctor and M. B. Garvie (*J. econ. Ent.*, 1967, **60**, 1570–1572).—DDT (2 lb active insecticide/acre) and lindane (0·2 and 0·4 lb active insecticide/acre) were added to each of the herbicides (2,4-D iso-octyl ester and 2,4,5-T iso-octyl ester) and sprayed along the roadside. There was a 91% reduction of *Dermacentor variabilis* after 19 days with the DDT-herbicide mixture; reductions with the lindane-herbicide mixtures (0·2 and 0·4 lb/acre of lindane) were 82% and 87% (after 4 days) and only 54% and 63% (after 19 days), respectively. C. M. HARDWICK.

Persistence of 2,4-D, 2,4,5-T, and dicamba in range forage grasses. H. L. Morton, E. D. Robinson and R. E. Meyer (*Weeds*, 1967, **15**, 268–271).—The persistence of 2,4-D (acid or amine salt), 2,4,5-T (acid or ester), and dicamba (2-methoxy-3,6-dichlorobenzoic acid) applied at 0·5–2·0 lb per acre to range forage grasses showed little difference between herbicides or between different formulations of the same herbicide. Disappearance of the herbicides increased with rainfall. A. H. CORNFIELD.

Influence of atrazine and simazine on forage yields and nitrogen components of maize. R. J. Fink and O. H. Fletchall (*Weeds*, 1967, **15**, 272–274).—Plots planted to maize were treated with atrazine (2-chloro-4-ethylamino-6-iso-propylamino-*s*-triazine) or simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] each at 2·5 to 10 lb per acre. The treatments, particularly with simazine, reduced forage yields 5 weeks after sowing. The effect was less in the second than in the first year. Reduction in forage yields due to the treatments decreased with time after planting, and maize grain yields were unaffected except where 10 lb simazine was applied. Where no fertiliser N was applied the treatments increased forage NO_3^-% during the first 5 weeks. The treatments increased forage N% in proportion to the amount of herbicides applied, more so where no N than where 100–200 lb N per acre had been applied. A. H. CORNFIELD.

Dinitroalkylphenol esters. Farbwerke Hoechst A.-G. (B.P. 1,049,061, 10.4.63. Ger., 11.4.62).—Successful esterification of dinitroalkyl- and -cycloalkyl-phenols is brought about by passing an inert gas (e.g. N_2) through the reactants at 75–145 (110–135)° and/or by adding one or more of PX_3, PX_5, POX_3, H_3PO_3 (X is halogen) preferably PCl_3, or anhydr. $MgCl_2$, $SbCl_3$, $ZnCl_2$, $SnCl_4$, $AlCl_3$, $FeCl_3$ or $HgCl_2$ which act as catalysts. The products are acaricides, fungicides, ovicides and herbicides. E. ENOS JONES.

Fungicides. E. I. du Pont de Nemours & Co. (B.P. 1,053,839, 29.5.64. U.S., 7.6.63).—Bis(di-isopropylthiocarbamyl) sulphide is especially effective in controlling plant fungi of the order *Erysiphales*. It is prepared by stirring a mixture of aq. N-NaOH, $NHPr^i_2$, and CS_2 during 2·5 h, then adding NaCN, followed by an aq. solution of $(NH_4)_2S_2O_8$, keeping overnight, and recrystallising ppt. (m.p. 117·5–118·5°) from EtOH. F. R. BASFORD.

Amino azacycloheptanones. Agripat S.A. (Assee of K. Gaetzi) (B.P. 1,058,521, 12.7.65. Switz., 13.7.64).—Compounds claimed are active against phytopathogenic fungi and comprise 7-NRR^1-2-azacycloheptanones (R is alkyl of 4–16 C, dialkylamino of >18 C, cyclohexyl, or optionally substituted Ph or aralkyl; R^1 is H or alkyl of 1–4 C), optionally substituted elsewhere by alkyl of 1–6 C. Thus, a mixture of 7-chloro-2-azacycloheptanone and n-hexylamine is heated at 120° for 14 h, then worked up, to give *7-hexylamino-2-azacycloheptanone*, b.p. 128–132°/0·01 mm. Acid-addition salts of the bases are also claimed. F. R. BASFORD.

Thiazolidine derivative. U.S. Rubber Co. (B.P. 1,059,677, 15.10.65. U.S. 2.11.64).—*3,3′-Methylene bis-thiazolidine*, m.p. 48·5–50·0°, useful as a fungicide against e.g. *Alternaria solani*, is made by reacting at least 1·5 moles CH_2O with 1 mole of an acid salt of $HSCH_2CH_2NH_2$ (e.g. the hydrochloride) in an aq. medium. E. ENOS JONES.

Sulpholanyl phenylhydrazines. Whiffen & Sons Ltd. (Inventor: K. G. Mason) (B.P. 1,047,525, 30.5.62).—The compounds have fungicidal activity (especially against certain rusts, e.g., *Uromyces phaseoli*) and the formula $NRR^1 \cdot NR^{II}R^{III}$ wherein R and R^I are H or alkyl of 1–8 C (but preferably at least one of them is H); R^{II} is Ph optionally substituted by halogen, NO_2, alkyl or alkoxy of 1–8 C, NH_2, alkylamino or dialkylamino; R^{III} is the group 2-R^{IV}-2-R^V-3-R^{VI}-4-R^{VII}-5-R^{VIII}-5-R^{IX}-sulpholan-3-yl; R^{IV}–R^{VIII} are H or C_{1-8}-alkyl (optionally substituted) or alkoxy; R^{IX} is H or alkyl of 1–8 C. E.g., a mixture of butadiene sulphone, $PhNHNH_2$, and 40% aq. KOH is kept at 60° for 10 h; the ppt. is recrystallised from MeOH to give 1-(*sulpholan-3′-yl*)-1-*phenylhydrazine*, m.p. 119–120°(*hydrochloride*, m.p. 189°). F. R. BASFORD.

Quaternary salts of aromatic disulphonic acids for combating phytopathogenic micro-organisms. Farbwerke Hoechst A.-G. (B.P. 1,058,548, 10.9.64. Ger., 10.9.63).—The compounds are of formula $[(NR^1R^{II}R^{III}R)^+]_2X_2$ wherein R–R^{II} are alkyl of 1–4 C or together with N form a heterocyclic ring; R^{III} is alkyl of 10–18 C; and X is anion of an aromatic disulphonic acid. Thus, didodecyl benzene-1,3-disulphonate (prep. described) is heated to 90°, then pyridine is added during 1 h, temp. rising to 130°. After a further 2 h the mixture is cooled and washed with ether to give *bis-(N-dodecylpyridinium)benzene-1,3-disulphonate*, effective against

brown rust (of wheat), powdery mildew (of cereals and grasses), etc. F. R. BASFORD.

Quaternary ammonium compounds for combating phytopathogenic micro-organisms. CIBA Ltd. (B.P. 1,056,546, 6.5.64. Switz., 30.5.63).—The active agent is adduct of a quaternary NH_4 compound and a compound of I_2 with an iodide. E.g., an aq. solution of I_2 and KI is quickly added to an aq. solution of the compound $PhCH_2 \cdot NMe_2(C_{12}H_{25}) \cdot Cl$; the product solidifies and is comminuted, washed with water, and recrystallised from EtOH, to give an adduct, m.p. 68–72°. The products are potent fungicides, especially effective against leaf spot fungi on plants. F. R. BASFORD.

5-Isoxazolones and derivatives. Imperial Chemical Industries Ltd. (Inventors: M. B. Green and R. Roberts) (B.P. 1,049,103, 1.5.64).—A β-keto ester (e.g. Et acetoacetate) or a diketene is reacted with NH_2OH or a deriv. (e.g. hydrochloride) at 30–70°. The 5-isoxazolone product is subsequently reacted with a diazo-compound (e.g. *o*-chlorobenzenediazonium compound) at a temp. < 15° to produce, e.g., 3-methyl-4-(*o*-chlorophenylhydrazono)-5-isoxazolone, useful as a fungicide. E. ENOS JONES.

Fungicidal tetrachloro-nitroaniline derivatives. Fisons Pest Control Ltd. (Inventors: A. J. Lambie, G. T. Newbold and M. B. Purdew) (B.P. 1,056,862, 20.8.63).—*o*-Nitroanilines especially effective against *Erysiphe cichoracearum* and rice blast have the formula $o\text{-}NO_2 \cdot C_6Cl_4 \cdot NH \cdot CR^{II}R^{III} \cdot CHR^{IV}R^{V}$ in which R^{II} and R^{III} are H, alkyl, or CH_2OR^{I} (R^{I} is H, alkyl, or acyl); R^{IV} and R^{V} are H, alkyl, acyloxy, alkoxy, hydroxyalkyl, acyloxyalkyl, alkoxyalkyl, hydroxyalkoxy, or alkoxyalkoxy, or R^{IV} is OH. E.g., a solution of NH_2Et in EtOH is added at < 0° to a suspension of $1,2\text{-}(NO_2)_2C_6Cl_4$ in EtOH, then after 24 h/> 1 atm. the mixture is worked up, to give 3,4,5,6-tetrachloro-2-nitro-*N*-ethylaniline, m.p. 75–77·5° (aq. EtOH). F. R. BASFORD.

Protecting plants against fungi. E. Lilly & Co. (B.P. 1,055,535, 13.4.64. U.S., 16.4.63).—Compounds of general formula $C_6H_3R^{I}R^{II} \cdot CO_2 \cdot R \cdot NR^{III}R^{IV}$ and acid-addition salts thereof are effective against fungi which attack food crops and plants, e.g., *Phytophthora infestans*, *Uromyces phaseoli*, *Colletotrichum lagenarium*, *Erysiphe polygoni*. R is saturated or unsaturated alkylene, oxa-alkylene, or thia-alkylene of 2–10 C; R^{I} and R^{II} are halogen; R^{III} and R^{IV} are alkyl of 1–8 C, alkenyl of 2–4 C, or together represent morpholino or C_4-C_5-polymethyleneimino which may contain alkyl 1–8 C. E.g. a mixture of γ-(2-methylpiperidino) propyl alcohol, $CHCl_3$ and $SOCl_2$ is boiled for 3 h to give γ-(2-methylpiperidino) propyl chloride hydrochloride, m.p. 170–174·5°. The free base from this is boiled with $3,4,1,C_6H_3Cl_2 \cdot CO_2H$ and $Pr^{i}OH$ for 24 h to give γ-(2-methylpiperidino)propyl 3,4-dichlorobenzoate hydrochloride, m.p. 172–174° (from EtOH). F. R. BASFORD.

Fungicidal compositions. VEB Berlin-Chemie (Inventors: W. Harnack and J. Schwarz) (B.P. 1,048,507, 20.7.65).—Compositions for combating plant fungi (*Phytophthora infestans*, *Septoria apii*) contain $NHR \cdot CH_2 \cdot CO_2R^{I}$ (or its salts) as active ingredients (R is Et, Pr, Pr^{i}, Bu, or Bu^{i}; and R^{I} is H; or R is allyl or $(CH_2)_2OH$ and R^{I} is H, Me or Et). F. R. BASFORD.

Dithiophosphonic acid esters having pesticidal properties. Stauffer Chemical Co. (B.P. 1,052,991, 17.6.64. U.S., 2.7.63).—Compounds claimed have low toxicity to mammals and the formula $R^{I}(OR)PS_2 \cdot C_6H_3Cl \cdot Me\text{-}1,4,3$ (R is alkyl of 1–4 C and R^{I} is monochlorinated R). An example is *O*-Et *S*-*p*-chloro-*m*-tolyl ethyldithiophosphonate, b.p. 150°/0·7 mm, obtained in 87% yield by boiling a mixture of $1,4,3\text{-}SH \cdot C_6H_3Cl \cdot Me$, $Et(OEt)PS_2Cl$, benzene and NEt_3 for 1 h. Its effect on housefly, American roach, salt-marsh caterpillar, is tabulated. F. R. BASFORD.

Phosphonic and thionophosphonic acid esters. Farbenfabriken Bayer A.-G. (B.P. 1,058,457, 1.12.65. Ger., 30.1.65).—3-Hydroxypyridines react with phosphonic or thionophosphonic acid ester halides in presence of an acid-binding agent to give products having insecticidal and acaricidal activity. Thus, equiv. amounts of 3-hydroxypyridine in MeEtCO and MeONa are stirred at 50° for 1 h, when an equiv. quantity of $Ph(OEt)P(S)Cl$ is added. After heating at 60° for ~5 h and stirring at room temp. for several h, the filtered solution is extracted with water–benzene; the benzene layer affords a 57% yield of phenylthionophosphonic acid-*O*-ethyl-[pyridyl-(3)] ester. S. D. HUGGINS.

Dialkylacetamidoethylphosphorodithioates. Nippon Soda K.K. (Inventors: Y. Uchiyama, Y. Arima, K. Taniguchi, N. Sato and M. Asada) (B.P. 1,062,952, 22.1.65).—Used as pesticides particularly for orchard and field crops, the claimed *O*,*O*-dialkyl-*S*-(2-acetamidoethyl)phosphorodithioates are obtained by reacting compounds of formula $(RO) \cdot R^{I}O \cdot P(S)\text{-}S \cdot M$, where R and R^{I} are lower alkyl radicals with 1–4 C and M is NH_4 or alkali metal, with compounds of formula $X \cdot CH_2 \cdot CH_2 \cdot NH \cdot CO \cdot CH_3$, where X is Cl, Br, a methane-sulphonic acid residue or a 4-toluenesulphonic acid residue at 40–100° in an inert solvent. Thus, methane sulphonyl chloride is added to 2-acetamidoethanol in $CHCl_3$ and the mixture refluxed for 11 h. Distillation of volatile components leaves the oily product 2-acetamidoethylmethane sulphonate which is added to NH_4 dimethyl phosphorodithioate in $CHCl_3$ solution and refluxed for 6 h, cooled, filtered and worked up to give *O*,*O*-dimethyl *S*-(2-acetamidoethyl)phosphorodithioate. S. D. HUGGINS.

Phosphorus-containing sulphonamides [insecticides]. Stauffer Chemical Co. (B.P. 1,048,320, 27.8.65. U.S., 25.9.64).—Title compounds (**I**) have the formula $RR^{I}PX \cdot S \cdot Y \cdot NR^{II} \cdot SO_2R^{III}$ where R and R^{I} are alkyl or alkoxy of 1–5 C; R^{II} is H or C_{1-5} alkyl or haloalkyl; when R^{II} is H then R^{III} is C_{1-5} alkyl, and when R^{II} is not H then R^{III} is C_{1-5} alkyl, Ph, or halophenyl; X is O or S; and Y is alkylene of 1–5 C. E.g., a mixture of $(OEt)_2PS_2K$, $MeSO_2NH \cdot CH_2CHClMe$ and COMeEt is boiled for 3 h, cooled, and filtered. Filtrate is washed with 5% aq. $NaHCO_3$ and water, then stripped at 110°/20 mm, to give *OO*-Et_2 *S*-3-(methanesulphonamido)propyl thiothionophosphate, n_D^{25} 1·4957. Effects of **I** on housefly and two-spotted mite are tabulated; some compounds also showed fungicidal properties. F. R. BASFORD.

Insecticidal compositions comprising phosphorodithioate esters. Roumanian Ministry of Petroleum and Chemical Industry (Inventors: L. Almasi, L. Paskucz, E. Radulescu, E. Kolosy and A. Hantz) (B.P. 1,054,437, 21.10.63).—A mixture of chlorinated benzenes obtained as residue in the production of PhCl is (after removal of latter) converted into corresponding sulphenyl chlorides (with $ClSO_3H$, followed by reduction with Zn–H_2SO_4 and chlorination) which are treated with the reaction product of P_4S_7 and EtOH, to give a mixed insecticidal composition containing $(OEt)_2PS_2R$, $(OEt)_2P(S)S_2R$, and $(OEt)_2P(S)SEt$ (R is a mixture of $C_6H_4Cl\text{-}p$ and 3,4- and 2,5-dichlorophenyl), with activity against *Aporia crategi* larvae Stage 4–5, *Hyponomeuta evonymellus* larvae, *Doralis favae*, *Hyalopterus pruni*, and *Leptinatarsa decemlineata* larvae Stage 2–3, almost equal to that of parathion but which is less toxic to warm blooded animals. F. R. BASFORD.

2-Hydroxy-*s*-triazines. Farbenfabriken Bayer A.-G. (Inventors: E. Degener, H. Holtschmidt and H.-G. Schmelzer) (B.P. 1,049,513, 3.12.63. Ger., 14.12.62).—2-Hydroxy-4-R^{I}-6-R^{II}-*s*-triazines are prepared in good yield by causing $R^{I}C(:NH)NH_2$ to react at 0–50° with $R^{II}CCl:N \cdot COCl$ (R^{I} is alkyl, alkenyl, cycloalkyl, cycloalkenyl, CCl_3, aralkyl, aryl, chloroaryl, alkaryl, alkoxyaryl, thioalkyl, arylamino, or $NH \cdot N:CHPh$; R^{II} is aryl, CCl_3, or Cl; at least one of R^{I} and R^{II} is Ph). E.g., $PhCCl:N \cdot COCl$ is added at room temp. to an aq. solution of *S*-Me isothiuronium sulphate and Na_2CO_3; the ppt. is recrystallised to give 2-hydroxy-4-methylthio-6-phenyl-*s*-triazine, m.p. 275–276°. It has insecticidal properties. F. R. BASFORD.

Phenyl-*N*-methyl carbamates. Farbenfabriken Bayer A.-G. (B.P. 1,055,603, 11.5.65. Ger., 27.6.64).—The title compounds, viz., $NHMe \cdot CO_2 \cdot C_6H_{4-n}R_n \cdot CH_2XR^{I}$ (X is O or S; R and R^{I} are alkyl of 1–4 C; *n* is 0–2) are insecticides. E.g., a solution of $o\text{-}OH \cdot C_6H_4 \cdot CH_2OMe$ in benzene is mixed with MeNCO, then NEt_3 is added. After 2 h at 50° the solution is cooled to 0°, with separation of o-(*methoxymethyl*)-N-*methylcarbamate* (90%), m.p. 50° (light petroleum). The compounds have low toxicity towards plants and mammals. F. R. BASFORD.

4-(*N*,*N*-Methyl-allylamino)phenyl-*N'*-methyl carbamates. Farbenfabriken Bayer A.-G. (B.P. 1,061,856, 9.9.65. Ger., 18.9.64).—The carbamates $CH_2:CHCH_2NMe \cdot C_6H_2 \cdot 2,5,4\text{-}R,R^{I},O_2CNHMe$ (where R and R^{I} are Me, or R is Me and R^{I} is Pr^{i}) are produced by reacting the corresponding phenol with MeNCO (**I**). Thus, 3,5-dimethyl-4(*N*,*N*-methyl-allylamino)phenyl-*N*-methyl carbamate is obtained by reacting 3,5-dimethyl-4(*N*,*N*-methyl-allylamino) phenol with **I** in presence of Et_3N. The products have insecticidal and acaricidal activity. E. ENOS JONES.

Benzofurazan derivatives. Shell Internationale Research Mij N.V. (B.P. 1,054,068, 14.9.64).—The title compounds are obtained by reacting $R^{I}R^{II}P(X) \cdot Y$, where R^{I} and R^{II} are alkyl or alkoxy, X is O and Y is Cl or Br, with the appropriate benzofurazan in presence of alkali or alkaline earth metal carbonate or e.g. Et_3N. Thus, 4-chloro-5 or 7-nitrobenzofuran, is obtained from 4-chlorobenzofuran in conc. H_2SO_4 and conc. HNO_3 and the purified solid is dissolved in Me_2CO for reaction with Na diethyl dithiophosphate

for 4 h at room temp. After filtration and evaporation of filtrate, the residual oil is dissolved in CH_2Cl_2, washed, dried and evaporated to give a residue that, after chromatography on SiO_2 gel, gives a 90% yield of a light amber oil, 4-diethoxyphosphinothioyl-thio-5 or 7-nitrobenzofurazan. The compounds are insecticides (tested against housefly, moth larvae, aphids, mustard beetles and spider mite). S. D. HUGGINS.

***N*-Acylhydroquinoline derivatives.** Monsanto Chemical Ltd. (Inventor: J. P. Brown) (B.P. 1,052,308, 25.8.64).—1-R-8-R^I-2,2,4-trialkyl-1,2-dihydroquinolines are claimed and are herbicides; R is acyl residue of a halo-aliphatic acid, R^I is aliphatic, aliphatic-oxy, aromatic, or aromatic-oxy substituent. E.g., a solution of 2,2,4,8-tetramethyl-1,2-dihydroquinoline and $PhNEt_2$ in benzene is added during 10 min. to a solution of $CH_2Cl{\cdot}COCl$ in benzene; after 4 days at room temp. the filtered liquor is evaporated, leaving oily 1-chloroacetyl-2,2,4,8-tetramethyl 1,2-dihydroquinoline in ~100% yield. F. R. BASFORD.

4-Alkylmercaptophenols. Allied Chemical Corp. (B.P. 1,051,110, 28.10.65. U.S., 5.11.64).—The claimed insecticides are prepared by reacting a dialkyl disulphide and BF_3 phenolate with Cl_2 at −10° or below, followed by hydrolysis of the product. Thus, Cl_2 gas is added to a stirred mixture of Me_2S_2 and BF_3 phenolate at −10 to −20°; the mixture is then allowed to reach room temp. when water is stirred in for 10 min., the temp. being 33–38°. CH_2Cl_2 is added and washed org. layer is distilled to give a 50% yield of *p*-$MeS{\cdot}C_6H_4OH$, b.p. 122°/3 mm Hg. S. D. HUGGINS.

Cyclopropanecarboxylic acid esters. Sumitomo Chemical Co. (B.P. 1,076,579, 2.12.64. Japan, 3, 5, 17 and 19.12.63, 18.3 and 14.4.64).—Those esters, which are active insecticides of low toxicity to warm blooded animals and to plants, are compounds $XNCH_2OC(O)CH(R)CHCH{:}CMe{\cdot}R^{III}$ where XN is a maleimide residue bearing optional substituents R^I and R^{II} in the ring (R^I and R^{II} are Me, Et, Pr, Pr^i; R^I can also be Ph with up to 2 Me or MeO groups); R is a CMe_2 group, which together with adjacent C atoms forms a cyclopropyl ring; R^{III} is Me or MeOC(O). They are obtained by esterifying a compound $XNCH_2OH$, such as *N*(hydroxymethyl)mono-Me-maleimide with the anhydride of an acid $HOOCCH(R)CHCH{:}CMeR^{III}$, e.g. chrysanthemic acid. The compound *N*(chrysanthemoxymethyl)-methyl-maleimide of n_D^{25} 1·5051, was tested as a 2% prep. in kerosene against houseflies. Mortality after 10 min. exposure was >90% (elapsed time 20 h). H. L. WHITEHEAD.

