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APPLICATION OF CHEMICAL METHODS FOR THE ASSESSMENT OF BEEF QUALITY

III.*—Methods related to fat spoilage

By D. PEARSON

Various methods for assessing the extent to which the fat has deteriorated in stored beef are considered in relation to various criteria discussed in Part I. From the point of view of routine quality control, the titrimetric determination of free fatty acids (FFA) appears to be the most reliable method. From statistical correlations with odour scores, most meats were considered acceptable provided the FFA did not exceed 1·2% (calculated as oleic acid on the extracted fat). Several of the other fat spoilage methods considered gave values which rose during the storage period to a maximum and then either remained constant or decreased steadily to a very low figure. Most fresh samples gave peroxide values of 0·0–1·0 mequiv./kg (on the extracted fat), but a critical limit of 5 might be applied under practical conditions.

Introduction

The deterioration of the fat in meat limits its storage life as its presence soon affects the odour and taste. The adipose tissue contains, in addition to the fat itself, blood and varying amounts of connective tissue, together with enzymes such as lipase and oxidase. As many of the minor constituents enter into the reactions contributing to spoilage, the problem of fat spoilage is considerably more complex than with a pure oil or fat. The main factors which are capable of contributing to the deterioration in meat fat quality are:

Atmospheric oxidation of the unsaturated bonds (beef fat contains about 42% oleic acid).

Attack by micro-organisms causing hydrolysis, and the appearance of metabolic products which influence flavour.

The development of free acidity due to the presence of tissue enzymes, which may also play a part in certain oxidative changes.

The acquirement of odours and taints due to adjacent odoriferous materials or strains of actinomycetes.

The various methods available for determining free fatty acids (FFA) in oils and fats have been reviewed by Mehlenbacher.[1] Rather surprisingly, few workers have reported results of FFA determinations applied to stored beef. Lea[2] reported a figure of 1·5% (as oleic acid) after 25 days at 0°, rising after 42 days to 5–11% which then coincided with unpleasant flavours in the cooked material. Unpleasantness was apparently first noticeable at a level of 2–3%. Broumand, Ball & Stier[3] reported a progressive increase in the FFA figure as storage progressed.

A large number of iodimetric methods have been described for determining peroxide values.[4] By employing a long reflux condenser, Sully[5] overcame side effects due to access of oxygen. Colorimetric[6–8] and polarographic[9] methods have also been described for the estimation. Although several workers have shown that the general trend of peroxide values is to rise following an induction period there is little published evidence of recommended critical limits.

Several workers have suggested that the thiobarbituric acid (TBA) number can be used as an empirical measure of the deterioration of fatty foods. The earlier methods[10,11] involved heating a slurry of the flesh with the TBA reagent and measuring the optical density of the pigment after extraction with amyl alcohol–pyridine mixture. More recently Tarladgis *et al.*[12] have applied the reaction to a distillate obtained from the acidified meat. The result is then calculated as mg malonaldehyde/kg meat (the compound probably responsible for the TBA reaction). Unlike other methods related to oxidative spoilage, the TBA test has the advantage that it apparently measures the deterioration in both extractable and non-extractable lipids. Keskinel, Ayres & Snyder[13] have found high TBA levels in heavily pigmented beef and suggest that the high TBA numbers found in lean beef (compared with pork) are due to the comparatively high phospholipid content.

The various modifications of the Kreis test, in which there was an increase in the red colour produced under acidic conditions with phloroglucinol as fats deteriorated, have been reviewed by Mehlenbacher[1]. No reference to its application to the fat extracted from stored beef however was found in the literature.

Broumand, Ball & Stier[3] determined the iodine value on one sample of canned ground beef, but observed no regular pattern of change during spoilage.

Experimental

It was decided to apply the following determinations, each of which is related to the spoilage of lipid material, to the stored samples of beef, and to consider them in relation to the criteria in Part I:[14] free fatty acids of the extracted fat; peroxide value of the extracted fat; Kreis test as applied to the extracted fat; thiobarbituric acid value as applied to distillates obtained from the acidified meat; iodine value of the extracted fat.

In order to reduce sampling errors and give greater consistency from one determination to the next, various fat values were estimated on a common chloroform extract.

* Part II. *J. Sci. Fd Agric.*, 1968, **19**, 366.