Extermination of insects. W. R. Grace & Co. (B.P. 1,050,858. 10.7.64. U.S., 12.7.63).—The insects, e.g. German cockroaches, are exposed to a mixture comprising ≤5 wt.-% of polyoxymethylene fibres ≥50 (<10) μ in length, and having a length : dia. ratio of >10 : 1, which are produced by polymerisation initiated by irradiation (Cf. B.P. 984,097) and at least one other finely-divided solid insecticidal material (particle size 2–10 μ), e.g., a SiO_2 aerogel (fluoridated SiO_2 having a F content of ≥10 wt.-%). J. M. JACOBS.

Insecticidal cultures of micro-organisms. Vsesoyuznyĭ Nauchno-Issledovatel'skiĭ Institut Zashchity Rasteniĭ (Inventors: O. I. Shvetzova, M. G. Gandman, E. R. Zurabova, N. P. Isakova, A. Ya. Leskova and N. S. Fedorinchik) (B.P. 1,056,292, 2.3.64).—*Bacillus cereus var. galleriae* is cultured in an initially sterile liquid medium, then the culture is freeze-dried (at −20° to −25°/0·01 mm), to give a powder which causes septicemia and paralysis of harmful pests. F. R. BASFORD.

Agricultural miticidal compositions containing chlorodiphenylazosulphide compounds. Nippon Soda K.K. (Inventors: K. Taniguchi, Y. Uebayashi, R. Sakimoto, K. Ishii and M. Asada) (B.P. 1,054,941, 7.8.63).—The active compounds have the formula $C_6H_{5-n}Cl_n{\cdot}S{\cdot}N_2{\cdot}C_6H_{5-m}Cl_m$ wherein *m* and *n* are 0–5 but $m+n$ is ≤1, e.g., $2,4\text{-}Cl_2{\cdot}C_6H_3{\cdot}S{\cdot}N_2Ph$. F. R. BASFORD.

3,5-Dihalo-4-alkylthioanilines, their derivatives and acaricidal compositions containing them. Farbenfabriken Bayer A.-G. (B.P. 1,053,690, 25.11.65. Ger., 31.12.64).—Compounds with acaricidal activity have the formula $1,3,5,4\text{-}NR^{II}R^{III}{\cdot}C_6H_2X_2{\cdot}SR^I$ wherein X is Cl or Br; R^I is optionally substituted aliphatic hydrocarbon radical of 1–12 C; R^{II} is H or alkyl of 1–5 C; and R^{III} is H or optionally substituted alkyl, alkenyl, acyl, or carbalkoxy, or $NR^{II}R^{III}$ is NCO. E.g., KCNS is added to a solution of $1,3,5\text{-}NH_2{\cdot}C_6H_3Cl_2$ in KBr-saturated MeOH (**I**), followed by a solution of Br_2 in **I** during 1·5 h at 3–10°. After a further 1 h the mixture is poured into water, the ppt is recrystallised from MeOH, to give 3,5-dichloro-4-thiocyanatoaniline, m.p. 167–168°. This is heated with NaOH and MeOH for 3 h at 110–120°, the mixture is then cooled, and filtered; the filtrate gives 3,5-dichloro-4-methylthioaniline, m.p. 125–126° (from CCl_4). F. R. BASFORD.

Substituted-*s*-tetrazines as herbicides. American Cyanamid Co. (B.P. 1,052,877, 26.11.63. U.S., 3.12.62).—The herbicides claimed 3-R-6-R^I-*s*-tetrazines (**I**) and -1,2-dihydro-derivatives thereof, wherein R is H, halogen, $NR^{II}R^{III}$, or SR^{IV} and R^I is SR^{IV} or $C_6H_{5-n}R^V_n$; R^{II}, R^{III} are H, alkyl of 1–4 C (which may contain OH or CO_2H), alkenyl of 2–4 C, aryl, or $NR^{II}R^{III}$ is a 3- to 6-membered heterocyclic radical; R^{IV} is alkyl of 1–4 C or alkenyl of 2–4 C; R^V is H, halo, OH, alkyl of 1–4 C, alkoxy, alkylthio, CO_2H, carbalkoxy, or NH_2 optionally substituted by alkyl of 1–4 C; and *n* is 1–3; but in the case of **I** then R is $NR^{II}R^{III}$ and R^I is SR^{IV}, and R^{II} and R^{III} are not both H, alkyl of 1–4 C, or alkenyl of 2–4 C. F. R. BASFORD.

Herbicidal compositions employing disubstituted tetrazines. American Cyanamid Co. (B.P. 1,052,876, 25.11.63. U.S., 3.12.62). —Active agents of compositions are *s*-tetrazines substituted in the 6-position by NRR^I and in the 3-position by SR^{III} or $NR^{II}R^{III}$ ($R\text{–}R^{III}$ are C_{1-4}-alkyl or C_{2-4}-alkenyl or $R\text{–}R^{II}$ are H). E.g., from a mixture of 3,6,di-(methylthio)-*s*-tetrazine, $NHMe_2$, and EtOH, heated in a sealed vessel at 70–75° for 7 h, there is obtained *6-dimethylamino-3-methylthio-s-tetrazine*, m.p. 37–38·5°. F. R. BASFORD.

Amino-azido-triazine derivatives. Deutsche Gold-u. Silber-Scheideanstalt (B.P. 1,054,073, 16.9.63. Ger., 15.9.62, 11 and 13.4 and 3.5.63).—Used as pesticides, herbicides and plant growth regulants, the title compounds are of the general formula $R^IR^{II}N{\cdot}A(N_3){\cdot}NR^{III}X$, where $R^I\text{–}R^{III}$ are H, alkyl; X is H, alkyl, $CONR^{IV}R^V$, $SO_2NR^{IV}R^V$, SO_2R^{VI}, $PO(NR^{IV}R^V)_2$, or $PS(NR^{IV}R^V)_2$ where $R^{IV}\text{–}R^V$ are H or alkyl and R^{VI} is alkyl; A is a triazinyl ring. E.g., 2,4-bis-isopropylamino-1,3,5-triazinyl-6-trimethylammonium chloride in water is added to NaN_3, with stirring to give a thick ppt. that crystallises immediately. After 1 h, the substance is filtered, washed and dried *in vacuo* giving 2,4-bis-isopropylamino-6-azido-1,3,5-triazine, m.p. 94–95°. S. D. HUGGINS.

Herbicidal asphalt emulsion. Esso Research and Engng Co. (B.P. 1,051,138, 13.9.63. U.S., 18.9.62).—Increased crop yields and the destruction of weeds are obtained by applying a film of an asphalt-in-water emulsion of a hydrochloride of a herbicide, such as 2-chloro-4-ethylamino-6-isopropylamino-1,3-5-triazine. The emulsions which are stable, and do not gel, are applied after seeding and maintain the moisture content of the seed bed at a desirable level. S. D. HUGGINS.

Sulphur-containing phenylureas and their use as herbicides. Shell Internationale Research Mij N.V. (B.P. 1,059,608, 23.9.65. U.S., 25.9.64).—The herbicides have the formula $NR^IR^{II}{\cdot}CO{\cdot}NRR^{IV}$ wherein R and R^I are H or C_{1-4}-alkyl or alkenyl; R^{II} is C_{1-4}-alkyl, alkenyl or alkoxy; and R^{IV} is Ph containing $(O)_mSR^{III}$ and optionally 1 halogen, or alkyl, alkoxy, mono- or poly-haloalkyl, alkyl- or dialkylamino, alkylamido or NH_2 (*m* is 0–2 and R^{III} is C_{1-12}-alkyl which may contain halogen, NO_2, CN, NH_2, alkoxy or C_{1-4}-alkylamino, dialkylamino of 2–8 C, alkylamido of 2–4 C, alkenyl or C_{2-4}-mono- or poly-haloalkenyl). Thus, a solution of $1,3,4\text{-}NH_2C_6H_3Cl{\cdot}SMe$ in EtOAc is added during 40 min. to EtOAc containing phosgene; the temp. rises to 38°. The cooled mixture is freed from solvent; the residue, in ether, is treated with $NHMe_2$, then after 15 min. the ppt. is recrystallised from benzene, to give 3-(m-*chloro*-p-*methylthiophenyl*)-1,1-*dimethylurea*, m.p. 127–129°. F. R. BASFORD.

Urea derivatives. Badische Anilin- u. Soda-Fabrik A.-G. (Inventors: W. Jentzsch and M. Seefelder) (B.P. 1,057,379, 20.5.64. Ger., 12.6.63).—A urea deriv. salt, $[R^INH{:}CH{\cdot}NR^{II}{\cdot}CO{\cdot}NR^{III}R^{IV}]Cl^-$ in which R^I is alkyl, cycloalkyl, aryl or aralkyl, R^{II} is H, alkoxy, dialkylamino, dibenzylamino or as R^I, R^{III} and R^{IV} are H or as R^I (but R^{II}, R^{III} and R^{IV} cannot all be aryl radicals) and any two of R^{II}, R^{III} and R^{IV} may form a heterocyclic ring, are prepared by the reaction of a *N*-monosubstituted acid amide R^INHCO with eg. $SOCl_2$ and with an urea $R^INHCONR^{III}R^{IV}$. The corresponding free urea deriv. $R^IN{:}CHNR^{II}CONR^{III}R^{IV}$ is obtained by treating the salt with alkali. The products are useful as herbicides and as intermediates in synthesis. J. M. JACOBS.

Carbamates and herbicidal compositions containing them. Union Carbide Corp., Assee. of R. A. Herrett and R. V. Berthold (B.P. 1,055,721, 16.3.64. U.S., 2.4.63).—A benzyl *N*-methylcarbamate in which the Ph is substituted by ≤1 halogen and/or NO_2 has herbicidal properties. An example is 3,4-dichlorobenzyl *N*-methylcarbamate, m.p. 53–54°, b.p. 139°/0·5 mm, obtained in 83% yield by keeping a mixture of $3,4\text{-}C_6H_3Cl_2{\cdot}CH_2OH$ (prep. described),

MeNCO, and ether in presence of 1 drop of $SnBu_2(AcO)_2$ for 48 h, then distilling. F. R. BASFORD.

Substituted pyridines. Imperial Chemical Industries Ltd. (Inventors: C. D. S. Tomlin, J. W. Slater, D. Hatley and C. J. Clayton) (B.P. 1,059,990, 18.12.64 and 19.8.65).—Compounds having activity against unwanted vegetation and helminths (especially liver fluke in animals) comprise tetrahalo (chloro or fluoro)-4-mercaptopyridines and salts thereof. E.g. H_2S is passed through a solution of KOH in EtOH at 0°, then a solution of pentachloropyridine in EtOH is added. After 5 h at 50° while continuing introduction of H_2S, the mixture is poured into water and acidified with aq. HCl. The ppt. comprises tetrachloro-4-mercaptopyridine, m.p. 165–166° (acetone). F. R. BASFORD.

Bipyridylium quaternary compounds. Imperial Chemical Industries Ltd. (Inventor: J. T. Braunholz) (B.P. 1,054,397, 1.10.62).—The title compounds have herbicidal properties and are prepared, e.g., by reacting a bipyridylium monoalkyl quaternary salt with an alkylene dihalide. E.g., a solution of trimethylene dibromide in $OBu[CH_2]_2 \cdot O[CH_2]_2 \cdot OH$ (**I**) is added during 45 min. to a boiling solution of 1-methyl-4-(pyrid-4′-yl)-pyridinium chloride in **I**, then after a further 1 h the mixture is filtered at 80°. Ppt. is recrystallised from aq. EtOH to give trimethylene bis[4-(1-methylpyrid-4-yl)-pyridinium] chloride tribromide dihydrate, m.p. 288–289° (decomp.). F. R. BASFORD.

Herbicidal composition. D. O. Guth (B.P. 1,055,620, 18.12.64).—There is claimed a storage-stable, non-caking, herbicidal composition comprising a particulate mixture of anhyd. Li 2,4-dichlorophenoxyacetate and the hydrated salt, the mixture containing 1·8–10·2% of water of hydration. F. R. BASFORD.

Herbicidal and/or defoliant compositions. H. Schwartz (B.P. 1,059,468, 7.7.64. U.S., 9.7.63).—The active agents have the formula $R \cdot CO \cdot NH \cdot C_6H_4CF_3$ wherein R is H, optionally halogenated aliphatic radical of 1–10 C in which the longest chain has 6 C, aryl, or aryl-C_{1-6}-alkyl; further substituents (e.g. NO_2) in R may be present. Examples are *m*-$CF_3 \cdot C_6H_4 \cdot NH \cdot COEt$, m.p. 70–71° (active against Johnson grass but not tomato plants or cotton) and many other tabulated compounds. F. R. BASFORD.

Oil-soluble tertiary amine salts. Armour & Co., Assee of B. E. Bartrum (B.P. 1,076,144, 8.4.64).—The salt is derived from an oil-soluble aliphatic t-amine and a 3,6,7-oxabicyclo(2.2.1) heptane-2,3-dicarboxylic acid (**I**), and has one C_{12}-C_{22} organic radical affixed to the N-atom. The salts, e.g., dimethyldodecyl t-amino salt of **I** are useful as plant growth regulators, herbicides and tree-killing agents. E. ENOS-JONES.

Hydroxybenzonitrile glucamine salts and herbicidal compositions containing them. May & Baker Ltd. (Inventors: B. J. Heywood, W. G. Leeds and R. F. Collins) (B.P. 1,058,609, 26.5.64).—Salts of $1,3,5,4\text{-}CN \cdot C_6H_2X^IX^{II} \cdot OH$ (**I**) (selective herbicides, wherein X^I and X^{II} are Br or I) and $NR^IR^{II} \cdot [CH(OH)]_4 \cdot CH_2OH$ (R^I and R^{II} are H, Me, Et, or **hydroxy**ethyl or -propyl) are very sol. in water and render prep. of aq. concentrates of 40% concn. very simple. E.g., a mixture of **I** ($X^I = X^{II} = I$) (40), *N*-methyl-glucamine (23·2 g), and water (to 100 cc) is shaken, then filtered, to give a clear solution, pH 8, which at 0° gels without separation of solid. F. R. BASFORD.

Herbicidal 4-(methylsulphonyl)-2,6-dinitro-*N*,*N*-substituted anilines. Shell Internationale Research Mij N.V. (B.P. 1,056,199, 11.11.65. U.S., 13.11.64).—The herbicides, e.g. 4-*methylsulphonyl*-2,6-*dinitro*-N,N-*dipropylaniline*, m.p. 150–151°, are produced by nitrating $4,3\text{-}Cl \cdot NO_2C_6H_3SO_2Me$ (optionally substituted in 2-position by Me) with fuming H_2SO_4 and conc. HNO_3 and reacting the product, e.g., $4,3,5\text{-}Cl \cdot (NO_2)_2C_6H_2SO_2Me$, with an alkyl- or alkenyl-amine NHR^IR^{II} (where R^I and R^{II} are alkyl or alkenyl $\geq$3C or together form an alkylene radical). E. ENOS JONES.

Control of undesired vegetation. Dow Chemical Co. (Inventor: M. J. Josephs) (B.P. 1,060,439, 27.8.64).—A method of regulating growth and increasing sugar content of plants or parts thereof comprises exposure to R_3PO, R_3PS, $R^1{}_3S$, or $R_2(OR^{II})PO$ (R is ethyleneimino; R^1 is 2-Me-ethyleneimino; R^{II} is low-mol. alkyl). Thus, a mixture of R_3PO, fuller's earth, Nacconol NR, and Daxad No. 27 is dispersed in water, to form a composition which applied to soil at the rate of 20 lb of R_3PO per acre is highly lethal to Japanese millet, wild oats, Sudan grass and meadow fescue grown subsequently therein. F. R. BASFORD.

Oxazinone derivatives. E. I. du Pont de Nemours and Co. (B.P. 1,060,741, 26.2.64. U.S., 27.2.63 and 6.2.64).—Herbicidal activity is claimed for the products of reaction of a substituted β-aminocrotonamide, $R^3 \cdot C(NH_2):CR^2 \cdot CO \cdot NHR^1$ (R^1 is H, aliphatic or aromatic group or furfuryl group, R^2 is H, Cl, Br, Me, Et, Pr^n, Pr^i, Bu^n, Bu^s, Bu^t, MeO, EtO, Pr^nO, Pr^iO, Bu^nO, Bu^sO or Bu^tO and R^3 is alkyl, Cl-alkyl or Br-alkyl) with $COCl_2$ at 40–100° for 30 min.–6 h. Thus, β-amino-*N*-s-butylcrotonamide is reacted with $COCl_2$ in tetrahydrofuran, to give a ppt. as the temp. rises to 45°. The mixture is refluxed for 2 h, cooled and filtered to give 6-s-butylimino-3,6-dihydro-4-methyl-2*H*-1,3-oxazin-2-one hydrochloride, m.p. 151·5–162·5° (with decomp.). S. D. HUGGINS.

Herbicidal cyclopropylformamide and cyclobutylformamide compounds. Chemical Investors S.A. (Inventors: R. P. Neighbors and T. R. Hopkins) (B.P. 1,061,718, 14.11.63).—Compounds (**I**) with herbicidal and fungicidal activity of the general formula $3,4,1\text{-}C_6H_3Y_2 \cdot NH \cdot CXR$ wherein R is **cyclo**propyl or -butyl; X is O or S; Y is H, Cl, F, or Me (at least one being Cl or F) are prepared by reacting, e.g., cyclopropanecarbonyl chloride in benzene solution with $1,3,4\text{-}NH_2 \cdot C_6H_3Cl_2$ and pyridine, then after 2 h at room temp. the filtered solution is diluted with hexane and cooled to 0° to give cyclopropane-3,4-dichlorocarbanilide, m.p. 129·5–130° (from EtOH). The cyclobutane compounds are prepared similarly. The effect of a number of **I** on oats, wheat, peas, radish, flax, millet, alfalfa, tomato, sugar-beet, brome, corn, coxcomb, cotton, crab-grass, and soyabean is tabulated. F. R. BASFORD.

Herbicidal compositions. May & Baker Ltd. (Inventors: K. Carpenter, B. J. Heywood, E. W. Parnell, J. Metivier and R. Boesch) (B.P. 1,052,881, 5.3.63. Addn. to B.P. 1,040,541).—Alkali metal, alkaline-earth metal, NH_4, and amine salts of herbicidal compounds of B.P. 1,040,541 are claimed and are equally active. E.g., $p\text{-}NH_2 \cdot C_6H_4SO_2NH \cdot CO_2Me$ (**I**) is added to aq. K_2CO_3 at 25°, then the mixture is filtered through Hyflo; the filtrate is stirred with KCl for 30 min., to give the K salt of **I**. F. R. BASFORD.

Animal Husbandry

Metabolisable energy of the anatomical parts and other fractions of barley and the effect of enzymes and water treatment. E. J. Novacek and C. F. Petersen (*Poult. Sci.*, 1967, **46**, 1008–1015).—The husk, pericarp, germ, and aleurone, but not the endosperm, of barley showed increased metabolisable energy (*ME*) when treated with water or enzymes. However, the endosperm without treatment had a *ME* value equal to that predicted for treated endosperm and for water- and enzyme-treated by-products from which the endosperm was obtained. *ME* values calculated from component parts were compared with those obtained by chick bio-assay. A. H. CORNFIELD.

Rapid test for nitrogen dioxide in silage gases. S. T. Dexter (*Agron. J.*, 1967, **59**, 483–484).—The presence of NO_2, which is sometimes formed during silage fermentation, could be detected by exposing starch–iodide paper to the air in the silo or silo chute. In the presence of moderate concn. of NO_2 the paper turns blue-black almost instantly, whilst with very low concn. the colour change is slower. A. H. CORNFIELD.

Evaluation of an improved artificial rumen technique for the study of rumen fermentation. T. W. Griffiths (*J. agric. Sci., Camb.*, 1967, **69**, 355–366).—An apparatus of the permeable type incorporating multiple fermentation units and a method of operation are described. The apparatus is best used in a comparative manner to measure treatment differences between individual fermentation units. The effects of a range of diets on rumen fermentation were investigated and the results compared with those from a rumen fistulated, non-lactating cow. Whilst correlation between treatment, differences in NH_3 and total volatile fatty acids was low, correlation coeff. for the mol. proportions of acetic, propionic and butyric acids were high and significant. M. LONG.

Use of chromic oxide incorporated in a feed to estimate faecal output in ruminants. M. K. Curran, J. D. Leaver and E. W. Weston (*Anim. Prod.*, 1967, **9**, 561–564).—The administration of Cr_2O_3 in the form of capsules tends to give recoveries of Cr_2O_3 significantly less than 100% with both cattle and sheep, whereas incorporation of Cr_2O_3 into cubed concentrates gives recoveries not significantly different from 100%. Administration of Cr_2O_3 by the second method is also considered preferable on the grounds of easier administration, fewer refusals and no risk of danger with careless workers. M. LONG.

Relationship of circulating glucose, ketones, and free fatty acids to milk production in Awassi ewes. J. H. Adler and E. Lotan (*J. agric. Sci. Camb.*, 1967, **69**, 349–354).—Increased milk production is associated with diminished blood-glucose (**I**) and increased blood serum-free fatty acids (**II**). A group of ewes showing blood-ketone

(III) levels of > 11% did not have higher values of II or lower values of I with increased milk production, whilst a group with levels of III < 11% did so. Ketonemia of the high-III group was associated with relatively low values of II. It is suggested that these ketones originate from caloric homeostasis rather than from depôt fat via II. M. LONG.

Nutrition of the dairy heifer. VI. Effect on milk production of the level of feeding during the last six months of pregnancy and the first eight weeks of lactation. W. H. Broster and V. J. Tuck (*J. agric. Sci., Camb.*, 1967, **69**, 465–477).—One group of Friesian heifers was offered herbage at a rate of 3·5 lb dry matter per 100 lb live wt. per day, of which they consumed 2·1 lb. Another group was offered 2·4 lb of which 1·8 lb was consumed. The rates of gross live wt. gain prior to calving were 2·2 and 1·7 lb/day respectively of which 0·89 and 0·49 lb were due to increases in dam live-wt. During the first 8 weeks after calving half the heifers of each group received 10 lb hay, 20 lb brewers grains and either 10 or 16 lb pellets per day. The pellets contained 17% crude protein and had a starch equiv. of 64. The higher level of precalving feeding caused a non-significant increase in yield during early lactation and significantly reduced the rate of decline in yield at mid-lactation. The higher level of feeding after calving caused significant increases of 6·2 lb/day in early lactation and reduced the rate of decline after peak yield. Solids-not-fat yield was increased by 173 lb and live wt. losses were reduced by 50 lb. A non-significant increase in milk fat yield was found. M. LONG.

Voluntary intake of low-protein diets by ruminants. I. Intake of food by cattle. II. Intake of food by sheep. R. C. Elliott (*J. agric. Sci., Camb.*, 1967, **69**, 375–382, 383–390).—I. The voluntary intakes of Rhodes-grass hay containing 3·4% crude protein by Africander and Mashona heifers are similar and increase as the levels of crude protein increase in the concentrates offered. However, with increasing amounts of concentrates offered intake of hay falls. Increasing allowances of dietary protein and of concentrate are generally connected with higher food intakes and digestible energy, although at low crude protein levels these appear to be depressed when liberal amounts of concentrate are fed. When diets which supply min. needs of protein for maintenance are fed the voluntary intake of digestible energy is also adequate for maintenance.

II. The behaviour of sheep corresponds to that of cattle. Cattle eat relatively more than sheep, although the response surfaces of food and digestible energy intakes for the two species to changes in protein and concentrate levels are different. Sheep need less protein and concentrate to reach max. intake levels and are less tolerant of high protein and concentrate levels. M. LONG.

Effect of ammonium acetate on the yield and composition of milk from heifers. M. Kay, T. Walker and G. McKiddie (*Anim. Prod.*, 1967, **9**, 482).—Where NH_4 acetate (I) was added to a cereal diet which had a fat-depressing effect on milk fat (II) a non-significant increase in II was found. Total rumen volatile fatty acids (*VFA*) and pH were unaffected, although the proportions of acetic and isobutyric acids were higher with acetate treatment. I, given with a silage–sugar beet pulp diet very slightly depressed II and total solids. Total *VFA* concn. was higher, although differences in fatty acid ratio occurred. M. LONG.

Relations between apparent digestibility of roughages in the rumen and lower gut of sheep, the volume of fluid in the rumen and voluntary feed intake. M. J. Ulyatt, K. L. Blaxter and I. McDonald (*Anim. Prod.*, 1967, **9**, 463–470).—With increasing amounts of the poorest hay given the proportion digested in the rumen fell and that distal to the rumen increased. Voluntary intakes of dry matter varied from 1·94 kg/day for dried grass to 1·28 for the poorest hay. The mean vol. of rumen fluid when feed was offered *ad lib* varied between 14·1 and 15·2 l regardless of feed type. The results support the view that sheep voluntarily consume roughages to achieve a constant fill of the rumen regardless of quality. Distention of the hind gut has little effect on voluntary intake. M. LONG.

Efficiency of utilisation of grass by lactating ewes. J. B. Owen D. A. R. Davies and W. J. Ridgman (*J. agric. Sci., Camb.*, 1967, **69**, 399–404).—A method for the estimation of comparative grazing intake using the administration of Cr_2O_3 and sampling the pasture with the min. of labour, is described. Efficiency of production of lamb up to 5 weeks of age is not associated with body-wt. of the ewe nor with wither height. It is negatively related to grass intake by the ewe and to some extent to ewe body-wt. gain during lactation. M. LONG.

Effect of source and level of dietary protein on the performance of in-lamb ewes. T. J. Forbes and J. J. Robinson (*Anim. Prod.*, 1967, **9**, 521–530).—Grass meal was compared with soya-bean meal as a source of protein for in-lamb ewes wintered indoors. Both were included at two levels, 90 g and 45 g digestible crude protein/day. The soya-bean diet resulted in a higher dry matter digestibility but level of intake had no effect. Digestibility of crude protein was not related either to source or to level of intake. Lamb birth wt. were not affected by source or level of dietary protein, whilst ewe gains were thus affected. Mean N retention was higher with the soya-bean diet. No correlation was found between N retention and lamb birth wt. M. LONG.

Retention of calcium, phosphorus, magnesium, sodium and potassium by the developing sheep foetus. A. C. Field and N. F. Suttle (*J. agric. Sci., Camb.*, 1967, **69**, 417–423).—Highly significant rectilinear relationships exist between foetal wet wt. and foetal age, although the regression coeff. are significantly less for triplets than for twins. Below 103 days the amount of fat in the foetus is too small to measure, thereafter fat increases with foetal age. Overall mean % (fat-free wet basis) of Ca, P, Mg, Na and K are 0·824, 0·521, 0·0258, 0·245 and 0·233 respectively. Ca, P and Mg concn. increase with foetal age whilst K concn. decreases. The deposition of minerals increases throughout gestation, being greatest for triplets and smallest for singles. The estimates of deposition of Ca, P and Mg given in a report of the Agricultural Research Council (1965) underestimate the requirements of Ca and P for ewes with twins at the later stages of gestation. M. LONG.

Artificial rearing of lambs on cold reconstituted whole milk and on milk substitute. R. V. Large and P. D. Penning (*J. agric. Sci., Camb.*, 1967, **69**, 405–409).—The use of cold milk poses only a few health problems and results in high growth rates with satisfactory feed conversion efficiencies. During the first 3 weeks both growth rate and food conversion efficiency of the substitute are similar to those obtained with full cream milk. M. LONG.

Soluble and structural components of lucerne and reed canary-grass clones in relation to acceptability to lambs. P. B. O'Donovan, R. F. Barnes, M. P. Plumlee, L. V. Packett, and G. O. Mott (*Agron. J.*, 1967 **59**, 478–481).—Samples of lucerne and two clones of reed canary-grass were analysed for sol. carbohydrates, sol. N, hexosans, pentosans, and crude lignin. Lucerne samples obtained from oesophageal-fistulated lambs contained significantly more carbohydrates than did either of the canary-grass clones. Lucerne was also consistently higher in sol. N, as % of total N, than was either clone. Despite differences in *in vivo* acceptability, there was no significant difference in chemical composition between the canary-grass clones. Lucerne was significantly lower in pentosans than was the canary-grass; it is considered that this may account for the better cellulose digestion in lucerne. A. H. CORNFIELD.

Comparison of the effects of white clover (*Trifolium repens*) and of perennial ryegrass (*Lolium perenne*) on fat composition and flavour of lamb. D. A. Cramer, R. A. Barton, F. B. Shorland and Z. Czochanska (*J. agric. Sci., Camb.*, 1967, **69**, 367–373).—The slaughter wt. of lambs fed white clover (*C*) was ~50% higher than that of lambs fed perennial ryegrass (*R*); the latter carcasses had more fat. The I values of subcutaneous fat of *C* were significantly higher than those of *R*. The extra fatness of *C* was consistent with greater production of volatile fatty acids in the rumen-reticulum. *R* produced significantly more shorter chain-saturated and C_{15}-branched-chain acids. The subcutaneous fat of this group contained more octadecadienic and octadecatrienic acids and the *longissimus dorsi* fat contained more stearic and oleic acids than that of *C*. The 12th rib chops of *C* had more flavour and odour than those from the *R* group. M. LONG.

Effect of pattern of daily feeding of pregnant sows on apparent digestibility. D. L. Frape and R. W. Hocken (*Anim. Prod.*, 1967, **9**, 547–552).—Trials conducted to measure the effects on ration digestibility of a pelleted compound fed once or twice per day (I), of a barley ration fed at a separate time to a concentrate ration (II) and reversal of II (III) showed that I, fed twice a day, as opposed to once gives a slight improvement in dry matter, gross energy, crude fibre and P digestibilities. II gives a slightly higher P digestibility than III. I twice a day is superior to the others with regard to dry matter and crude protein. M. LONG.

Utilisation of phytate-phosphorus by poultry. T. S. Nelson (*Poult. Sci.*, 1967, **46**, 862–871).—A review. A. H. CORNFIELD.

Importance of the source of isolated soya-bean protein in nutrition experiments. P. Vohra and F. H. Kratzer (*Poult. Sci.*, 1967, **46**, 1016–1017).—Different sources of isolated soya-bean protein differed considerably when supplied in purified diets, in the extent of wt. gains by poults. The changes in the extent of wt. gains due to addition of Zn and EDTA to diets containing different sources of

isolated soya-bean protein also varied considerably depending on the source of the protein. The data indicate the importance of using the same source of isolated soya-bean protein when comparing results obtained by different workers or when repeating experiments.
A. H. CORNFIELD.

Metabolisable energy content of durum wheat and wheat cereal using chicks. W. C. Lockhart, R. L. Bryant and D. W. Bolin (*Poult. Sci.*, 1967, **46**, 805–810).—Three methods of assay gave very similar results for metabolisable energy (*ME*) content of durum wheat and wheat cereal. Wheat had an *ME* content of 3,153 and 3,502 kcal per kg on a 90% and 100% dry-matter basis respectively, whilst wheat cereal (11·1% moisture, 12·7% protein) had an *ME* content of 3,400 kcal per kg. A. H. CORNFIELD.

Protein reserves and survival of cocks on a protein-free diet. H. Fisher and J. H. Ashley (*Poult. Sci.*, 1967, **46**, 991–994).—Chicks and adult cocks were fed either a normal diet or a high-protein, though balanced, diet for 4 weeks. After putting the birds on a protein-free diet both the young and old birds pre-fed the high protein diet showed less early mortality than did those pre-fed the normal diet. As mortality increased with time of depletion of protein so the difference due to initial protein level disappeared.
A. H. CORNFIELD.

Utilisation of calcium and phosphorus from soft rock phosphate by chicks. I. Motzok, D. Arthur and S. J. Slinger (*Poult. Sci.*, 1967, **46**, 985–991).—Max. growth and bone ash of chicks to 4 weeks of age was obtained when the diet contained about 1·05% Ca (0·2% as $CaCO_3$) and sufficient soft rock phosphate (containing < 1·5% F) to raise the inorg. P in the diet from 0·16% to 0·48%. Not more than 900 i.u. of vitamin D_3 per kg of diet was required for max. wt. gains. Even when sufficient soft rock phosphate was added to supply 1·12% dietary Ca, extra Ca (0·15% as $CaCO_3$) was required to obtain max. wt. gains. A. H. CORNFIELD.

Comparison of phosphorus assay techniques with chicks. II. Development of a calcium standard curve for monosodium phosphate. R. H. Harms, P. W. Waldroup and B. L. Damron (*Poult. Sci.*, 1967, **46**, 981–985).—Various levels of supplemental P (0–0·25% as NaH_2PO_4) were added to a degerminated maize-soya-bean meal diet with various Ca levels for each P level. Tibia ash and body wt. of chicks fed the diets for 21 days were used to determine the Ca requirement at each P level. The Ca requirement increased from 0·44% to 0·59% as total P level in the diet increased from 0·30% to 0·55%. Increasing the optimum P/Ca ratio by the addition of extra P to diets containing 0·29–0·53% Ca did not depress growth rate or tibia ash. A. H. CORNFIELD.

Availability of calcium from Mexican and Californian sesame meals. M. Cuca and M. L. Sunde (*Poult. Sci.*, 1967, **46**, 994–1002).—When soya-bean meal provided the major portion of protein in the diet of chicks to 4 weeks of age normal bone ash was obtained with 0·8% Ca in the diet. When Californian sesame meal supplied most of the protein 1·05% Ca, and when Mexican sesame meal was used 1·50% Ca respectively was required for normal bone ash. Low levels of Ca in sesame diets resulted in poor growth and abnormal feathering. Although sesame meal is high in Ca compared with other protein feedstuffs only 76% of its Ca in Californian meal and 53% in Mexican meal was available to the chick. A. H. CORNFIELD.

Dietary inorganic phosphorus effects on retention and deposition of ^{89}Sr. L. B. Colvin, C. R. Creger, M. N. A. Ansari, W. S. Allen and J. R. Couch (*Poult. Sci.*, 1967, **46**, 895–899).—The activity of ^{89}Sr injected into chicks receiving 1% dietary Ca increased in the bones, eyes and brain with level of dietary inorg. P (0·1–2·0%). Max. activity was found in bones of birds receiving 2% inorg. P 7–12 days following injection with Sr. The % of injected activity found in the excreta decreased with increasing level of inorg. P in the diet. A. H. CORNFIELD.

Effect of adding sugar or starch to the diet on biological responses of the chick to raw and autoclaved soya-bean meal. G. Dal Borgo, M. H. Pubols and J. McGinnis (*Poult. Sci.*, 1967, **46**, 885–889).—The growth response of chicks to autoclaving (5 lb per sq. in. for 30 min.) soya-bean meal was much higher when starch than when glucose or sucrose was used as the main source of carbohydrate. Chicks fed raw soya-bean meal had significantly lower pancreatic amylase than those fed autoclaved meal. Liver-glycogen was higher where starch than where glucose was supplied with either raw or autoclaved meal. A. H. CORNFIELD.

Coffee oil meal in diets for growing chicks. L. B. Carew, jun., H. Alvarez and O. M. Marin (*Poult. Sci.*, 1967, **46**, 930–935).—Addition of even 2·5% coffee oil meal to the diet of chicks reduced wt. gains, whilst 10% or higher levels in the diet resulted in marked toxicity effects. Autoclaving coffee meal reduced its mortality effects but had little effect in increasing its nutritional value.
A. H. CORNFIELD.

Linoleic acid requirement of chicks. D. T. Hopkins and M. C. Nesheim (*Poult. Sci.*, 1967, **46**, 872–881).—Chicks required 0·8–1·4% linoleic acid in their diets for normal growth. When the dams were depleted of linoleic acid chick response to the acid was greater than when the dams received safflower oil. Liver-linoleic acid fell to very low levels in chicks fed deficient diets, and 5,8,11-eicosatrienoic acid accumulated in the liver lipids. Deficiency of linoleic acid resulted in reduced growth rate, enlarged livers, and increased liver-fat. Oleic acid improved the growth rate of chicks fed linoleic acid-deficient diets, but did not prevent the other symptoms of linoleic acid deficiency. A. H. CORNFIELD.

Comparison of the growth-stimulating properties of Vigofac and streptomycin in broiler diets with and without fish meal. P. W. Waldroup, D. R. Landes, R. D. Kealy, D. E. Greene and E. L. Stephenson (*Poult. Sci.*, 1967, **46**, 974–976).—The addition of 0·2–2·5% Vigofac (a conc. extract from *Streptomyces* fermentations and maize germ meal) to maize-soya-bean meal diets increased wt. gains of chicks to 2 weeks of age. There was no response to Vigofac when the diets contained 4% fish meal, and wt. gains where fish meal was present were no better than where Vigofac was added in the absence of fish meal. The addition of streptomycin at levels equal to that supplied by Vigofac did not improve wt. gains of chicks whether or not fish meal was added to the diet.
A. H. CORNFIELD.

Effect of cycloserine on chick growth. B. E. Haskell and U. Wallnofer (*Poult. Sci.*, 1967, **46**, 977–980).—Cycloserine was added to chick diets at levels 1–100 times that which would be supplied by the presence of 30% linseed meal in the diet. Cycloserine had no effect on wt. gains or feed intake and did not produce any symptoms of vitamin B_6 deficiency. Cycloserine is therefore not the pyridoxine antagonist reported elsewhere in linseed meal.
A. H. CORNFIELD.

Interactions of dietary zinc and vitamin D in laying hens. D. K. Schisler and E. W. Kienholz (*Poult. Sci.*, 1967, **46**, 918–924).—Laying hens put onto a diet deficient in vitamin D showed greatly reduced egg production, decreased body size, reduced bone Zn% and increased liver-Zn%. Addition of 300 ppm Zn ($ZnCO_3$) to the diet did not eliminate these effects of vitamin D deficiency. Zn% in the yolk, gizzard, kidney, and femur was not affected by addition of Zn or vitamin D_3 (750 i.u.) to the diet. Bone- and liver-Zn% were increased by addition of Zn to the diet to a greater extent when vitamin-D was also added to the diet.
A. H. CORNFIELD.

Effect of *Eimeria tenella* infection on zinc absorption by chicks. D. E. Turk and J. F. Stephens (*Poult. Sci.*, 1967, **46**, 939–943).—In only one of three trials did a significant increase in Zn absorption (as measured by blood-Zn levels from orally administered ^{65}Zn) occur as a result of infecting the chicks with *Eimeria tenella*. This increase occurred during the first days following inoculation, and thereafter the pattern of absorption was similar for infected and uninfected birds. A. H. CORNFIELD.

Gross abnormalities in chicks fed amino-acid-deficient diets. J. O. Anderson and R. E. Wernick (*Poult. Sci.*, 1967, **46**, 856–860).—Chicks were fed to 2 weeks of age with diets containing about half the normal requirement of each of 16 NH_2-acids. Wt. gains were reduced by 50–60%. Abnormal feathering occurred when the ration was deficient in leucine, arginine, valine, isoleucine, tryptophan, or alanine + tyrosin. The types of abnormalities were similar with all the deficient NH_2-acids, but the extent of abnormalities differed. Reduced feather pigmentation occurred when diets deficient in phenylalanine and tyrosine were fed to coloured chicks.
A. H. CORNFIELD.

Mode of action of the growth-stimulating properties of antibiotics. B. E. March and J. Biely (*Poult. Sci.*, 1967, **46**, 831–838).—Chlortetracycline and oleandomycin separately or in combination, did not reduce the dietary requirements of chicks for riboflavin, pyridoxine, or folacin. Addition of antibiotics to diets sufficient or deficient in one or more B-complex vitamins indicated that growth stimulation does not necessarily result from an increase in vitamin availability due to enhanced bacterial synthesis in the intestine. Increasing absorptive capacity of the intestine is probably a more reasonable explanation of the 'vitamin-sparing' effect of antibiotics.
A. H. CORNFIELD.

Effect of amino-acid supplementation of low protein maize and grain sorghum diets on performance of egg production stock. J. W. Deaton and J. J. Quisenberry (*Poult. Sci.*, 1967, **46**, 924–929).—14% Protein diets based on either maize or grain sorghum were fed without and with supplementation with 11 NH_2-acids at levels which would make them equal to each other and to the positive control 16% protein maize–soyabean–fish meal diet. The maize diet was significantly better than the sorghum diet in egg production and feed efficiency with respect to egg production. Supplementing the sorghum diet with NH_2-acids up to the 16% protein control diet increased egg production and wt. and feed efficiency, but not up to the level of the control diet. Egg production of birds receiving the 14% protein maize diet was equal to those receiving the 16% protein control diet. A. H. CORNFIELD.

Influence of vitamin B_6 deficiency on serum components in mature female chickens. M. W. Attar, N. J. Gaghir and J. Asmar (*Poult. Sci.*, 1967, **46**, 838–843).—Vitamin B_6 deficiency, induced in mature female chickens by feeding a pyridoxine-deficient diet, resulted in loss in body wt. and reduced feed intake, cessation of egg production and reduction in serum-glutamic-oxalacetic transaminase activity. Pyridoxine deficiency did not affect serum-total N, but reduced serum-non-protein-N. The deficiency had no effect on albumin or total globulin levels, but reduced the γ-globulin fraction. A. H. CORNFIELD.

Effect of reserpine in the diet on adult male chickens. G. H. Arscott (*Poult. Sci.*, 1967, **46**, 1019–1021).—The addition of 1 or 7 ppm reserpine to the diet of adult male chickens for 16 weeks had no significant effect on semen vol. or on fertility or hatchability of eggs from hens inseminated with semen from the treated males. A. H. CORNFIELD.

Factors affecting growth response of turkeys administered œstradiol-17-monopalmitate. R. E. Moreng (*Poult. Sci.*, 1967, **46**, 910–918).—The greatest response to subcutaneous injection with œstradiol-17-monopalmitate (0·03 g) by both male and female turkeys occurred between 16 and 22 weeks of age for heavy-bodied birds and between 11 and 14 weeks of age for medium birds. A grower diet was superior to a high-energy finishing diet in giving a response to the injection treatment. A further injection treatment of B.B. Bronze turkeys at 24 weeks of age had no effect on wt. gains to 30 weeks of age. A. H. CORNFIELD.

Egg yolk pigmentation. III. Effect of origin and storage conditions of yellow maize on the utilisation of its xanthophyll. I. Bartov and S. Bornstein (*Poult. Sci.*, 1967, **46**, 796–805).—Maize stored under unfavourable conditions (in cans exposed to the sun during the day) showed a reduction in content of xanthophyll and carotenoid pigments, an increase in free fatty acids and a decrease in the rate of xanthophyll utilisation by laying hens. Although unfavourable storage of fresh, local and old, imported maize resulted in reduced xanthophyll contents in both, xanthophyll utilisation by hens was reduced only in the fresh maize. A. H. CORNFIELD.

Effect of physical factors on yolk mottling and albumen quality of eggs. C. D. Blackshear, M. R. Parkes and K. N. May (*Poult. Sci.*, 1967, **46**, 952–955).—Shaking eggs in an arc along their long axis for 5–30 min. increased yolk mottling and slightly reduced Haugh units. Spinning eggs along their long axis had little effect on mottling, but reduced Haugh units. Mottling increased and Haugh units decreased with increasing temp. of storage (4·4–38°) for 5 days. A. H. CORNFIELD.

Hatchability of fertile eggs from hens receiving a linoleic acid-deficient diet. C. C. Calvert (*Poult. Sci.*, 1967, **46**, 967–973).—Hatchability of fertile eggs from hens receiving linoleic acid-deficient diets was nearly zero, whilst birds receiving 3% linoleic acid in their diets produced eggs with 75% hatchability. Mortality of embryos during incubation, particularly in the 0–4 and 20–22 day periods, was much greater with deficient than with normal embryos. Most of the surviving embryos from linoleic acid-deficient hens were found in abnormal positions within the shell after 22 days of incubation. Extension of the incubation period to 29 days resulted in only 8% of the deficient embryos hatching. Deficient eggs were lower than normal eggs in wt., yolk%, dry matter and lipid content, and in linoleic and arachidonic acid content of the yolk lipid. A. H. CORNFIELD.

Some electrophoretic components of egg white and their effect on egg quality. E. A. Sauter, jun. (*Diss. Abstr.* B., 1967, **27**, 2282).—Relationships between the electrophoretic pattern of thick egg white and the interior quality of eggs, as measured by Haugh units, are examined. Sources of eggs were 20 hens producing eggs having ⩽90 Haugh units and 20 hens giving eggs with ⩾80 units. From each group, hens giving eggs which deteriorated rapidly in storage were separated from those whose eggs deteriorated slowly. The eggs were stored at 50°F and electrophoretic patterns were determined on fresh eggs and after monthly intervals during 5 months' storage. Protein fractions were determined by paper electrophoresis. Lysozyme (**I**) was the only fraction of the thick white closely associated with rate of deterioration of eggs. The **I** content of fresh eggs deteriorating slowly was 13–18% and that of eggs deteriorating rapidly was 10–13% of the total protein. Ovalbumin was separated into three fractions. The A_1 component, the largest constituent of the thick white, represented ~30% of the total protein; during storage this value showed a consistent but non-significant decrease. The A_2 and A_3 fractions also showed decreases but of smaller magnitude. The ovoglobulin–ovomucoid fraction, comprising 18–19% of the total protein, gave no distinct electrophoretic pattern. Conalbumin and the non-mobile fraction (16 and 5% respectively of the total protein) were stable during storage. A. G. POLLARD.

Laboratory tests with promising insecticides for control of adult and larval stable flies. G. A. Mount, J. B. Gahan and C. S. Lofgren (*J. econ. Ent.*, 1967, **60**, 1600–1602).—Many compounds (146) were evaluated against adult and 212 against larval *Stomoxys calcitrans*. The 19 most effective of these were tested as sprays in a wind tunnel and by addition to CSMA medium. Four had low oral toxicity to rats and mice. C. M. HARDWICK.

Controlling lice and chorioptic mange mites on dairy cattle. J. G. Matthysse, R. F. Pendleton, A. Padula and G. R. Nielsen (*J. econ. Ent.*, 1967, **60**, 1615–1623).—Conventional high-vol. sprays were compared with low-vol. sprays applied by an electric mist blower, using Ciodrin, diazinon, coumaphos and carbaryl. Ruelene and fenthion were also tested as pour-on treatments. (24 references.) C. M. HARDWICK.

Comparative efficiency of coumaphos applications on various body areas by brush-on or pour-on for the control of cattle grubs. A. R. Roth and W. M. Rogoff (*J. econ. Ent.*, 1967, **60**, 1754–1755).—The control animals had 9·2 grubs/head. Pour-on treatments gave 98% control. Brush-on treatment varied from 97% for the back and 46% for the belly. C. M. HARDWICK.

Control of lone star tick on cattle. R. O. Drummond, T. M. Whetstone and S. E. Ernst (*J. econ. Ent.*, 1967, **60**, 1735–1738).—Twenty compounds were applied to cattle as sprays and two also as pour-on treatments. Their effectiveness against *Amblyomma americanum* 1 day and 1, 2 and 3 weeks after is given. None was more effective than the standard 0·5% Toxaphene. C. M. HARDWICK.

Horn fly and face fly control on range cattle with aerial applications of ultra-low-volume malathion sprays. B. H. Kantack, W. L. Berndt and E. U. Balsbaugh, jun. (*J. econ. Ent.*, 1967, **60**, 1766–1767).—Four applications in 1965 and six in 1966 gave good control of *Haematobia irritans* and *Musca autumnalis*. Control was better when both cattle and pasture were sprayed. C. M. HARDWICK.

Sheep ectoparasite control. I. Insecticides and application methods for keds and biting lice. J. G. Matthysse (*J. econ. Ent.*, 1967, **60**, 1645–1650).—Of eight high-pressure sprays tested, DDT, malathion, dieldrin, ronnel, coumaphos or diazinon killed all *Melophagus ovinus*. Ronnel and diazinon were effective when applied with a sprinkler can together with dieldrin as a low-vol. spray. Control of keds lasted >1 year. Only DDT as a high-vol. spray and ronnel from a sprinkler can control all *Bovicola ovis*. (16 references.) C. M. HARDWICK.

Dimethoate residues in eggs and tissues of laying hens. M. Sherman and M. T. Y. Chang (*J. econ. Ent.*, 1967, **60**, 1552–1554).—Hens were given drinking water containing technical and emulsifiable dimethoate(30 ppm), for 59 weeks. Analytical prodecures are discussed. No residues were detected in eggs, liver or fat while on treatment or 1 month afterwards. Some residues were found in breast muscle for up to 7 days after treatment with technical but not emulsifiable dimethoate. C. M. HARDWICK.

Control of chicken body, shaft and wing lice on laying hens by self treatment with insecticide dusts and granules. R. A. Hoffman and B. F. Hogan (*J. econ. Ent.*, 1967, **60**, 1703–1705).—Of the seven toxicants tested, Zytron (*O*-2,4-dichlorophenyl *O*-methyl isopropylphosphoramidothioate) granules, and Imidan and carbophenothion dusts, applied to the litter eliminated lice within 10 days. Ronnel and bromophos granules reduced but did not eliminate lice. Zytron granules in dust baths were less effective. C. M. HARDWICK.

Ethyl-4-hydroxy-6,7-di-isobutoxy-3-quinolinecarboxylate (Buquinolate), a new broad spectrum coccidiostat. A. T. Engle, R. P. Humphrey and C. A. Johnson (*Poult. Sci.*, 1967, **46**, 810–818).—The addition of 0·00825–0·011% Buquinolate to the diet of chickens at various ages prevented mortality and reduced wt. gains in birds with severe and moderate coccidiosis caused by *Eimeria tenella*. 0·00275% Buquinolate in the feed controlled single infections of *E. acervulina* and *E. necatrix*, mixed infections of *E. brunetti* and *E. acervulina*, and mixed infections of five species of *Eimeria*, whilst 0·00825% permitted nearly normal growth and completely prevented mortality. A. H. CORNFIELD.

Furaltadone hydrochloride for the treatment of avian vibrionic hepatitis and chronic respiratory disease complex in chickens. T. H. Eleazar and B. W. Bierer (*Poult. Sci.*, 1967, **46**, 819–822).—The addition of 0·0264% furaltadone hydrochloride to drinking water for 7 days followed by 0·0132% for another 7 days increased wt. gains and reduced the incidence of air-sacculitis in broilers affected by chronic respiratory disease complex. Layers medicated for 21 days with 0·0264% of the drug in drinking water returned to egg production more quickly than did controls. The same treatment delayed sexual maturity of growing pullets but did not affect egg production or egg wt. subsequently. The same treatment given to 7-week-old pullets affected with avian vibrionic hepatitis resulted in more rapid remission of clinical symptoms than in control birds. A. H. CORNFIELD.

Evaluation of blackhead preventive drugs with regard to growth and feed efficiency of turkeys. R. L. Atkinson, J. W. Bradley, J. R. Couch and J. H. Quisenberry (*Poult. Sci.*, 1967, **46**, 1002–1008).—Addition of 0·015% 1.2-dimethyl-5-nitroimidazole to the diet of turkeys improved wt. gains and feed efficiency to 8 weeks of age. Addition of 0·01875% 4-nitrophenylarsonic acid and 0·0365% *p*-ureidobenzenearsonic acid had no effect on wt. gains or feed efficiency. Mortality was not affected by any of the drugs. A. H. CORNFIELD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Annual Rep. for 1966. Fed. Res. Stn for Cereal Processing, Detmold. [A] **I. Bakery.** (*Brot Gebäck*, 1967, **21**, 101–124). [B] **II. Milling.** (*Getreide Mehl*, 1967, **17**, 61–72).—[A] The report lists lectures delivered and research projects in progress, together with brief reports on completed studies, covering (*i*) flour and dough, (*ii*) bread investigations, (*iii*) fine and long-life baked goods, and (*iv*) starch. (39 references.)

[B] The report lists research projects in progress and includes brief reports on completed studies, covering (*i*) grain properties, (*ii*) durum wheat and macaroni goods, (*iii*) milling technique, and (*iv*) rice quality. The same references are included. E. C. APLING.

Detection and estimation of ochratoxin A in some cereal products. P. M. Scott and T. B. Hand (*J. Ass. off. analyt. Chem.*, 1967, **50**, 366–370).—Ochratoxin A, from *Aspergillus ochraceus*, in cereals is extracted with aq. MeOH and n-hexane and partitioned on a Celite column. It is then separated by TLC and determined by measurement of fluorescence under long and short wave u.v. radiation. The detection limit is ~25 μg per kg of cereal. A. A. ELDRIDGE.

Losses of vitamin during technological processing of oats. L. Tunger (*Getreide Mehl*, 1967, **17**, 55–59).—A survey of the thiamin (**I**) content of barley samples for 1963 and 1964, and of losses during flaking at a number of different plants, showed original contents of 0·66–1·16 (mean 0·83) mg per 100 g (dry basis) and processing losses varied from 5 to 65%. Kinetic studies of the effect of moisture content and time and temp. of heating on the destruction of **I** are reported, analysed and summarised graphically. Rate of destruction of vitamin increased with temp. ($Q_{10} = 1·8$) and also with moisture content (up to 11% moisture content only). The results show that by careful choice of time/temp., it should be possible to produce flaked oats containing ⩽0·6 mg **I** per 100 g (dry basis). (13 references.) E. C. APLING.

Evaluation of the modulus of elasticity of the wheat grain. L. Shelef and N. M. Mohsenin (*Cereal Chem.*, 1967, **44**, 392–402).—Studies of the mechanical properties of Seneca wheat grains subjected to uniaxial compression in an Instron table model testing machine with a 50-lb load cell, are reported. Loads were applied to whole grains by means of parallel plates, a smooth cylindrical indenter and a cylindrical indenter, and also core specimens (prepared by cutting off both ends of the grain) were loaded by parallel plates. At a constant rate of deformation all tests showed linear relation between load and deformation, partly recoverable and partly residual (up to a certain load), and then non-linear at higher loads. Calculated values of apparent modulus of elasticity for Seneca wheat of 9·1% moisture content ranged from $1·6 \times 10^3$ to $8·3 \times 10^5$ psi. (15 references.) E. C. APLING.

Influence of commercial processing on composition and properties of corn zein. J. A. Boundy, J. E. Turner, J. S. Wall and R. J. Dimler (*Cereal Chem.*, 1967, **44**, 281–287).—Chemical and physical properties of two commercial prep. of zein (**I**) were compared with those of **I** prepared in the laboratory by extraction of the grain with 70% ethanol, and the effects of treatment of native **I** with SO_2 or alkali were studied. Results show that commercial steeping in SO_2 solution cleaves S–S bonds, with disruption of the intramol. aggregation of the native **I** and possible formation of *S*-sulphocysteine residues (high S content, low electrophoretic mobility). Alkali-treated **I** contained less cystine and total S than the native protein, but showed similar electrophoretic mobility. (13 references.) E. C. APLING.

Studies of rice quality. R. Garcia Faure and J. M. Vallejo Acevedo (*Boln Inst. nac. Invest. agron., Madr.*, 1967, **26**, 217–231).—Laboratory results (protein content, alkali test, starch–iodine blue test, Amylograph, and cooking quality) are reported for white rice of varieties grown in Spain during 1963/4 (21 samples) and 1964/5 (24 samples). The range of results was found to be small and differences between seasons were generally greater than differences between varieties. Amylograph characteristics showed association with variety, but cooking quality was unrelated. (13 references.) E. C. APLING.

Effect of oxygen concentration on deteriorative mechanisms of rice during storage. T. Iwasika and T. Tani (*Cereal Chem.*, 1967, **44**, 233–237).—In two experiments Brown short-grain rice was stored (*i*) for 1 year under N_2, CO_2 or air at ambient temp. (33° to −2°, mean 14·7°) and (*ii*) for 200 days at 23° in CO_2, CO_2/air (4 : 1), CO_2/air (1 : 1), air, and air enriched to twice the normal content of O_2. Germination % was highest in rice stored in normal air, but fall in diastatic activity was unaffected by atm. composition. Storage in atm. low in O_2 led to a decrease in the acidity of the water-extract, to some production of alcohol and to a greater increase in reducing sugars during storage. Possible mechanisms involved are briefly discussed, and differences are explained as due to depression of normal respiration and production of alcohol by the anaerobic respiratory system proposed by Taylor (*Am. J. Bot.*, 1942, **29**, 721) at reduced levels of O_2. Evaporation of alcohol is suggested as an additional cause of wt. loss during storage of rice. (11 references.) E. C. APLING.

Reduction of microbial populations in flours incorporated into refrigerated foods. L. Wiseblatt (*Cereal Chem.*, 1967, **44**, 269–280).—The use of heat and/or propylene oxide (**I**) vapour, for the reduction of microbial population, using flours inoculated with *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus flavus* and *Escherichia coli*, is discussed. Effective reductions were achieved by heat treatment or with **I** followed by heat (45 min. at 130° or 1,000 ppm **I** followed by 20 min. at 130°) without loss of functional or organoleptic properties in the treated flour, apart from some reduction in diastatic activity. A simple analytical method for determination of residual **I** in flour is described, involving methanolic extraction, distillation of the extract, and colorimetric determination of **I** in the distillate by the method of Jones and Riddick. (*Analyt. Chem.*, 1957, **29**, 1214). (11 references.) E. C. APLING.

Protein alteration in flour damaged by ball-milling and roller-milling. B. L. D'Appolonia and K. A. Gilles (*Cereal Chem.*, 1967, **44**, 324–331).—Flour was overground to varying degrees by ball or roller mill treatment, and changes in the protein were studied by means of determinations of non-protein nitrogen (*NPN*), specific colour reactions, Sephadex column chromatography, gel electrophoresis and -SH group determinations. The major effects were observed in the water-sol. fraction of the protein in which increases in *NPN*, amino-groups and in the low mol. wt. fraction separated by gel filtration, paralleled the increasing severity of the milling treatment. The possibility of protein denaturation in highly damaged flour was indicated by a decrease in the proportion of the total flour N contained in the water-sol. fraction, but gel electrophoresis patterns were only slightly varied and there was no appreciable change in -SH content. (19 references.) E. C. APLING.

Determination of salt in self-raising flour. E. J. Malec and J. F. Conn (*Cereal Chem.*, 1967, **44**, 344–345).—A simple, rapid titrimetric (Mohr) procedure is described. The method is applicable in presence of $NaHCO_3$ and ortho- (but not pyro-) phosphate, and is suitable for control purposes in the mill. Blank values vary with flour type and amount of leavening agents; they must be determined on the flour containing all ingredients except salt.

E. C. APLING.

State of iron in flour, dough and bread. J. Leichter and M. A. Joslyn (*Cereal Chem.*, 1967, **44**, 346–352).—Determinations of available (extractable) Fe in three different types of bread, flour and dough, by the modified 2,2′-dipyridyl method are reported. Available Fe in flour or dough, whether enriched or not, was approx. 80% of the total Fe, but total and extractable Fe in bread were substantially identical. Investigation of the Fe-binding capacity of bread crumb and crust, gluten, starch and pentosans showed that Fe^{3+} forms complexes with bread more readily than does Fe^{2+} and that the Fe-binding capacity of flour components increased in the order pentosans < starch < gluten. The Fe in bread was found to be mainly in the ferric state. (16 references.)

E. C. APLING.

Recent investigations on wheat flour pentosans. H. Neukom, L. Providoli, H. Gremli and P. A. Hui (*Cereal Chem.*, 1967, **44**, 238–244).—Further investigation of fractions separated by chromatography of wheat flour pentosans (**I**) on DEAE-cellulose (Kundig, W., *et al.*, *Helv. chim. Acta*, 1961, **44**, 823) are reported. The prep. of highly purified α-L-arabinofuranosidase from Pectinol R-10 is described; this enzyme liberated arabinose from the arabinoxylan (Fraction 1), yielding an insoluble xylan. Glycoprotein 2 (Fraction 2) was degraded with Pronase (a protease from *Streptomyces griseus*), yielding an alcohol-insol. arabinoxylan (**II**) containing residual protein but no galactose, and an alcohol-sol. arabinogalactan (**III**) (70% galactose and 30% arabinose), containing residual protein but no xylose. Results indicate that in Fraction 2 the **II** and **III** portions are linked *via* a polypeptide bridge, and a hypothetical structure is proposed. A caffeic acid ester of starch was prepared as a model compound for study of oxidative gelation of flour **I**; no gelation was observed, but it is suggested that such compounds could serve as special antioxidants, or as protein or metal precipitants by complex formation. (20 references.)

E. C. APLING.

Isolation and chromatographic fractionation of hemicelluloses from wheat flour. E. W. Cole (*Cereal Chem.*, 1967, **44**, 411–415).—Hemicelluloses from the tailings fraction of wheat flour were dissolved in 0·5 N NaOH, purified by alcohol pptn, treatment with α-amylase and dialysis and fractionated on a column of DAEA-cellulose (borate form) to give five fractions: xylose, arabinose and glucose were found in the acid hydrolysates of all fractions and galactose in the hydrolysates of fractions 4 and 5. Fraction 5 alone contained protein in addition to the sugars. (11 references.)

E. C. APLING.

Amylograph *vs.* falling number values compared. B. E. Patterson and L. G. Grandall (*Cereal Sci. Today*, 1967, **12**, 332–335).—The effects on the Amylograph and the Hagberg falling no. values of progressive additions of barley malt to different wheat bread flours are illustrated by curvilinear graphs. Both methods show good reproducibility, the Hagberg method being more rapid. Although the two sets of readings are not rectilinearly correlated, data could probably be established for different wheat types that would enable the use of either method with equally satisfactory results.

P. S. ARUP.

Determination of barium and strontium in maize meal and bread flour by atomic absorption spectrometry after separation by ion exchange chromatography. A. Strasheim, F. W. E. Strelow and E. Norval (*Jl S. Afr. chem. Inst.*, 1967, **20**, 25–31).—The procedure is described in detail. The final eluate is evaporated to dryness and Ba and Sr separated from Ca etc. as in the case of bread flour. The extracted Ba and Sr as chlorides are dissolved in 0·5N-HCl (2 ml) and diluted to 10 ml with MeOH. For atomic absorption determination of Sr the solution is diluted 1 in 5, keeping HCl and MeOH concn. constant. Atomic absorption is determined with an C_2H_2–air flow at 5536Å for Ba and 4607Å for Sr. Reproducibility tests gave coeff. of variation of 10·5% for 12 determinations of Ba and 13·1% for 21 determinations of Sr. Recovery experiments gave 97 ± 5% for Ba (6 determinations) and 95 ± 9% for Sr (5 determinations) with additions of 1–2 ppm Ba and 0·5–1 ppm Sr.

J. I. M. JONES.

Polysaccharide components of soya-beans. G. O. Aspinall, R. Begbie and J. E. McKay (*Cereal Sci. Today*, 1967, **12**, 223, 226–228, 260–261).—Galactomannans, acidic polysaccharides (of the pectin type), and xylans were extracted from the defatted hulls with various solvents. Acidic polysaccharides and arabinogalactans were extracted from the defatted and deproteinised cotyledons. The composition of these substances and their hydrolysis products was investigated and partly elucidated. (14 references.)

P. S. ARUP.

Method for determining very low α-amylase activities. S. Winkler and G. Luckow (*Stärke*, 1967, **19**, 159–165).—In order to study the amylolytic effects of natural extracts, especially from yeast, a colorimetric method for measuring very low activities has been developed. 10 ml of sample or standard enzyme prep. is mixed with 30 ml of a 0·05% solution of pure potato starch and incubated at 23°. After 8 min., 2 ml samples are removed each min. and mixed with 5 drops of 0·01 N I_2 solution in a cuvette, for colour comparison in an amyloscope. The time to reach the standard colour is 10–20 min. A determination takes from 0·5 to 1 h and the average deviation for a standard enzyme solution at different concn. is ±3%.

J. B. WOOF.

Determination of α-amylase activity in cereals by paper capillarographic viscosity measurement ('PcV' method). M. Rohrlich, S. Winkler and W. Hitze (*Stärke*, 1967, **19**, 166–169).—The substrate used for the assay is a 0·4% solution of Na starch prepared by treating acid washed potato starch with alkali and drying the washed product. The enzyme for assay is dissolved in acetate buffer, pH 4·8, centrifuged and incubated with the substrate at 23°. After 8 min., strips of filter paper (14 × 2 cm) previously washed and dried under vacuum, are dipped and suspended in the solution, which rises by capillary action at a rate dependent on the η; after 4 min. the height is measured. A linear relationship exists between height and amount of cryst. enzyme added; an 18 mm rise is $\equiv 5 \times 10^{-2}$ μg/ml enzyme. Values are lower than those obtained by other methods but are obtained rapidly on very small quantities of material, even of low activity.

J. B. WOOF.

Starch-complexed protein. I. Spectrophotometric determination of free amino groups and total nitrogen. S. Rogols and J. E. Green (*Stärke*, 1967, **19**, 169–173).—Protein in starch (**I**) is measured by estimation of the α-amino groups present with ninhydrin. 0·5 g **I** is placed in an acid-washed flask with 10 ml water and sufficient 0·1% I_2 solution to give a blue colour. Ninhydrin in citrate buffer, pH 5·0, containing $SnCl_2$ is added and the flask placed in a boiling water bath for 45 min. The cooled mixture is diluted to 50 ml with Pr^nOH and flocculated **I** removed by centrifuging. The absorption of the supernatant is read at 570 mμ. A different standard curve is required for each type of starch, the N content being determined by Kjeldahl. (20 references.) (In English.)

J. B. WOOF.

Modern analytical methods for starch derivatives and hydrolysates. J. R. van der Bij (*Stärke*, 1967, **19**, 256–263) —A review of methods currently in use for determination of functional groupings in starch prep. and the sugar composition of hydrolysates. For the former, i.r. spectroscopy can replace some of the conventional methods like titration. The analysis of three types of starch hydrolysate, and of hydrolysates of plant gums by gas chromatography and by thin layer chromatography is discussed.

J. B. WOOF.

Relation of starch damage and related characteristics to kernel hardness in Australian wheat varieties. P. C. Williams (*Cereal Chem.*, 1967, **44**, 383–391).—Results of the particle size index (*PSI*) test for kernel hardness (cf. Symes, *Aust. J. agric. Res.*, 1965, **16**, 113) are shown to be closely related to the damaged starch (**I**) content of flour milled from the wheat by a standardised milling procedure, and to be associated with water absorption, diastatic activity, and gassing power. Regression formulae for the calculation of these factors from *PSI* index are presented, and it is shown that difference between predicted and calculated diastatic activity gives a measure of α-amylase activity. Results indicate that **I** from hard wheat is more susceptible to diastatic attack than is **I** from soft wheat flours, but there appears to be no relation between the distribution of **I** granule sizes within and between Australian varieties, and the **I** damage incurred during milling. (17 references.)

E. C. APLING.

Thermogravimetric behaviour of starches. B. Carrol and J. W. Liskowitz (*J. agric. Fd Chem.*, 1967, **15**, 701–703).—Results obtained by the method of Morita (*Analyt. Chem.*, 1956, **28**, 64) could not be related to any functional group or structural characteristic of starches, starch fractions, or modified starches; the results could only be used for purposes of identification.

P. S. ARUP.

New methods for specification and appraisal of raw materials for dough products. L. Capol (*Mitt. Geb. Lebensmittelunters. u. Hyg.*

1967, **57**, 453–458).—A review covering the evaluation of durum wheat and durum products (with special reference to the method of Gilles and Youngs), Swiss standards, ash content for semolinas and other milling products, and standards of cleanliness. P. S. ARUP.

Changes in flour proteins during dough mixing. C. C. Tsen (*Cereal Chem.*, 1967, **44**, 308–317).—Acetic acid (0·05 N) extracts of various flours and doughs were fractionated by gel filtration on a column of Bio-Gel P-150, yielding four major u.v.-absorbing components, representing primarily glutenins (**I**), gliadins, albumins and non-proteins. During mixing of dough or wet gluten the amount of **I** extracted increased without significant changes in the other components. The rate of increase of **I** was intensified by higher mixing speeds, and was greater with weak than with strong flours. The increase in **I** is presumed to arise by disaggregation of large protein aggregates in the nondispersible fraction of the protein, and possible mechanisms are discussed. Extracts of soft wheat flour contained more **I** than extracts from hard wheat flour, suggesting that the protein aggregates in soft flour are smaller in size and/or more liable to disaggregation than those in hard flours. (15 references.) E. C. APLING.

Importance of the dough rest period in the straight dough process. J. Höpfner (*Brot Gebäck*, 1967, **21**, 84–87).—The effect of variations in the period between dough-making and moulding on the vol., crumb structure, colour, texture and aroma of rye and wheaten breads is discussed. The differences are illustrated. (10 references.) E. C. APLING.

An electronic recording dough mixer. V. Measurement of energy used in a mixograph-type mixer. P. W. Voisey, H. Miller and M. Kloek (*Cereal Chem.*, 1967, **44**, 359–372).—The use of an electronic integrator to record the energy used to mix dough in a Mixograph is described, and the results obtained with two flour types are reported and discussed. In recording the rate of energy input, a development curve consisting of a single line is obtained which is considered to be easier to interpret than conventional mixing curves. (15 references.) E. C. APLING.

Study of gas production and retention in doughs with a modified Brabender Oven-Rise Recorder. C. J. Marek and W. Bushuk (*Cereal Chem.*, 1967, **44**, 300–307).—The Brabender Oven-Rise Recorder was used at a constant temp. of 30° to record a curve indicating the gas production (*GP*) and gas retention (*GR*) capacity of fermenting dough, and the effects of flour type and quantity of yeast, malt, sugar, NaCl and improvers added were studied. Increased yeast addition increased, and salt decreased, both *GP* and *GR* capacity. Malt slightly reduced production without affecting *GR*. Sugar had little effect on retention, but at low levels increased, and above 5% decreased, *GP*. High levels of KIO_3 decreased *GR*, but additions of ≯40 ppm of KIO_3 or $KBrO_3$, or increases in the time or rate of mixing increased *GR* capacity without affecting *GP*. *GP* was independent of flour strength, but *GR* increased with increasing strength. E. C. APLING.

The possible application of analytical methods for elucidation of faults in quality of bakery raw materials and finished bakery products. A. Rotsch (*Brot Gebäck*, 1967, **21**, 81–84).—A brief review of present and future possibilities. E. C. APLING.

Study of amylolytic activity under conditions resembling those in the baking process. K. Möttönen (*Getreide Mehl*, 1967, **17**, 53–55).—Preliminary studies are reported on the use of a modified falling no. apparatus. This provides a continuous record of the movement of the falling probe and of the temp. of the suspension immediately beneath the probe, which carries a thermocouple attached to its base. Doughs of Finnish whole rye meal were tested, dispersed in either water or 0·1 N HCl, after spontaneous fermentation at 25, 30, 35 and 40° for periods of 0–72 h. Falling no. results are tabulated, and representative probe movement–temp.–time profiles are plotted three-dimensionally and discussed. The results suggest that the method will prove useful in studying the effects of fermentation, dough temp., pH, etc. on amylolysis at baking temp. (13 references.) E. C. APLING.

Comparison of methods for determining lactic acid in bakery products. J.-M. Brümmer and U. Klempin (*Dt. LebensmittRdsch.*, 1968, **64**, 15–19).— A review of the methods available for analysing bread for lactic acid. Details are quoted for three methods (colorimetric, distillative and enzymic) found in practice to be the most suitable. When applied to rye bread, the three methods gave widely varying results; use of the distillative method is recommended. (91 references.) J. B. WOOF.

Optimisation of white layer cake formulations by a multiple-factor experimental design. L. T. Kissell (*Cereal Chem.*, 1967, **44**, 253–268).—The experimental design previously reported (*ibid.*, 1962, **39**, 16) is extended to study the effects of simultaneous variations in the proportions of seven ingredients in full-formula white layer cake. Treatment combinations (77) were baked to sample responses in terms of cake vol., shape and internal score; multiple regression analysis provided second-order equations for computing response surfaces. In the analysis of variance, significant effects on cake vol. were found for water, sugar, baking powder and flour ratios and for five interaction terms. Top contour was responsive to the water × sugar interaction, and internal score was affected by baking powder, egg albumin and flour ratios and several interactions. Several response surfaces are presented to show the range of variables in which superior cake qualities were indicated. Test baking generally confirmed the predicted cake performance. Examples show that no single optimum combination of ingredients exists, but the utility of a relatively small, controlled multiple-factor experimental design, for location of areas of satisfactory performance is demonstrated. (11 references.) E. C. APLING.

Rôle of emulsifiers in the incorporation of air into layer cake batter systems. J. C. Wootton, N. B. Howard, J. B. Martin, D. E. McOsker and J. Holme (*Cereal Chem.*, 1967, **44**, 333–343).—Microscopical and aeration studies of the effect of alpha-tending surface active lipids, particularly propylene glycol monostearate, lactoylated glyceride and 1-acetyl-3-monostearin, in high-ratio white cake batters and yellow cake batters are reported. Results are discussed in relation to measurements of the effects of these additives on the interfacial tension at an oil–water interface. The beneficial effect in cake batter systems is shown to arise under conditions where the α-additive forms strong interfacial films, which can be demonstrated microscopically. The amount of air which can be incorporated into the batter is shown to increase with the 'interfacial strength' of these films as estimated with the DuNuöy tensiometer. The incorporation of air demands the presence of sol. protein in the batter and the suggested mechanism is that a film of additive, crystallised in the α-polymeric form, reduces contact between the glyceride oil and the protein-stabilised foam of the batter. E. C. APLING.

Storage of raw materials in bread and biscuit factories. K. Weber (*Brot. Gebäck*, 1967, **21**, 88–92).—The advantages of bulk storage, automatic weighing and feeding systems are discussed; representative installations are described and are illustrated with layout diagrams and photographs. E. C. APLING.

Fermentation and Alcoholic Beverages

Simultaneous determination of volatile acids and sulphur dioxide in alcoholic beverages by microdiffusion. J. L. Owades and J. M. Dono (*J. Ass. off. analyt. Chem.*, 1967, **50**, 307–311).—By the use of a modified Conway cell the determination is rapid and requires only small samples. SO_2 may be eliminated by complexing it with a Hg salt in the cell. Recoveries were: acetic acid 92 to 106%, SO_2 90 to 107·4%. A. A. ELDRIDGE.

Use of atomic absorption spectrophotometry for the determination of copper in alcoholic products. D. H. Strunk and A. A. Andreasen (*J. Ass. off. analyt. Chem.*, 1967, **50**, 338–339).—The sample is aspirated directly into the burner of the spectrophotometer through a capillary tube. Results are precise and comparable with those obtained by the ZDBT method. A. A. ELDRIDGE.

Changes in redox potentials of wines treated with cation exchanger resins. II. J. Ganewa, N. Goranow and L. Litschew (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 180–184).—In comparison with untreated samples, treated wines (*ibid.* 1965, **15**, 135) showed no difference as regards changes in redox potential during maturation in bottles; owing to the increased acidity of the treated wines, and to their decreased content of heavy metals, they were less prone to oxidation. The treatment did not necessitate any increase in the use of SO_2. P. S. ARUP.

Examination and judgment of bentonites for treatment of wine. II. Determination of effectiveness on wine. W. Kain (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1967, **17**, 201–222).—Further to Part I (*ibid.*, 1967, **17**, 10) eight wines of different origins behaved similarly when treated with nine different samples of bentonite (**I**). The best criterion of stabilising efficiency was found to be the swelling capacity (in water) of **I**, those of high capacity being classed as 'very good', and those of lower capacity as 'fair'. Swelling vol. was 10–49 ml per 2 g of sample. The efficiency of **I** was improved by swelling in water for 15 min. before use. A good **I** should show a comparatively low capacity for

removal of the colour of red wine; this property was evaluated by the method of Wobisch and Schneyder, using the $CoSO_4$ standard. (30 references.) P. S. ARUP.

Determination of aldehydes in wines by the direct bisulphite method. J. F. Guymon and D. L. Wright (*J. Ass. off. analyt. Chem.*, 1967, **50**, 305–307).—Satisfactory collaborative results were obtained by using the method of Guymon and Crowell (*ibid.*, 1963, **46**, 276). The method determines aldehydes free or bound with bisulphites, but not acetals. A. A. ELDRIDGE.

Effect of acetaldehyde–sulphurous acid on the colorimetric determination of lactic acid. H. Rebelein (*Dt. LebensmittRdsch.*, 1968, **64**, 9–11).—Lactic acid (**I**) in wine can be measured by the method of Rebelein (*ibid.*, 1961, **57**, 36) in which it is adsorbed on an ion exchange resin, eluted and oxidised with ceric sulphate; MeCHO formed is measured colorimetrically. MeCHO present in the wine in combination with SO_2 acts as an acid and is adsorbed and eluted with **I**, so that a correction must be made as follows: 15 ml of column eluate is treated with 5 ml of 27% Na acetate, 2 ml of 1·55 N H_2SO_4, 50 ml of Na nitroprusside and 5 ml of 10% piperidine. The extinction of the resulting violet solution gives the apparent '**I** value' by reference to a calibration curve. This value multiplied by 0·25 is then subtracted from the total **I** determined in the original method. J. B. WOOF.

Evaluation of gas-liquid chromatography for the determination of fusel oil in distilled spirits. R. L. Brunelle (*J. Ass. off. analyt. Chem.*, 1967, **50**, 322–329).—By means of the GLC procedure described results at least as accurate as those afforded by the A.O.A.C. method (*Official Methods of Analysis*, 10th ed., 1965, 9·037, 9·040) were obtained. In the 0–10 g per 100 l range results were reproducible to ±0·03 g per 100 l. Columns packed with 30% Carbowax 1500 or 1-docosanol on Chromosorb W were used. A. A. ELDRIDGE.

Use of gas chromatography in distilling research and control. G. J. de Becze, H. F. Smith and T. E. Vaughn (*J. Ass. off. analyt. Chem.*, 1967, **50**, 311–319).—Details are given of the construction and contents of four chromatographic columns used (*a*) to measure the broadest spectrum of volatile trace components, (*b*) to determine acetaldehyde and methanol, (*c*) for gin samples and fractions, and (*d*) to separate ethyl formate from acetone. In most cases the presence of 0·1 g per 100 l could be detected. The ratios of individual components are often characteristic of a given product. A. A. ELDRIDGE.

Modified assay for α-amylase in germinating barley. D. E. Briggs (*J. Inst. Brew.*, 1967, **73**, 361–370).—The activity of α-amylase (**I**) is defined as the reciprocal of the time taken by a heat-treated malt extract to reduce the iodine-colouring capacity of a solution of sol. starch to half its initial value, under standardised conditions. Reasons are given for preferring starch–iodine colour to reducing power measurements for following enzyme activity. **I** is extracted from grain and contaminating enzymes are largely inactivated by heating a pulverised sample in a solution of Ca acetate. Boiled extracts of coloured malt make solutions of **I** less stable to heat. Precautions taken to achieve accurate sampling of enzyme digests and in measuring the starch–iodine colour improve the precision of the method. Results are calculated graphically using standard graph principles. (49 references.) S. A. BROOKS.

Proteolytic activity based on a malt flour substrate interaction. P. R. Witt, jun. and E. A. Tousignant (*Cereal Chem.*, 1967, **44**, 403–410).—A procedure for the estimation of total proteolytic activity of malt is described. Finely-ground brewers' malt (50 to 100 mg) is extracted for 5 min. at 40° with 2 ml of cysteine–versenate–phosphate buffer (Weissler and Garza, *Proc. Am. Soc. Brew. Chem.*, 1965, **225**) and incubated for 15 to 30 min. at 40° with 1% casein solution (5 ml; pH 6·0). Proteolysis is then arrested with CCl_3CO_2H (3 ml) and liberated tyrosine (**I**) is determined in the filtrate by spectrometry at 280 nm. The reaction is linear for digestion periods of 15–30 min., and the unit of activity is defined as that quantity of enzyme which liberates 10 μg of **I** per ml (of final filtrate) in 15 min. Results obtained for a series of differently processed malts are reported. (14 references.) E. C. APLING.

Malt peptidase activity. M. Jones and J. S. Pierce (*J. Inst. Brew.*, 1967, **73**, 347–349).—Activity of individual mash tun peptidases has been estimated under varying pH conditions. Max. activity at 65° for 2 h lies between pH 4·3 and 4·6. In most cases this pH optimum is well defined with activity falling off rapidly on either side of these values. However, the pH optima for the production of glycine, threonine and proline are more diffuse. There is no significant indication that in a malt substrate a second pH optimum occurs in the alkaline pH range. S. A. BROOKS.

Malt peptidases. M. Jones and J. S. Pierce (*J. Inst. Brew.*, 1967, **73**, 349–351).—Malt contains some peptidases (**I**) which are inactivated by oxidation of the thiol groups in their mol. but only slightly activated by reducing agents. **I** with a trypsin type of specificity, restricted to the hydrolysis of basic bonds linking carbonyl groups of arginine, or lysine, to the amino groups of other amino-acids, do not appear to be significantly active under the defined brewery mashing conditions. There is evidence that some proline is produced by an enzyme system different to that producing other amino-acids. S. A. BROOKS.

Brewing liquor—a review. A. A. D. Comrie (*J. Inst. Brew.*, 1967, **73**, 335–341).—Of the ions present in brewing liquor, Ca^{2+} is one of the most important, in conjunction with PO_4^{3-}, because of its beneficial lowering of pH in the mash tun; HCO_3^- has the opposite effect. Na^+, Mg^{2+}, Cl^- and SO_4^{2-} are thought to have an important effect on flavour. Liquor treatment, involving prior removal of ions followed by addition of hardening salts, is discussed in detail with respect to modern plant operation. S. A. BROOKS.

Determination of coagulable nitrogen in wort and beer. C. Kremkow and A. Karst (*Mschr. Brau.*, 1967, **20**, 414–416).—The method of Esser, Mueller and Rolink has been compared with two modifications involving boiling with dithionite (*i*) in a salt or glycerine bath and (*ii*) by direct heating, in a series of interchange analyses carried out by the Sub-Committee for Quality Control of the Technical Scientific Committee of the Versuchs- u. Lehranstalt für Brauerei. Agreement between laboratories was best for modification (*i*); more coagulable N in wort (modification *ii*) and less in beer (modifications *i* and *ii*) were found than by the standard method. The original method is recommended because of its speed. J. B. WOOF.

Malt analysis and clarification of beer in the lager cellar. G. Krauss and C. Kremkow (*Mschr. Brau.*, 1967, **20**, 413–414).—Analytical values for fine grind extract, protein content and degree of degradation, turbidities of Congress wort and beers before and after filtration and lagering time from 43 routine brewery brews have been investigated, using a Siemens 2002 data processing system and an ALGOL programme previously described by Silbereisen and Kremkow. With an equation relating turbidity before filtration, sol. N of the malt, protein content of the malt (% dry wt.) and lagering time in days, the behaviour of beer in cellar could be predicted to within 32% . No relation could be found between turbidity of Congress wort and cellar clarification or between malt analysis and turbidity after filtration. J. B. WOOF.

Rapid gas chromatographic examination of beer flavour. D. R. Maule (*J. Inst. Brew.*, 1967, **73**, 351–361).—Gas chromatographic analysis of the headspace vapour of beer permits rapid measurement of the concn. of myrcene (**I**) and the principal volatile alcohols, esters and carbonyl compounds in beer. In some cases the results obtained can be correlated with differences in flavour associated with changes in brewing procedure. The strength of the hop aroma of beers which had been dry-hopped, or to which hop oil had been added, was broadly reflected by the content of **I**, which varied from 7 to 120 μg/l. A similar range was found in wort, the content depending on the conditions of boiling. However, the major part of this was lost during fermentation. (19 references.) S. A. BROOKS.

Continuous fermentation process. Brewing Patents Ltd. (Inventors: D. J. Millin and M. A. Pinnegar) (B.P. 1,079,517, 18.6 and 7.9.65).—Beer is obtained by maintaining a circulating body of yeast-containing liquor on one side of a porous partition and a moving body of wort on the other side, the pores of the partition being able to prevent the passage of yeast cells, while allowing the sol. materials to pass through. An advantage of the process is a reduction of loss by adsorption on the yeast of beer-flavouring substances derived from the hops. S. D. HUGGINS.

Fruits, Vegetables, etc.

Gas-liquid chromatography of fruit acids. E. Hautala (*J. Ass. off. analyt. Chem.*, 1967, **50**, 287–288).—Seven methyl esters of fruit acids were chromatographed. The benzoate appeared as a shoulder on the fumarate peak; the other esters gave separate peaks. Quant. results were not achieved. A. A. ELDRIDGE.

Translucency as index of ripeness in pineapples. R. P. Bowden (*Fd Technol. Aust.*, 1967, **19**, 424–425, 427).—An apparatus for translucency (*T*) measurements by means of a photoelectric cell, is described. Fresh slices of medium *T* achieved the highest quality scores. Low *T* was correlated with undue sourness and lack of

flavour and high *T* with flatness and over-ripe flavours. After slices from the same fruits had been canned in syrup, no significant differences were observed between the medium and low *T* samples, but the inferiority of the high *T* slices was still fully evident. The findings are supported by chemical analysis showing that *T* is correlated with acidity, pH, Brix/acid ratio and ester concn.
P. S. ARUP.

Stability of moist-pack apricots in storage. D. McG. McBean and J. J. Wallace (*C.S.I.R.O. Fd Preserv. Q.*, 1967, **27**, 29–35).—The fruits containing ~22% of moisture can be satisfactorily stored provided that their content of SO_2 is ⩽3000 ppm. Estimated storage life varies from 5 months at 30° to >1 year at 10°. Loss of moisture and SO_2 should be prevented by plastic foil wrappings. P. S. ARUP.

Technology of banana marketing. E. G. Hall (*C.S.I.R.O. Fd Preserv. Q.*, 1967, **27**, 36–42).—A review with special reference to conditions in Australia, problems of harvesting, transport, and the technology of ripening. P. S. ARUP.

Influence of nitrogen on quality, respiration and storage life of Washington navel oranges. R. A. de Fossard and F. H. Lenz (*Qualitas Pl. Mater. veg.*, 1967, **14**, 289–303).—Trees that did not receive $(NH_4)_2SO_4$ (**I**) produced fruit with the best quality juice but only gave ¼ total yield (by wt.) of trees receiving 2 kg **I** per annum. Higher levels of **I** did not increase yield or quality. Organoleptic tests favoured juice with highest total sol. solids (*TSS*)/acid ratio and lowest % titratable acid. Fruit stored at 0° had least fungal wastage but that at 15° had less rind breakdown (*RB*) and total wastage, the *RB* being specially evident at 7·5°; most oranges were affected irrespective of N treatment. Conditions arising at higher temp. are also discussed but respiration rate at 20° is similar irrespective of treatment, a climatic rise in CO_2 evolution not being noted. Yield, internal quality and storage life with the 2 kg concn. **I** treatment shows that this is superior to all the other variations.
C.V.

Isolation and identification of an antifungal active substance in walnuts. T. Ikekawa, E. L. Wang, M. Hamada, T. Takeuchi and H. Umezawa (*Chem. pharm. Bull., Tokyo*, 1967, **15**, 242–245).—The substance, identical with juglone (5-hydroxy-1,4-naphthoquinone) was isolated from *Juglans regia* Linn and *J. Sieboldeana* Maxim. It prevented growth of *Tricophyton mentagrophytes* and numerous other bacteria and fungi. C.V.

Properties of spray-dried combinations of milk and fruits and vegetables. W. M. Breen and S. T. Coulter (*J. Dairy Sci.*, 1967, **50**, 1049–1054).—Data on flavour preservation, shelf life, moisture contents and bulk densities are presented for products made by spray drying purées of 17 different fruits and 16 different vegetables alone, or combined with, skim milk. M. O'LEARY.

Pigment changes during maturation of tomato fruit. R. A. Edwards and F. H. Reuter (*Fd Technol. Aust.*, 1967, **19**, 352–357).—The principal change during the maturing of 11 varieties was an increase in the content of lycopene (**I**) and decreases in the chlorophylls and carotenes. The **I** content at maturity varied from 12·1 to 198·6 μg/g of fruit. The variety San Marzano showed the max. content of **I**; the contents of 21 pigments at six different stages of maturity of this variety are tabulated and considered.
P. S. ARUP.

[Analysis of] processed vegetable products. L. M. Beacham (*J. Ass. off. analyt. Chem.*, 1967, **50**, 689–690).—Investigations in progress under the auspices of the A.O.A.C. are briefly reviewed.
A. A. ELDRIDGE.

Determination of moisture in dried vegetables. B. R. Rader (*J. Ass. off. analyt. Chem.*, 1967, **50**, 701–703).—The Karl Fischer and near i.r. methods, using dimethylformamide for extraction of the water, are more specific than the vacuum oven method, being applicable to samples which lose volatile oils when heated.
A. A. ELDRIDGE.

Irradiation of potatoes in Pakistan. W. A. Farooqi, M. Mohyuddin and M. A. Hamid (*Fd Irrad.*, 1967, **7**, No. 3, 41–43).—With increase in radiation dose, loss of water content and incidence of storage rot were greatly reduced. With a radiation dose of 2 krad there was a stimulating effect upon sprouting. C.V.

Properties of cross-linked potato starch. K. F. Gotlieb and P. Woldendorp (*Stärke*, 1967, **19**, 263–271).—Potato starch is usually used after gelatinisation or some form of chemical treatment. The properties of gelatinised starch depend on the rate and extent of heating and agitation. Starch has been treated with increasing amounts of phosphorus oxychloride and the effects of degree of cross-linking on the behaviour of the starch during and after gelatinisation studied. The changes in temp. at which swelling begins, changes in the Brabender η during heating and cooling and the rheological properties after cooling have been followed.
J. B. WOOF.

Potato processing. M. J. Willard (B.P. 1,079,418, 20.7.65, U.S., 20.7.64).—A crisp snack food is prepared by keeping potatoes at a moisture of 20–40% for ⩽2 h, blanching, pressure cooking or otherwise weakening the potato cell wall bonds, then frying in hot fat. F. R. BASFORD.

Fried or partially fried potato products. Dow Chemical Co. (B.P. 1,079,628, 6.6 66. U.S., 9.6.65).—The potato pieces are treated prior to frying, with an aq. solution of water-sol. cellulose deriv. such as an hydroxy alkyl cellulose ether, at 50–95° for 0·1–5·0 min. This treatment, after French frying, gives superior colour, enhanced texture, higher moisture and reduced oil content. By appropriate choice of film-forming hydrocolloid and process conditions, the improved properties can be achieved individually or preferably concurrently. S. D. HUGGINS.

Non-alcoholic beverages

Methods for determination of oxygenated terpene, aldehyde and ester concentrations in aqueous citrus essences. J. A. Attaway, R. W. Wolford, M. H. Dougherty and G. J. Edwards (*J. agric. Fd Chem.*, 1967, **15**, 688–692).—Photometric determinations of (*a*) oxygenated terpenes (as $C_{10}H_{18}O$), (*b*) saturated aldehydes, and (*c*) unsaturated aldehydes, used colour reactions with: (*a*) vanillin dissolved in H_2SO_4, (*b*) H_2O_2 and *p*-phenylenediamine, and (*c*) *o*-dianisidine; the standard solutions for quant. comparison contained for (*a*) a mixture in equal pt. of citronellal, linalool, terpinen-4-ol, and α-terpineol, for (*b*) octanol, and for (*c*) citral; the solvent in each case was 80% EtOH. A standard procedure for the determination of esters as ethyl butyrate was adopted; the sample is treated with solutions of hydroxylamine hydrochloride and NaOH for 5 min., acidified, and then treated with aq. $FeCl_3$ for optical measurement at 525 mμ. Analyses of 15 citrus essences are tabulated. (12 references.) P. S. ARUP.

Paper chromatographic detection of adulteration in Concord grape juice. J. Fitelson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 293–299).—Anthocyanins, precipitated with Pb acetate, are hydrolysed with HCl and the anthocyanidins, dissolved in isoamyl alcohol, are subjected to ascending paper chromatography. The spots are eluted with ethanol containing HCl and oxalic acid, and extinction is measured at 445, 545 and 645 nm. The presence in Concord grape juice of the juice of other varieties of red grape or of grape skin extracts can thus be detected. A. A. ELDRIDGE.

Detection of adulterated Concord grape juice with other anthocyanin-containing products. L. R. Mattick, L. D. Weirs and W. B. Robinson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 299–303).—Paper chromatography is used to separate the acylated monoglycosides, monoglycosides, acylated diglycosides, diglycosides and sugars from aq. solution in that order. Densitometer tracings of Concord grape juice with, and without the addition of other grape pigments, are reproduced. An increase in the malvidin monoglucoside content of the juice indicates the presence of the juice of other grape varieties. A. A. ELDRIDGE.

Relation between refractive index, specific gravity and total solids of tomato juice, purée and paste. F. C. Lamb (*J. Ass. off. analyt. Chem.*, 1967, **50**, 690–700).—Relationships between total solids and (*a*) refractive index and (*b*) sp. gr. depart slightly from linearity. Regression equations have been computed for the regions 4–10, 10–20 and 20–40% of total solids. Comments on the precision of the known analytical methods used are offered.
A. A. ELDRIDGE.

[Coffee, etc.] Beverage brewing apparatus. Rudd-Melikian, Inc. (B.P. 1,079,384, 23.4.65. U.S., 24.4.64).—An apparatus for brewing beverages, e.g., coffee, by forcing hot water through a mass of beverage material is illustrated and claimed. F. R. BASFORD.

Milk, Dairy Products, Eggs

Effect of ion exchange resins on composition of milk and its fractions. G. K. Murthy (*J. Dairy Sci.*, 1967, **50**, 809–813).—Passage of milk through ion exchange resins to remove radionuclides was shown to increase the concn. of casein N and acid sol. P in the serum and to decrease the amount of sedimented casein.

There was also a decrease in the quantity of Ca and acid sol. P associated with the sedimented caseinates. (15 references.)
M. O'LEARY.

Pilot plant fixed-bed ion exchange resin system for removing iodine-131 and radiostrontium from milk. H. E. Walter, A. M. Sadler, D. G. Easterly and L. F. Edmonson (*J. Dairy Sci.*, 1967, **50,** 1221–1225).—A description is given of an ion exchange system in which ^{131}I is removed from milk by passage through an anion resin column; radiostrontium is subsequently removed by passage of the milk, acidified to pH 5·3, through the cation column. Over 93% of ^{131}I and 90% of ^{90}Sr were removed by this technique. Flavour of the resin-treated milk was rated by a trained panel as being slightly lower than untreated milk. At the end of an 8 h run a slight loss in milk fat % occurred because of adsorption by the anion resin. M. O'LEARY.

Triglyceride structure of cows' milk fat. I. Preliminary observations on the fatty acid composition of positions 1, 2, and 3. R. E. Pitas, J. Sampugna and R. G. Jensen (*J. Dairy Sci.*, 1967, **50,** 1332–1336).—Data obtained on the fatty acid composition of positions 1, 2, and 3 of milk fat triglyceride, using Brockerhoff's technique for stereospecific analysis are presented. (13 references.)
M. O'LEARY.

Fatty acid composition and flavour of autoxidised milk fat. K. G. Raghuveer and E. G. Hammond (*J. Dairy Sci.*, 1967, **50,** 1200–1205).—Typical flavours developed during autoxidation of milk fat were unaffected by deodorisation, randomisation, hydrolysis and resynthesis. This is considered to indicate that the flavour of autoxidised milk fat is dependent only on the fatty acid composition. A mixture of 1·5% linoleic, 0·5% linolenic, and 0·2% arachidonic acids in tridecanoin satisfactorily reproduced the flavour typical of the early stages of autoxidation. Incorporation of a mixture of polyunsaturated fatty acids, isolated from milk fat, into tridecanoin at the 3% level gave improved reproduction of the later stages of autoxidation. (17 references.) M. O'LEARY.

Pasteurisation treatment and consumer acceptance of milk. D. D. Deane, J. A. Chelesvig and W. R. Thomas (*J. Dairy Sci.*, 1967, **50,** 1216–1220).—Fifty randomly selected households were used to evaluate the flavour of homogenised, standardised whole milk pasteurised for 17 seconds at 72·2, 75·6, 78·9, 82·2, or 85·6°. Milk pasteurised at 78·9° was preferred by the group. Ability of panel members to note differences in flavour of milk appeared to increase with age of the panellist. Cooked flavour of the milk decreased on storage at 1·7–4·4°. (18 references.)
M. O'LEARY.

Effect of temperature on stability of hydrogen peroxide in milk. V. M. Amin and N. F. Olson (*J. Dairy Sci.*, 1967, **50,** 1336–1337).—Data showing the rate of decomposition of H_2O_2 in redistilled water, phosphate buffer, sterilised reconstituted non-fat dry milk, sterilised homogenised whole milk, and raw whole milk at 37·8, 48·9, 54·4, 57·2° are presented. The significance of the results for treatment of milk for destruction of coagulase-positive staphylococci is discussed. Little H_2O_2 is decomposed in the treatment time at 54·4 and 57·2° (0·9–6·9 and 0·6–6·3 min. respectively) and it would appear that 37·8° is not a satisfactory temp. for treating raw milk for cheese making since significant destruction of H_2O_2 would occur during treatment (51·7–140·3 min.), which would increase the duration of treatment even beyond that obtained in sterile milk. (10 references.) M. O'LEARY.

Hydrogen peroxide treatment of milk. E. J. Mann (*Dairy Inds*, 1967, **32,** 674–675).—A brief review of recent literature. (17 references.) C.V.

Effect of hydrogen peroxide on whey protein nitrogen value of heated skimmilk. N. L. Fish and R. Mickelsen (*J. Dairy Sci.*, 1967, **50,** 1045–1048).—Possible reasons for the reduction of whey protein nitrogen denaturation on heating by prior treatment with H_2O_2 are advanced, based on evidence obtained from disc electrophoresis patterns. (13 references.) M. O'LEARY.

Seasonal variation in deposit formation from whole milk on a heated surface. H. Burton (*J. Dairy Res.*, 1967, **34,** 137–143).—Experiments with bulk milk from two herds, using a hot wire laboratory apparatus, showed that in a 12 month period deposit formation during the period (Sept.–Apr.) was double that which occurred in May–June. The amount of deposit was positively correlated with fat content but not with the mineral or protein contents of the milk. (14 references.) M. O'LEARY.

Some effects of pyrophosphate and citrate ions upon the colloidal caseinate-phosphate micelles and ultrafiltrate of raw and heated skimmilk. G. V. Morr (*J. Dairy Sci.*, 1967, **50,** 1038–1044).—Heating of skimmilk containing added pyrophosphate (TSPP) (**I**) or citrate ions (**II**) caused substantial disintegration of the colloidal caseinate-phosphate micelles. Micelles of heated **I**-skimmilk contained greater proportions of Ca-phosphate (**III**) while micelles of heated **II**-skimmilk contained lower proportions of **III** than micelles sedimented from control skimmilk. Addition of **I** resulted in lower levels of ultrafilterable Ca and higher levels of ultrafiltrable inorg. phosphate whereas addition of **II** caused an increase in both ultrafiltrable Ca and inorg. phosphate as compared with control skimmilk. Sedimentation experiments showed that **I** disaggregated the colloidal caseinate-phosphate micelles from heated **I**-skimmilk more effectively than those of heated control skimmilk and had a much greater effect than that caused by the addition of oxalate. (26 references.) M. O'LEARY.

Kinetics of thermal destruction of bacteriophages active against *Streptococcus cremoris*. M. Koka and E. M. Mikolajcik (*J. Dairy Sci.*, 1967, **50,** 1025–1031).—Heat inactivation of two bacteriophage races (cl and rl), active against *S. cremoris* was determined. For cl phage a single linear heat destruction curve with D values at 60° of 14 min. in skimmilk and 3·7 min. in broth was obtained. For rl phage a nonlinear curve, capable of integration into two components with varying inactivation rates and having D values at 65° of 16 and 64 min. in skimmilk and 3·4 and 6·8 min. in broth, was obtained. The heat protective properties of skimmilk towards the second component of rl phage decreased with increase in temp. so that at 87° the rate of inactivation was the same in both skimmilk and broth. (18 references.) M. O'LEARY.

Acetaldehyde and diacetyl production by *Streptococcus thermophilus* and other lactic streptococci. V. Bottazzi and F. Dellaglio (*J. Dairy Res.*, 1967, **34,** 109–113).—Strains of *Streptococcus thermophilus* were shown to form more MeCHO and diacetyl in both skimmilk and MRS medium than other homofermentative lactic streptococci. The diacetyl : MeCHO ratio in skimmilk was 0·1 : 1 for *S. lactis* and *S. cremoris*, 0·5 : 1 for *S. diacetilactis* and 0·3 : 1 for *S. thermophilus*. (10 references.)
M. O'LEARY.

Relationships between Wisconsin mastitis test scores and cell counts in milk. D. Kroger and D. E. Jasper (*J. Dairy Sci.*, 1967, **50,** 1226–1233).—The relationship between Wisconsin mastitis test scores and cell content was determined on milk samples of quarter and tank origin over a 5-day period. Regression equations for milks of various ages are presented. M. O'LEARY.

Relationships between California mastitis test reaction and composition of milk from opposite quarters. U. S. Ashworth, T. L. Forster and L. O. Luedecke (*J. Dairy Sci.*, 1967, **50,** 1078–1082).—Tests with milk from opposite quarters showed that subclinical mastitis (*SCM*), as determined by the California Mastitis Test, caused the production of abnormal milk, the degree of abnormality being proportional to the severity of the test reaction. *SCM* caused a decrease in total solids, fat, nonfat solids, and lactose and an increase in chloride content and pH of the milk. (11 references.)
M. O'LEARY.

Identification of yellow material remaining on discs after filtration of milk. E. V. Caruolo and R. D. Mochrie (*J. Dairy Sci.*, 1967, **50,** 1170–1171).—The yellow material remaining on discs after filtration of pooled milk from five cows, demonstrating varying degrees of mastitis, was identified as being mainly β-carotene.
M. O'LEARY.

Estimation of aflatoxin M in milk. I. F. H. Purchase and M. Steyn (*J. Ass. off. analyt. Chem.*, 1967, **50,** 363–366).—Of the solvent mixtures tested acetone–chloroform–water (38 : 58 : 4) extracted most aflatoxin M from dried milk powder. A. A. ELDRIDGE

Rapid determination of protein in milk by dye binding. J. W. Sherbon (*J. Ass. off. analyt. Chem.*, 1967, **50,** 542–547).—A procedure based on Udy's method (*Nature, Lond.*, 1956, **178,** 314) has been used in collaborative studies. Results were subject to a smaller variation than were those obtained by the Kjeldahl method. The amount of dye bound by the milk did not correlate with the non-protein N content of the milk (about 5% of the N determined by the Kjeldahl method). A. A. ELDRIDGE.

Comparison of the reproducibilities of the Kjeldahl and dye binding methods for measuring protein in milk. J. W. Sherbon and B. Hemphill (*J. Ass. off. analyt. Chem.*, 1967, **50,** 557–560).—The Orange G dye binding method resulted in less variation between replicates of the same sample than did the Kjeldahl method, but, unlike the latter, the dye binding method gave results which were affected by the time elapsing between duplicate determinations.
A. A. ELDRIDGE.

Dye binding method for [determining] milk protein. H. A. Luke (*J. Ass. off. analyt. Chem.*, 1967, **50**, 560–564).—10 samples were examined in quadruplicate in seven laboratories. For each sample laboratory means were between 98·56 and 101·53% of the overall mean. Inter-laboratory coefficients of variation were 0·52 to 1·13%. A. A. ELDRIDGE.

Ultra-violet spectrophotometric determination of protein content in milk. M. Iwaida, Y. Kawaguchi and T. Tsugo (*J. Dairy Sci.*, 1967, **50**, 1322–1327).—A description is given of a technique for the u.v. spectrophotometric determination of protein content in milk. With individual Holstein milk, standard deviations from Kjeldahl figures of less than 0·02% were obtained. Much lower accuracy was obtained with Jersey milk due to the fluorescent effect of fat in the u.v. region. Preservatives such as $HgCl_2$, formalin, or $CHCl_3$ did not affect the results significantly. M. O'LEARY.

Rapid methods for determining copper content of milk. A. C. Smith (*J. Dairy Sci.*, 1967, **50**, 664–668).—Two rapid methods for determining the Cu content of milk are given. The conventional dry-ashing procedure (*DAP*) is replaced by pptn. with the use of zinc dibenzyldithiocarbamate which eliminates the need for pH adjustment. Both methods agree with *DAP*, and are repeatable. (16 references.) M. O'LEARY.

New substrate for [determining] alkaline phosphatase in milk. A. L. Babson and S. J. Greeley (*J. Ass. off. analyt. Chem.*, 1967, **50**, 555–557).—The red solution obtained when phenolphthalein monophosphate is hydrolysed and NaOH is added can be compared directly with a standard prepared from the same milk. A. A. ELDRIDGE.

Determination of the freezing point of milk by thermistor cryoscopy. R. W. Henningson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 533–537).—Detailed procedural directions, which incorporate the suggestions of the major manufacturers of thermistor cryoscopes are given. A. A. ELDRIDGE.

Isolation and detection of dioctyl phthalate from milk lipids. J. Cerbulis and J. S. Ard (*J. Ass. off. analyt. Chem.*, 1967, **50**, 646–650).—Dioctyl phthalate was found in the lipids from an individual sample of milk. The milk was dialysed and evaporated to dryness: a light petroleum extract of the dried milk was chromatographed on Al_2O_3, dioctyl phthalate being separated by TLC and identified by i.r. spectroscopy. The procedure is applicable to other natural lipids. The possible source of the contamination in the milk examined is discussed. A. A. ELDRIDGE.

Micro-measurement of milk fat. G. W. Molnar and N. F. Poole (*Microchem. J.*, 1967, **12**, 94–98).—Commercial homogenised cow's milk was centrifuged and % cream was measured with a microhematocrit reader. Milk fat % was determined by the Roese-Gottlieb method and by a modified Babcock method in which dil. H_2SO_4 (95%) was used to prevent charring; isopentyl alcohol was added to facilitate the rise of fat in the neck of the Babcock bottle. The Roese-Gottlieb method was more accurate. The relation of % cream (X) to % fat (Y) was shown to be $Y = 0{\cdot}008 + 0{\cdot}610\ X$. This relation was used to determine % fat from % cream in twelve samples of milk, with a standard deviation of $\pm 0{\cdot}027$, and in one tenth of the time taken for standard methods. Correlation factors must be determined independently for each different combination of globule dimension, centrifugation time and centrifugal force. G. W. FLINN.

Comparison of the modified Stamm, iron, and iodometric peroxide determinations on milk fat. D. L. Hamm and E. G. Hammond (*J. Dairy Sci.*, 1967, **50**, 1166–1168).—Milk fat samples, with peroxide values ranging from 0·46 to 20·3, were analysed by the modified Stamm, iron, and iodometric methods. Correlation of the corrected iron and Stamm values with the iodometric values was greater than 99·9%. M. O'LEARY.

Investigation of teat and milk piping. H. Ostromow and W. Hofmann (*Dt. LebensmittRdsch.*, 1967, **63**, 359–365).—Carbon black used in rubber manufacture contains varying amounts of benzene-extractable polycyclic and other hydrocarbons and deriv. which may be extracted into milk when the rubber is used in milking equipment. Methods of extracting and identifying these compounds in milk are described. For 3,4-benzpyrene, 1,2,5,6-dibenzanthracene and 20-methylcholanthrene, milk containing K oxalate is extracted with ethanol–ether (1 : 2) and analysed by chromatography on silica gel paper. A similar extract from milk can be analysed for diphenylguanidine, 2-mercaptobenzothiazole and *N*-phenyl-*N*-isopropyl-*p*-phenylenediamine by TLC on silica gel. Di-(2-ethylhexyl)phthalate is determined by GLC; di-iso-nonylphthalate is added as internal standard. The behaviour of a number of types of rubber is described. J. B. WOOF.

Nutritive value of milk and milk products. Water-soluble vitamins in milk and milk products. M. E. Gregory (*J. Dairy Res.*, 1967, **34**, 169–181).—Literature published from 1962 to 1966 dealing with water-sol. vitamins in cow's milk and milk products is extensively reviewed. (103 references.) M. O'LEARY.

Constant speed, fixed angle torsiometer for measuring coagulation of milk by rennet. J. C. Oosthuizen and J. Van der Staay (*S. Afr. J. agric. Sci.*, 1967, **9**, 1011–1017).—The apparatus consists essentially of a stainless steel cylinder that oscillates at a constant speed through a fixed angle round its vertical axis. Changes in the restraining drag of the curd are measured by means of strain gauges and automatically recorded. P. S. ARUP.

Proteolysis and liberation of free amino-acids by lactic acid bacteria in milk. II. Thermobacteria. I. Miller and O. Kandler (*Milchwissenschaft*, 1967, **22**, 469–480).—Determination was carried out by column chromatography using an amino-acid analyser for the dialysable fractions. Up to the point of max. acidification, there was considerable variation but thereafter, constant results were obtained and under similar conditions a strain provided a reproducible spectrum of free amino-acids. The species however gave constant proportionate ratios of the various amino-acids. *Lactobacillus bulgaricus* always shows more serine (**I**) than threonine (**II**) and *L. jogurti* shows a higher concn. of alanine (**III**), apart from glutamic acid (**IV**) and proline (**V**). A small amount of **IV**, but more **V** and **III** are present in *L. helviticus* while in *L. acidophilus* there is always more **II** than **I**. The amino-acid spectrum of *L. lactis* is quite varied but generally similar to *L. bulgaricus*. *L. salivarius* liberates only **IV**, **V** and **III** together with basic amino-acids. (16 references.) C.V.

Composition of granules in evaporated milks stored at low temperatures. K. K. Fox, V. H. Holsinger, L. P. Posati, and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1032–1037).—Analysis of granules deposited from evaporated milks stored at 2–4° for several years showed that they consisted of about 50% phosphate and 21% calcium, present in molar ratios of approximately 1 : 1. An insol. matrix of peptide-like material containing glutamic acid was also present in the granules. (10 references.) M. O'LEARY.

Effect of temperature on porosity of dried whole milk powder granules. F. Berlin, E. D. DeVillbiss and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 655–658).—Measurement of densities of spray-dried whole milk foam at 25, −80, and −195° by pycnometric techniques, using He or N_2 as the displaced medium, indicated that marked thermal contraction of the powder particles occurred at the lower temp., with an associated closing of the pores on the powder surface. (12 references.) M. O'LEARY.

Possible inclusion of artifacts in flavours recovered directly from dried whole milk. F. E. Kurtz (*J. Dairy Sci.*, 1967, **50**, 814–817).—Volatiles from silicone high vacuum grease, Apiezon-N grease, and Teflon gaskets had no effect on milk flavour, whereas volatiles from silicone, Neoprene, and Buna-N gaskets and O-rings adversely affected milk flavour. Volatiles from Viton O-rings had a borderline effect on flavour. M. O'LEARY.

Attenuated total reflectance of infra-red energy by dairy products. P. G. Kliman and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1211–1215).—A description is given of the application of the attenuated total reflectance technique to the analysis of dairy products. The only area which appears to indicate a successful use of the technique is the measurement of total solids of milk e.g. the monitoring of total solids in the output of high speed evaporators. M. O'LEARY.

n-Pent-1-en-3-ol and n-pent-1-en-3-one in oxidised dairy products. W. Stark, J. F. Smith and D. A. Forss (*J. Dairy Res.*, 1967, **34**, 123–129).—Pent-1-en-3-ol (**I**) and pent-1-en-3-one (**II**) were isolated in about equal amounts from buttermilk obtained from oxidised cream. The flavour of **I** was shown to be detectable in water and skim-milk in a concentration of $3 : 10^6$ and in butter oil and butter at $1 : 10^5$. The flavour of **II** was about 1000 times stronger than that of **I**. (10 references.) M. O'LEARY.

[Determination of] butterfat in homogenised and chocolate milk. D. J. Mitchell and R. W. Weik (*J. Ass. off. analyt. Chem.*, 1967, **50**, 537–541).—Collaborative studies disclosed significant differences between results obtained by the Babcock and Roese-Gottlieb methods and the detergent method. Procedure for the detergent method is detailed. A. A. ELDRIDGE.

Identification of fatty acids from butterfat using a combined gas chromatograph–mass spectrometer. R. Ryhage (*J. Dairy Res.*, 1967, **34**, 115–121).—A description is given of a procedure in which

a combined gas chromatograph–mass spectrometer instrument was used to identify 52 components of butterfat fatty acids from a 2 g sample in one day. M. O'LEARY.

Volatile compounds in butter oil. I. Lower boiling compounds. D. A. Forss, W. Stark and G. Urback (*J. Dairy Res.*, 1967, **34**, 131–136).—High vacuum degassing, gas chromatography and mass spectrometry were used to isolate and identify the following compounds from best quality Australian butter oil: the $C_{3,5,7,9}$ alkan-2-ones, the $C_{2,4,6,8,10,12}$ n-alkanoic acids, the $C_{6,8,10}$ 5-lactones, dimethyl sulphone, butanone, undecan-2-one, 5-dodecalactone, diacetyl, n-nonanoic acid, a decenoic acid, methyl n-decanoate, toluene, *o*-methoxyphenol, a cresol, phenol, benzaldehyde, methyl benzoate, and benzothiazole. Though none of the compounds were isolated in an amount greater than flavour threshold, the distillate obtained on de-gassing produced an attractive creamy flavour when added to bland non-cultured butter. (17 references.) M. O'LEARY.

[Analysis of] dairy products. R. W. Weik (*J. Ass. off. analyt. Chem.*, 1967, **50**, 531–533).—Studies in progress under the auspices of the A.O.A.C. are briefly reviewed. A. A. ELDRIDGE.

Role of propionibacteria in split defect of Swiss cheese. H. S. Park, G. W. Reinbold and E. G. Hammond (*J. Dairy Sci.*, 1967, **50**, 820–823).—Swiss cheese, made with single strains of *Propionibacterium shermanii* (**I**), showed a much greater tendency to split when stored at 7·2° than similar cheese made with *P. arabinosum*. Attempts to relate the greater tendency towards splitting with greater gas production or proteolysis by **I** were unsuccessful. M. O'LEARY.

Effect of type of acid used in direct acidification procedures on moisture, firmness, and calcium levels of cheese. A. E. Shehata, M. Iyer, N. F. Olson and T. Richardson (*J. Dairy Sci.*, 1967, **50**, 824–825).—Ca content of Blue and Pizza cheeses, made by direct acidification of milk, were higher when phosphoric acid (**I**) was used than when hydrochloric (**II**) or lactic acids (**III**) were employed. Blue cheese made with citric acid (**IV**) had the lowest Ca level. Cheeses made with **I** and **II** had lower moisture contents and were firmer than those made with **III** or **IV**. (20 references.) M. O'LEARY.

Denaturation of cottage cheese whey proteins by heat. E. J. Guy, H. E. Vettel and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 828–832).—The proteins (*P*) in cottage cheese whey (*W*) were shown to be comparatively stable to heat; 30 min. at 91° was necessary to denature 80% of *P*. The Harland-Ashworth test gave a reasonably accurate index of the extent of heat denaturation, provided the pH of *W* was adjusted to 5·4–6·5 immediately prior to analysis. Stability of the *WP* was shown to be associated with the low *W* pH. *W* stability was not significantly affected by concn. prior to heating, but heating reduced the buffering capacity of the *W*. M. O'LEARY.

Rennin-like enzyme from *Mucor pusillus* for cheese manufacture. G. H. Richardson, J. H. Nelson, R. E. Lubnow and R. L. Schwarberg (*J. Dairy Sci.*, 1967, **50**, 1066–1072).—A description is given of the properties of a fungal rennet produced from *Mucor pusillus* as compared with those of veal rennet and swine pepsin. (18 references.) M. O'LEARY.

Greater utilisation of whey powder for human consumption and nutrition. F. V. Kosikowski (*J. Dairy Sci.*, 1967, **50**, 1343–1345).—Possible uses of whey powder (*WP*) as a constituent of manufactured foods are discussed and a description is given of a trial in which acceptable whole milk Ricotta cheese was manufactured using non-hygroscopic *WP* as the coagulant instead of lactic starter. M. O'LEARY.

Some effects of hydrogen peroxide on casein and its implications in cheese making. P. F. Fox and F. V. Kosikowski (*J. Dairy Sci.*, 1967, **50**, 1183–1188).—Treatment of casein with H_2O_2 and catalase was shown to increase solubility and proteolysis significantly, especially that by rennin. These results are considered at least partially to explain the increased softness of cheddar cheese made from H_2O_2–catalase-treated milk. M. O'LEARY.

Rapid determination of chloride concentration of cheese by use of a Pungor electrode. V. H. Holsinger, L. P. Posati and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1189–1193).—A description is given of a rapid method for the determination of chloride concn. in cheese using a conventional pH meter equipped with a chloride electrode. M. O'LEARY.

Determination of moisture in cheese. T. E. Strange (*J. Ass. off. analyt. Chem.*, 1967, **50**, 547–555).—Results obtained by the A.O.A.C. methods (*Official Methods of Analysis*, 10th ed., 1965, 15.157, 15.158), the International Dairy Federation oven method, and a distillation method are tabulated, together with some comparisons between the results obtained by the A.O.A.C. method I and the Karl Fischer titration method. The latter method is satisfactory if each step of the procedure is standardised. A. A. ELDRIDGE.

Cheese manufacture. Genvrain (B.P. 1,079,604, 18.1.66. Fr., 22.1.65).—Increased yields are obtained if the collected whey is brought to pH 4·6–7·0 and heated at approx. 90° for 30 sec.–30 min. so that proteins are precipitated, for separating at the same temp. The yield factor showed an increase of 13·3% over the yield from a milk not enriched with serum proteins. By varying the conditions of extraction (pH, temp., time), 15% can be obtained. An alternative consists in concentrating the whey prior to commencing the above process. This latter is specially advantageous when the clear whey is to be employed for bacterial culture, dessication, or for use in the prep. of animal foodstuffs. S. D. HUGGINS.

Edible Oils and Fats

Gas chromatographic determination of residual hydrocarbon solvents in solvent extracted edible oils. J. O. Watts and W. Holswade (*J. Ass off. analyt. Chem.*, 1967, **50**, 717–726).—The sample is injected directly on to a GLC column containing Chromosorb P. 60–80 mesh, coated with 10% didodecyl phthalate; the chromatograph is equipped with a ^{90}Sr capillary ionisation detector cell and A is used as carrier gas. For n-hexane and n-heptane in cottonseed oil recoveries of 85 to 96% are reported. A. A. ELDRIDGE.

Determination and enumeration of the associative microflora of edible emulsions. I. Mayonnaise, salad dressings and tomato ketchup. G. T. Muys, H. W. van Gils and P. de Vogel (*Lab. Pract.*, 1966, **15**, 648–652).—Use is made of a culture medium which is selective with respect to acetic acid-resistant microflora. This enables various resistant moulds, yeasts and lactobacilli to be isolated. A. A. ZIMMERMAN.

Multi-determination of antioxidants in lard. D. F. McCaulley, T. Fazio, J. W. Howard, F. M. DiCiurcio and J. Ives (*J. Ass. off. analyt. Chem.*, 1967, **50**, 243–250).—The antioxidants BHA, BHT, Ionox-100, PG, THBP and TDPA are removed by vacuum sublimation in an apparatus which is illustrated, and collected on a surface cooled with liquid N_2. Determination by GLC follows, with recoveries of 84 to 104%, u.v. and i.r. spectrophotometry being used for identification, by comparison with standards. PG and THBP were separated as their trisilyl derivatives and TDPA as the dimethyl ester. A. A. ELDRIDGE.

Meat and Poultry

Official meat products control by classical and modern analytical methods. O. D. Wyler (*J. Ass. off. analyt. Chem.*, 1967, **50**, 476–485).—A review, with special reference to methods used in Switzerland. A. A. ELDRIDGE.

Extraction of lipids from raw beef lean by using various solvent systems. S. N. Hagan, E. W. Murphy and L. M. Shelley (*J. Ass. off. analyt. Chem.*, 1967, **50**, 250–255).—$CHCl_3$–MeOH extraction (Bligh and Dyer, *Can. J. Biochem. Physiol.*, 1959, **37**, 911) yielded at least as much fat as the A.O.A.C. method, and the ratio of phospholipid to triglyceride was higher. A. A. ELDRIDGE.

Investigation and evaluation of liver paté. W. Stoya (*Dt. Lebensmittl Rdsch.*, 1968, **64**, 12–15).—In evaluating paté the external appearance, prep. and condition should first be noted, especially the surface fat coating (normally 1–2 mm thick). The internal appearance and colour should then be examined (consistency, aroma, taste and colour retention). Criteria for chemical evaluation are discussed with reference to analytical data. Moisture varied from 40 to 68% (average 54·2). Fat varied from 14–43·6% (30 ± 5% is recommended). Protein levels were between 11·1 and 17·7% (average 14·1). 80% of all samples examined had a fat: protein ratio <2·3 and most had less than 4% surface water. Collagen levels, determined by the hydroxyproline method were 7–20%. In some cases the limit of 50 mg % of KNO_3 was exceeded. (13 references.) J. B. WOOF.

Chopped meat products. Riviana Foods Inc. (B.P. 1,079,463, 23.4.65. U.S., 24.4.64).—A chopped meat product of excellent

texture, good keeping qualities, flavour, odour stability, colour fixation, resistance to shrinkage and retention of natural meat flavours, odours, juices and fats on cooking, comprises chopped meat and 1–20 wt.-% of pregelatinised rice particles of sp. gr. 0·25–0·8. F. R. BASFORD.

Fish

Disc electrophoresis method for the identification of fish species. R. R. Thompson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 282–285).—A collaborative study of the starch gel zone electrophoresis method for characterising fish protein, (Ornstein and Davis, *Disc Electrophoresis*, Rockville, Md., 1962), using Canalco apparatus, gave satisfactory results. The recommended procedure is described in detail. A. A. ELDRIDGE.

Determination of isopropyl alcohol in fish protein concentrate by solvent extraction and gas-liquid chromatography. R. G. Ackman, H. J. Hingley and H. E. Power (*J. Fish. Res. Bd Can.*, 1967, **24**, 1521–1529).—Isopropyl alcohol (**I**) residues in fish protein concentrates prepared with this solvent can be determined by a hot extraction technique employing EtOAc as the solvent, followed by GLC analysis of the extract. Studies on vacuum stripping of fish protein concentrates and analysis of various samples suggest that the **I** is trapped mechanically inside particles because of formation of an impervious shell during drying. (11 references.) S. A. BROOKS.

Amino-acid composition of myosin from trout muscle. H. Buttkus (*J. Fish. Res. Bd Can.*, 1967, **24**, 1607–1612).—The amino-acid composition of myosin (**I**) from the white muscle of trout has been found to be very similar to that of rabbit **I**. The concn. of 13 of the amino-acid residues were within 3% of those of rabbit **I**. Valine, glycine and methionine were slightly higher. Histidine and proline contents differed by three residues per 10^5 g of protein, representing the greatest differences. The possible relationship of these differences to the lower enzymatic activity and thermal stability of trout **I** is discussed. The apparent specific vol. of trout **I** calculated from the amino-acid composition (0·735 cm^3/g) was identical to that obtained by density measurements. An apparent mol. wt. calculated from the amino-acid analyses was 500,027 ± 44. (18 references.) S. A. BROOKS.

[Determination of] drained weight of frozen shrimp. J. C. Werren and R. W. Weik (*J. Ass. off. analyt. Chem.*, 1967, **50**, 275–278).—The shrimp is weighed after being thawed in water at 80° F flowing at 1–3 gal per min. A. A. ELDRIDGE.

Net weight and drained weight relationship of frozen shrimp products. J. C. Werren, A. R. Johnson and R. W. Weik (*J. Ass. off. analyt. Chem.*, 1967, **50**, 278–282).—The modified method used by Werren and Weik (previous abstract) is preferred to the A.O.A.C. method (*Official Methods of Analysis*, 10th ed., 1965, 10.008). A. A. ELDRIDGE.

Spices, Flavours, etc.

Detection and identification of dehydrated red beets in capsicum spices. W. G. Schwien and B. J. Miller (*J. Ass. off. analyt. Chem.*, 1967, **50**, 523–525).—After removal of oil with light petroleum the dried residue is examined microscopically. Red particles, after comparison with beet parenchyma, may be examined by paper chromatography of an extract in methanol containing 1% HCl, and by spectrophotometry at 700 to 400 nm. A. A. ELDRIDGE.

Microflora of black and red pepper. C. M. Christensen, H. A. Fanse, G. H. Nelson, F. Bates and C. J. Mirocha (*Appl. Microbiol.*, 1967, **15**, 622–626).—Dilution cultures of 30 samples of ground black pepper (*BP*) showed an average 39,000 colonies of fungi per g. The details are provided but amongst the bacteria isolated from *BP* were *Escherichia coli*, *E. freudii*, *Serratia* sp., *Klebsiella* sp., *Bacillus* sp., *Staphylococcus* sp. and *Streptococcus* sp. No cultures of *Shigella* or *Salmonella* were found. In both *BP* and red pepper, *Aspergillus flavus* and *A. ochraceus* were isolated. (10 references.) C.V.

Micro method for the determination of zirconium oxychloride in table salt. E. Kröller (*Z. analyt. Chem.*, 1967, **226**, 199–201).—A method for the determination of zirconium oxychloride in table salt is described, in which a red lake produced by zirconium in a 2% H_2SO_4 medium with xylenol orange is measured spectrophotometrically at 535 nm. G. P. MITCHELL.

Determination of benzaldehyde in flavours and cordials by ultraviolet spectrophotometry and 2,4-dinitrophenylhydrazine precipitation. R. L. Brunelle (*J. Ass. off. analyt. Chem.*, 1967, **50**, 319–322).—In collaborative work the u.v. spectrophotometric method gave average recoveries of 96·0 to 104·0%, whilst recoveries in the pptn. method were 84·7 to 97·0%. However, when evaporation losses were reduced by cooling the distillate, recoveries of 95·6 to 100·7% were reported for the latter method. A. A. ELDRIDGE.

Flavours, their uses and abuses. Citrus flavours. I. M. Wightwick (*Fd Technol. Aust.*, 1967, **19**, 404–405, 407).—A review. P. S. ARUP.

Colouring matters

Synthetic carotenoids in food, feeding stuff and pharmaceutical industries. U. Manz (*Chimia*, 1967, **21**, 329–335).—The importance and applications of commercial synthetic carotenoids, particularly β-carotene, β-apo-8′-carotenal, ethyl β-apo-8′-carotenoate and canthaxanthin, are discussed. The development of water-sol. carotenoids is pointed out. Examples of use include colouring foodstuffs such as edible vegetable oils, orange juice, confectionery, fruit preserves and various other food articles. The application in relation to pigmentation of egg yolk, broiler and plumage is surveyed. (88 references.) M. SULZBACHER.

[Identification and determination of] colour additives [in foods]. K. A. Freeman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 525).—19 programmes of study being undertaken under the auspices of the A.O.A.C. are listed. A. A. ELDRIDGE.

Identification of lactoflavin in bakery and confectionery products. K. Hildenbrand (*Dt. LebensmittRdsch.*, 1967, **63**, 372–373).—The food colourings lactoflavin (riboflavin) and quinoline yellow can be isolated and fractionated by the wool fibre and paper chromatographic method of Thaler or by the polyamide powder procedure of Davidek. A surer identification is possible from the u.v.–visible spectrum. The spectra of the two dyes separately and together in a mixture are shown. The fluorescence spectra of the dyes excited by daylight, artificial and u.v. light are shown. J. B. WOOF.

Pesticides in Food

Infra-red spectra of carbophenothion (Trithion) and some of its possible metabolic products. J. T. Chen and R. W. Dority (*J. Ass. off. analyt. Chem.*, 1967, **50**, 426–430).—The names and structural formulae of five possible metabolites of carbophenothion are given, together with curves of their i.r. spectra. Since the spectra are significantly different the curves can be used for the identification of residues. A. A. ELDRIDGE.

Multiple [determination of] organophosphorus pesticide residues in non-fatty foods. J. R. Wessel (*J. Ass. off. analyt. Chem.*, 1967, **50**, 430–439).—Residues were determined by GLC using electron capture (Burke and Giuffrida, *ibid.*, 1964, **47**, 326) and KCl thermionic detection (Giuffrida, Ives and Bostwick, *ibid.*, 1966, **49**, 8). Recoveries of ronnel, ethion, Trithion, diazinon, methyl parathion, parathion and malathion in lettuce and apples were 78·9 to 98·07. A. A. ELDRIDGE.

Extraction of chlorinated pesticide residues from non-fatty samples of low moisture content. P. F. Bertuzzi, L. Kamps, C. I. Miles and J. A. Burke (*J. Ass. off. analyt. Chem.*, 1967, **50**, 623–627).—A mixture of water and MeCN (35 : 65) is more effective than MeCN alone for extraction at ~10% moisture. A. A. ELDRIDGE.

Acetonitrile dilution: a possible source of error in the 'Rapid method for [determining] chlorinated pesticide residues in non-fatty foods'. M. L. Porter, J. A. Burke and P. F. Bertuzzi (*J. Ass. off. analyt. Chem.*, 1967, **50**, 644–645).—The method of mixing the diluted MeCN and light petroleum in the procedure of Mills, Onley and Gaither (*ibid.*, 1963, **46**, 186) is critical. A. A. ELDRIDGE.

Insecticides in human fat in New Zealand. H. V. Brewerton and H. J. W. McGrath (*N.Z. Jl Sci.*, 1967, **10**, 486–492).—Results of chromatographic analyses of 52 samples show mean concn. of 1·6 ppm DDT and 3·8 ppm DDE, in fair agreement with values recorded by other countries. The estimated daily intake of DDT per man in New Zealand is 0·03 mg, well within the FAO 'acceptable daily intake' of <0·01 mg per kg body-wt., and achieved through the established tolerance of 5 ppm DDT in meat fats, fruit and vegetables. Dieldrin concn. in the human fat were ~0·27 ppm. After extraction and clean-up the fats were chromatographed on 5-ft columns of 5% DCIl and 5% QFl, each on Chromosorb W at 200° and 185°, respectively, the peak-heights for each of the nine organo-chlorine compounds being averaged. (14 references.) W. J. BAKER.

Determination of mercury in cereal flours. I. Sarudi, jun. (*Getreide Mehl.*, 1967, **17**, 59–60).—Trace amounts of Hg (~0·4 ppm) in flour contaminated with seed dressing may be reliably determined colorimetrically using diphenylcarbazide; org. matter is destroyed by H_2SO_4 + HNO_3 using a Soxhlet. E. C. APLING.

Determination of chlorohydrocarbons, in particular lindane in vegetable foodstuffs by electron capture gas chromatography. R. Knoll and R. Engst (*Z. analyt. Chem.*, 1967, **227**, 424–431).—Using columns of Sterchamol impregnated with silicone gum preproduct the method is sensitive down to 1 ng lindane and 0·01 ppm can be determined in fruit and vegetable residues. Quant. determination of lindane, heptachlor, dieldrin and *p,p'*-DDT in mixtures is possible. The method is more rapid and precise than the TLC method. P. N. R. NICHOLS.

Removal of chlorinated insecticide residues from milk fat by molecular distillation. D. D. Bills and J. L. Sloan (*J. agric. Fd Chem.*, 1967, **15**, 676–678).—Lindane, heptachlor and its epoxide, aldrin, DDT, DDD, and DDE in concn. representing severe contamination were almost quant. removed from the fat by mol. distillation at 200° and 5×10^{-4} torr. Analytical and industrial uses of this technique are suggested. (10 references.) P. S. ARUP.

Sweep co-distillation clean-up of milk for determination of organophosphate and chlorinated hydrocarbon pesticides. R. R. Watts and R. W. Storherr (*J. Ass. off. analyt. Chem.*, 1967, **50**, 581–585).—The pesticide is extracted with Et acetate and methanol is added as a coagulant. An aliquot of the extract is concentrated and the concentrate is subjected to sweep co-distillation. Determinations made by GLC using a K thermionic detector or an electron capture detector gave satisfactory results. A. A. ELDRIDGE.

Simultaneous determination of selected chlorinated insecticide residues in milk. L. A. Richardson, J. R. Lane, J. T. Peeler and J. E. Campbell (*J. Dairy Sci.*, 1967, **50**, 1073–1077).—A description is given of a technique which permits the rapid determination of heptachlor, heptachlor-epoxide, DDT + DDE, dieldrin, and endrin in milk. The method is not suitable for detecting the insecticide lindane. (10 references.) M. O'LEARY.

Reductive dechlorination of DDT by *Escherichia coli*. B. F. Langlois (*J. Dairy Sci.*, 1967, **50**, 1168–1170).—*E. coli* ATCC 11775 was shown to be capable of dechlorinating over 90% of 99·3% *p,p'*-DDT after seven days growth at 37° in various broths containing the insecticide. Under similar conditions in skimmilk only a slight dechlorination was detected. The organism had no effect on 70% *p,p'*-DDD in either broth or skimmilk. M. O'LEARY.

Analysis of chlorinated pesticide residues in fats and oils, utilising dimethyl sulphoxide. M. Eidelman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 591–595).—Collaborative results obtained for lindane, heptachlor-epoxide, *p,p'*-DDT, dieldrin and endrin by Eidelman's method (*ibid.*, 1962, **45**, 673; 1963, **46**, 182) were not satisfactory. A. A. ELDRIDGE.

Residues in poultry tissues from low level feeding of five chlorinated hydrocarbon insecticides to hens. J. G. Cummings, M. Eidelman, V. Turner, D. Reed, K. T. Zee and R. E. Cook (*J. Ass. off. analyt. Chem.*, 1967, **50**, 418–425).—Residues of lindane, heptachlor-epoxide, dieldrin, endrin and DDT in abdominal fat (greatest storage), breast tissue (<0·1 ppm) and liver (<1 ppm) were determined by electron capture gas chromatography. A. A. ELDRIDGE.

[Determination of] residues of Zytron, *O*-2,4-dichlorophenyl *O*-methyl isopropylphosphoramidothioate, in chicken tissues and eggs. M. C. Ivey, H. V. Claborn and B. Brinkman (*J. Ass. off. analyt. Chem.*, 1967, **50**, 634–637).—In the gas chromatographic method used, a Pyrex glass tube was filled with 80–100 mesh Chromosorb W coated with SF-96; the carrier gas was N_2 and detection was by electron capture. The sample is extracted with hexane and MeCN and the solution remaining after the extraction and concn. procedures is cleaned up on a column of silicic acid. Recoveries of 86 to 95% are reported. A. A. ELDRIDGE.

Food Processing, Refrigeration

Isolated soya-bean protein as banana spray-drying aid. S. Mizrahi, Z. Berk and U. Cogan (*Cereal Sci. Today*, 1967, **12**, 322, 324–325).—The protein is extracted from soya-beans with 0·03 M-$Ca(OH)_2$ at 55° and precipitated by adding HCl to pH 4·5; a Na proteinate is prepared by adding 5% NaOH to pH 7·0. Additions of 4–20% (dry basis) of either of these prep. to banana purée facilitates spray-drying and prevents caking, browning; and moisture absorption of the powder. These products afford bases for nutritional beverages. P. S. ARUP.

White mineral oils. Review of food laws requirements. (*J. Inst. Petrol.*, 1967, **53**, 121–128).—National pharmacopoeia standards for medicinal grade mineral oils ('liquid paraffin') are commonly applied to such oils when used in food processing, and in some countries the statutory regulations prescribe additional tests; manufacturers must observe these in production. Six laboratories of English, German, and United States manufacturers have co-operated in trials of official methods. The presence and concn. of carcinogenic hydrocarbons were tested by paper-chromatography; this method proved laborious and inconclusive. Other countries require measurement of the u.v. absorbence at 275–400 nm. This may be misleading because absorbence at 275–299 nm is mainly due to innocuous mono- and di-cyclic aromatics; many known carcinogens absorb at 300–400 nm, but detection limits are low due to overlapping bands and interference by dicyclic hydroaromatics. The simple u.v. absorbence might be useful in production control. Sensitivity of this test is improved by extracting a solution of the oil in an equal vol. of n-C_6H_{14} with Me_2SO and measuring the absorbence of the extract. Precautions for ensuring the purity of the Me_2SO and protecting it from air-oxidation during the test are detailed. (21 references.) A. R. PEARSON.

Deep-freezing of bakery goods in the 'craft' bakery. F. O. Michel (*Brot Gebäck*, 1967, **21**, 92–99).—The advantages, in terms of labour economy and simplicity in organisation accruing from the installation of deep-freeze equipment are discussed. Detailed examples of costings and estimates of labour saving are presented and the results obtained with representative fancy goods, frozen before, or after, baking are briefly described and are illustrated. E. C. APLING.

Influence of drying techniques on some properties of nonfat dried milk. A. Tamsa, A. Kontson and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 1055–1060).—The bulk density, particle density, sinkability and dispersibility of nonfat dried milk were varied by vacuum shelf drying, instantising and foam-spray-drying techniques. Good sinking and dispersing properties were obtained only by vacuum shelf drying, instantising conventional spray-dried material and foam-spray-drying with low levels of CO_2 or N_2 incorporated prior to drying. High bulk density, dispersibility, and sinkability values for nonfat dried milk were obtained by the addition of detergents to the concentrate prior to conventional spray drying. (20 references.) M. O'LEARY.

Freeze-drying of Atlantic cod steaks. R. Legendre (*J. Fish. Res. Bd Can.*, 1967, **24**, 1461–1473).—A preliminary study was made of the freeze-drying of steaks 6–18 mm thick, at sample temp. from 38 to 98° and under total dryer pressures from 20 to 2000 μ, from pre- and post-rigor, quick (*QF*) and slow-frozen (*SF*) cod. The drying time varied directly with the thickness, inversely with the temp. of the sample, was not affected by total pressure within the dryer with the thinner samples, but appeared to vary inversely with pressure for those 12 mm thick. Total drying time was the same for 16 mm thick samples from pre- or post-rigor, *QF* or *SF* fish, but varied with 6 mm thick steaks. Evaluation of quality varied inversely with both thickness and temp. but was not affected by dryer pressure. Only the post-rigor, *SF* samples were consistently below the quality level of consumer acceptance. (13 references.) S. A. BROOKS.

Effects of ultra-rapid freezing on cod muscle. R. M. Love (*Chemy Ind.*, 1967, 2151).—Evidence advanced shows that ultra-rapid freezing (from −195° to ~0°) does not appear to damage cod-muscle proteins more than does slow freezing (*Idem, Bull. Jap. Soc. scient. Fish.*, 1967, **33**, 746). Damage was assessed by extraction of myofibrillar protein in 5% NaCl at 0°. W. J. BAKER.

Packaging

Comparison of adhesive tapes by neutron activation analysis. J. E. Scott, C. M. Hoffman, M. J. Pro and H. L. Schlesinger (*J. Ass. off. analyt. Chem.*, 1967, **50**, 371–376).—By the use of neutron activation analysis applied through Mn, Na, Zn and Sb, adhesive tapes produced by different manufacturers can be distinguished and batch-to-batch variation detected. A. A. ELDRIDGE.

Feeding experiments on rats to establish the toxicological harmlessness of packaging systems based on liquid epoxy resins. G. Wilmes and K. Frese (*Dt. LebensmittRdsch.*, 1967, **63**, 367–372).—

Male and female rats were fed over periods of 12 weeks on diets which contained either the epoxy resin lacquer itself or food which had been heated in contact with the lacquer. In a third set of experiments the lacquer foil was extracted under reflux with water, 3% acetic acid, 10% ethanol and ether and the residues added to the diet. The concn. were about 150 times higher than the max. possible human intake. Wt. loss, haemoglobin content and wt. of heart, liver, spleen, kidney and lungs of animals on the test diet were no different from those with no additives or those with harmless quartz powder added. Histological study of the organs showed no abnormalities. (22 references.) J. B. WOOF.

Polyethylene packaging for shell-less eggs. J. Klapka (*Dt. LebensmittRdsch.*, 1967, **63**, 365–367).—The equipment consists first of an electronic detector for automatically sorting eggs according to quality and rejecting bad ones. This is followed by automatic devices for washing and breaking the eggs, and a press for forming the packaging shell from polyethylene discs. The filled packages are then sealed and dispatched. The flow diagram, dimensions and operation of the process are discussed.
J. B. WOOF.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Explanation of the common relationships of diabetes mellitus and atherosclerosis with consumption of carbohydrates, saturated fats, alcohol and with smoking. S. R. Erlander (*Stärke*, 1967, **19**, 179–186).—A review of the inter-relationships of dietary factors, enzymes and the onset of certain diseases. Atherosclerosis is most readily formed when there is a deficiency of carbohydrate metabolising enzymes and high blood fat levels. Smoking affects the disease by destroying ascorbic acid and raising fat levels. Cirrhosis of the liver has the reverse effect by reducing the level. Alcoholics and diabetics are prone to cirrhosis and coronary disease because of low enzyme levels. Specific enzyme deficiencies can arise also from the consumption of undistilled alcoholic drinks because of the presence of some sugars and dextrins. (96 references.) (In English.) J. B. WOOF.

Nutritive value of coconut protein concentrates obtained by wet processing. G. R. Rao, G. Ramanatham, K. Indira, U. S. B. Rao, M. R. Chandrasekhara, K. J. Carpenter and D. S. Bhatia (*Indian J. exp. Biol.*, 1967, **5**, 114–117).—The digestibility and protein efficiency ratio (*PER*) of skim milk concentrates from fresh and autoclaved coconuts (*C*), as well as the acid and heat coagulates, and whey solids (*WS*) have been determined *in vitro*. The supplementary effects of *WS* and minerals on the poor *PER* of *C* have also been investigated. *PER* of skim milk concentrates from autoclaved *C* was negative while that from fresh *C* was fairly high (1·4–1·9); acid and heat coagulates also had a higher *PER* (1·9–2·4). The digestibility of both acid and heat coagulates *in vitro* was higher than that of concentrates from fresh *C*. The results suggest that the protein of skim milk suffers some damage during autoclaving and concn. (13 references.) S. A. BROOKS.

Amino-acids of processed seed meal proteins. C. R. Mitra and P. S. Misra (*J. agric. Fd Chem.*, 1967, **15**, 697–700).—The amino-acid composition and protein content of 29 seed meals (defatted and freed from EtOH-sol. lipids) were determined by two-dimensional paper chromatography. Most of the meals compared well with those in general use. (25 references.) P. S. ARUP.

Lysine and methionine availability in heated casein–glucose mixtures. M. N. Rao and J. M. McLaughlan (*J. Ass. off. analyt. Chem.*, 1967, **50**, 704–707).—The available lysine and methionine is considerably reduced when mixtures of casein, glucose and water (2 : 2 : 1) are autoclaved at 15 psi for 20 min. Plasma lysine levels correlated well, plasma methionine levels less well, with gross protein values. Plasma amino-acid levels may provide an index for measuring the nutritional availability of amino-acids.
A. A. ELDRIDGE.

Simple method for making full-fat soya flour. W. J. Albrecht, G. C. Mustakas, J. E. McGhee and E. L. Griffin, jun. (*Cereal Sci. Today*, 1967, **12**, 81–83).—The directions described for the production of a flour containing 40% of protein of satisfactory palatability can be carried out with hand-operated equipment. The process includes soaking in cold water for 16 h, followed by cooking for 10 min., air-drying, cracking, dehulling, and grinding.
P. S. ARUP.

Free nucleotides in resting and metabolising baker's yeast. E. Oura and H. Suomalainen (*J. Inst. Brew.*, 1967, **73**, 370–376).—The free nucleotides (*FN*) of semi-aerobically and aerobically cultured baker's yeast in different industrial stages have been investigated under different conditions. The amounts of protein and nucleic acids diminish progressively during industrial propagation, while the amount of *FN* falls only during the last stage of propagation. In yeasts cultured under different conditions of aeration and nutrient addition, the proportions of *FN* change considerably when the yeast passes from the resting state to growth or metabolism. A small addition of ethanol to a respiring yeast suspension caused clear alterations in the spectrum, the most obvious being the reduced amount of nucleotides with energy-rich phosphate bonds. (27 references.) S. A. BROOKS.

Evaluation of molasses for yeast production. V. Stuchlík (*Sb. vys. Sk. chem.-technol. Praze, Potravin. technol.*, 1964, [1967], **8**, 123–132).—The utility of various determinations (nitrogen assimilating factor *NAF* by the rapid biological or chemical micromethods or the biological macromethod, biotin activity, and of *NAF* and yield of yeast dry matter in parallel experiments) for the selection of molasses for yeast production is discussed in the light of factory results obtained during 1939–1945. (14 references.) (In German; English summary.) E. C. APLING.

Synthetic protein foodstuffs. General Mills Inc. (B.P. 1,079,625, 2.5.66. U.S., 28.5.65).—Fibrous protein products simulating the general characteristics of meat products are prepared from vegetable proteins (soya-bean, corn, peanut) or animal proteins (casein, keratin); they are prepared by forming a spinning dope and extruding through a perforated die into a precipitating bath (aq. acid and salt) and the formed filaments may be stretched to orient the mol. structure; neutralising or washing follows. Normally further processing follows to simulate the fibrous texture of meat products, their flavour and colour; binders, egg albumen, starch, gum, alginates, CMC, etc. are added to at least partially agglutinate the fibres. Vegetable and animal fats are normally added, also various smoke flavours. The processes are described in detail; the object is to obtain a product of crisp texture and relatively low moisture content. S. D. HUGGINS.

Unclassified

Colorimetric determination of volatile sulphur compounds in food. I. Reaction with *N.N*-dimethyl-*p*-phenylenediamine. H. G. Maier and W. Diemair (*Z. analyt. Chem.*, 1967, **227**, 187–196).—The colour reactions of the reagent with H_2S, methyl-, ethyl- and furfuryl-mercaptan, dimethyl disulphide, acetaldehyde-diethyl mercaptal, acetone-diethyl mercaptal, SO_2 and allyl mustard oil are described. A method is given for the determination of such compounds in foodstuffs, e.g. coffee. P. N. A. NICHOLS.

Rapid thin-layer chromatographic detection of flavatoxins; application to alimentary microbiology. J. Jaquet and P. Boutibonnes (*C. r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1244–1251).—Conc. $CHCl_3$ extracts of the samples (defatted, if necessary, with light petroleum) are submitted to TLC on microscope slides coated with silica gel; development, in parallel with reference compounds, is carried out with MeOH–$CHCl_3$ (1 : 19) as ascending solvent in a stoppered jar for exactly 4 min. Examination in u.v. light shows flavatoxins B and G as blue spots at R_F 0·5–0·6. The min. detectable amount of the two flavatoxins was 0·02 μg. (19 references.) P. S. ARUP.

Amperometric determination (by a single operation and without separation) of calcium and magnesium (both being present) in whisky, saliva, and milk. D. Monnier, A. Daïna and G. Delpin (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1967, **57**, 458–460).—Results are tabulated for analyses carried out by the authors' method (cf. *Analytica chim. Acta*, 1966, **35**, 231); the samples buffered with ethanolamine are titrated with EGTA for Ca, and with EDTA for Mg. P. S. ARUP.

Problems in sanitation analysis of low grade flours and cinnamon. O. L. Kurtz (*J. Ass. off. analyt. Chem.*, 1967, **50**, 521–523).—Minor modifications in A.O.A.C. methods for determining extraneous materials in flours and cinnamon are suggested.
A. A. ELDRIDGE.

Use of Howard mould count method. L. G. Clark (*Fd Technol. Aust.*, 1967, **19**, 408–409, 411, 413, 415, 417).—The essential details in determining mould counts are stressed and the necessity for proper training in the technique is emphasised. The value of this method in relation to good legislation is upheld. (12 references.)
P. S. ARUP.

Gas chromatographic identification of irradiated foods. B. Jansson and G. Löfroth (*Atompraxis*, 1967, **13**, 254–256).—Gas chromatography is used and, where applicable, this technique is highly sensitive and capable of standardisation. Irradiated egg powder can readily be distinguished from the untreated samples. Attempts to distinguish between unirradiated and irradiated wheat flour were unsuccessful but the authors suggested that this might be possible with improved techniques. (12 references.) C.V.

Problems confronting the food microbiologist. L. Leistner (*Fleischwirtschaft*, 1967, **47**, 25–28, 30).—A general review. (99 references.) C.V.

3.—SANITATION, WATER, etc.

Discussion on pesticides: benefits and dangers. (*Proc. R. Soc.*, B, 1967, **167**, 88–163).—**Introductory note,** W. Slater; **Present levels in food and possible sources of contamination,** D. T. Lewis; **Toxicity to man: risks from present levels,** W. J. Hayes, jun.; **Effects on wildlife,** N. W. Moore; **Importance in preventive medicine,** P. C. C. Garnham; **Importance in British food production,** H. Sanders; **Importance in Indian food production,** D. B. Reddy; **Possible new and more selective means of pest control,** R. A. E. Galley. C.V.

Mobile laboratory methods for determination of pesticides in air. III. Mevinphos. G. A. Lloyd and G. J. Bell (*Analyst, Lond.*, 1967, **92**, 578–580).—The collection of samples of airborne droplets or vapour is described; mevinphos (**I**) (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) is then determined by hydrolysis (20 h at 20°) in cold aq. NaOH–Pr^{i}OH to yield mainly the keto-isomer of Me acetoacetate, which slowly enolises. Addition of Br_2 and reaction of the bromoketone with CN^- results in liberation of CNBr, which is then determined spectrophotometrically at 520 nm by a modified Aldridge method (*ibid.*, 1945, **70**, 474), *p*-phenylenediamine being used instead of the carcinogenic benzidine; the colour is allowed to develop during 2 h at 40°. Necessary precautions and corrections for max. accuracy are indicated; the calibration graph is rectilinear for 0–200 μg of **I**. The error ranges from $\pm$10 to 20%. W. J. BAKER.

Effect of carbon monoxide on metabolism of insecticides *in vivo*. S. E. Lewis (*Nature, Lond.*, 1967, **215**, 1408–1409).—Reports and discusses the effect of CO and O_2 (in varying ratio) on formation of dieldrin (**I**) in **I**-resistant houseflies treated with aldrin (2 μ in 2 μl of acetone), and of CO on formation of **I** in housefly-larvae treated similarly. Results indicate that a component sensitive to CO is involved in the metabolism of some insecticides *in vivo* and that this component is most probably the microsomal cytochrome-like pigment *P*-450 (cf. Cooper *et al.*, *Science*, 1965, **147**, 400) which is involved in the inhibition by CO of the epoxidation of aldrin by fly microsomes *in vitro* (Ray, *Biochem. Pharmac.*, 1967, **16**, 99). W. J. BAKER.

Pest control in grain and flour storage. S. Lück (*Getreide Mehl*, 1967, **17**, 49–52).—Available physical and chemical means of pest control are briefly and comparatively surveyed; properties and uses of available fumigants are described in detail. The advantages of fumigation in terms of efficiency, economy and minimal residual effect on the flour and grain are emphasised. Problems of residual toxicity are briefly discussed, and it is concluded that the controlled use of chemical pest control is essential to restrict the serious world losses of food grains caused by insects and rodents. E. C. APLING.

Vinyl phosphate insecticide sorption to proteins and its effect on cholinesterase I_{50} values. A. C. Boyer (*J. agric. Fd Chem.*, 1967, **15**, 282–286).—Two such insecticides of low mammalian toxicity (SD 8447 and SD 7859) (both ^{14}C-labelled) were shown by a method of equilibrium dialysis to be adsorbed by mammalian blood plasma and housefly homogenates. The results were calculated to represent the concn. of insecticide available for 50% inhibition of cholinesterase. The question as to whether the low mammalian toxicity of the insecticides may be connected with their immobilisation by binding to proteins is considered. P. S. ARUP.

Alkaline hydrolysis, anticholinesterase, and insecticidal properties of some nitro-substituted phenyl carbamates. T. R. Fukuto, M. A. Fahmy and R. L. Metcalf (*J. agric. Fd Chem.*, 1967, **15**, 273–281).—A series of 27 of these compounds was studied. At pH 7–8, the nitrophenyl *N*-methylcarbamates were unstable, and their alkyl-substituted deriv. were almost equally so. The nitrophenyl *N,N*-dimethyl carbamates were stable at pH 8, and had higher anticholesterinase activity than the other two groups. Practically all the compounds used alone showed low toxicity to housefly and mosquito, but several are strongly synergised by piperonyl butoxide. A study of the alkaline hydrolysis of 4-nitrophenyl *N*-methylcarbamate was made and a mechanism for carbamylation of cholinesterase, consistent with the hydrolysis data is proposed. P. S. ARUP.

Behavioural responses to contact with DDT in *Anopheles atroparvus*. J. L. Gerold and J. J. Laarman (*Nature, Lond.*, 1967, **215**, 518–520).—Two colonies of *A. atroparvus* were obtained by selecting for escaping (colony *A*) and not escaping (colony *B*) from a tube lined with DDT-impregnated paper. Neither DDT nor the solvent oil was essential for the escape reaction, but each strongly increased the response. W. J. BAKER.

Pyrethrum as an insect repellent. I. Literature review. G. D. Glynne Jones and N. K. Sylvester (*Pyrethrum Post*, 1966, **8**, No. 4, 38–41).—Pyrethrins have been shown to have strong repellent properties against a wide range of pests including biting insects (mosquitoes, tsetse flies), pests of stored products (grain and flour weevils, blowflies) and insect pests of animals (horseflies, stableflies and ticks). (48 references.) J. L. WALPOLE.

Factors influencing the effectiveness of swimming pool bactericides. G. P. Fitzgerald and M. E. DerVartanian (*Appl. Microbiol.*, 1967, **15**, 504–509).—Techniques of evaluation are considered. It is shown that concn. 25, 50 and 100 mg of the chlorine stabiliser cyanuric acid (**I**) per litre increased the time required for a 99% kill of *Streptococcus faecalis* by 0·5 mg Cl_2/l at pH 7·4 and 20° from <0·25 min. without **I** to 4, 6 and 12 min. respectively in its presence. The effect of the presence of ammonia N was studied in a comparable series. In a concn. >0·05 mg/l faster rates of kill were attained with 100 mg **I** +0·5 mg Cl_2/l than when Cl_2 was used alone. (13 references.) C.V.

New method of predicting the biochemical oxygen demand of brewery waste. M. Søltoft (*J. Inst. Brew.*, 1967, **73**, 393–405).—The difficulties of obtaining reliable predictions of B.O.D. for brewery waste water are discussed. A method of estimation is demonstrated by reference to a practical example; calculations are based on the quantities of all materials going into or leaving the brewery. (21 references.) S. A. BROOKS.

Sublethal effects of surfactants on fish. R. Marchetti (*Riv. ital. Sostanze grasse*, 1968, **45**, 27–34).—The effect of concn. of various surfactants on the swimming power of fish has been studied. A C_{12}-branched alkyl benzene sulphonate had an effect only at high concn. but the C_{14}-branched and C_{12}- and C_{14}-linear alkyl benzene sulphonates affected swimming power at low concn. similar to those found today in Italian surface waters. (15 references.) L. A. O'NEILL.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JUNE, 1968

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

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

CONTENTS

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