Preparation of fat extract

After maceration of the prepared beef sample with chloroform and filtering,[15] the filtrate was washed with water and refiltered through a large fluted filter paper containing anhydrous sodium sulphate. The concentration of the filtrate was determined by evaporating a known volume.

Method 11 Free fatty acids

25 ml neutralised 95% alcohol was mixed with 25 ml chloroform extract and titrated with 0·1 N sodium hydroxide using phenolphthalein. The acidity was calculated as oleic acid.

Recoveries were checked by addition of known amounts of oleic acid.

Method 12 Peroxide value

A 150 ml round-bottomed flask was fitted on to a long reflux condenser (fitted with a water jacket in the upper portion only) by means of the ground glass joints as described by Sully. 30 ml glacial acetic acid and 5 ml chloroform were added down the condenser and the mixture was boiled. While the mixture was refluxing, potassium iodide solution (2 g dissolved in the minimum quantity of water) was added down the condenser. Then 25 ml of chloroform extract was carefully added by pipette, and the condenser water was shut off for a short time so that all the dissolved fat went down into the flask. After the condenser water had been turned on again, the mixture was boiled gently for 3–5 min. The flask was then removed and rapidly cooled and the contents were diluted with 50 ml water and titrated with 0·01 N sodium thiosulphate using starch as indicator. The peroxide value was then calculated as mequiv. of peroxide oxygen per kg of extracted fat:

$$\text{Peroxide value} = \frac{\text{Thiosulphate titration (ml)} \times 0{\cdot}01 \times 1000}{\text{weight of fat (g)}}$$

Method 13 Kreis value[15]

Two stoppered 25 ml graduated test-tubes were marked 'S' and 'B', and to each were added 5 ml chloroform extract (containing not more than 1·5 g fat) and 5 ml trichloracetic acid solution (30% in glacial acetic acid). Then 1 ml phloroglucinol solution (1% in glacial acetic acid) was added to the sample tube 'S' and 1 ml glacial acetic acid to the blank tube 'B'. After being stirred by air bubbled through each for 3 seconds, the tubes were placed in a water bath (controlled at 45°) for 15 min. At the end of the heating period 4 ml ethanol (95%) were added to each tube. The optical density of 'S' was determined at 545 nm in the 1 cm cell of a Unicam SP 600 spectrophotometer against 'B'.

$$\text{Kreis value} = \frac{\text{optical density at 545 nm} \times 15}{\text{wt. of fat in 5 ml extract taken}}$$

Method 14 Iodine value

The chloroform extract was accurately diluted so that the concentration of fat present was between 3·0–4·5% (w/v). 20 ml Wijs' solution was added to 10 ml extract for the B.P. method of determination (using 10 ml chloroform instead of carbon tetrachloride for the blank).

Method 15 Thiobarbituric acid number

The TBA number was determined by the distillation method of Tarladgis[12] and calculated as mg malonaldehyde/kg meat.

The data obtained from the application of the various methods to the samples of fresh beef and those stored at 5° are summarised in Table I. The TBA number was also determined separately on fresh and stored samples obtained by comminuting portions of lean tissue (with no visible fat) and the outside fat derived from the same piece of beef (Table II).

TABLE II

Data obtained from lean and fatty samples prepared from the same piece of meat (both stored at 5°C)

Lean sample — 11·2% fat
Fatty sample — 70·2% fat

Days of storage	*TBA number[12] Lean portion	Fatty portion
0	0·20	0·38
4	0·75	0·29
7	1·35	0·55
10	1·92	0·76
14	2·04	0·78
17	2·01	0·79
20	2·05	0·72

*mg malonaldehyde/kg meat

TABLE I

Data comparing the reliability of 5 different methods related to the spoilage of fat in beef (as applied to 12 samples stored at 5°C)

	Method	Coeff. of Variation between replicates (mean)	Recovery % (mean)	Values obtained with samples as received (fresh)[a] min.	max.	mean	C.V. (%)	Values obtained at 'just spoiling' stage[a] (mean)	Sensitivity Ratio 'just spoiling' value / 'fresh' value (mean)	Time of performance, h[d] 1 determination	4 determinations
11	FFA	3·3	99·5	0·24	1·1	0·70	35·5	1·77	2·53	½	½
12	Peroxide value	35[c]	—	0·0	2·1	0·8(9)	20·3	4·5	5·6	½	1
13	Kreis value	6·7	—	0·8	6·8	2·1	46·8	4·7	2·24	½	¾
14	Iodine value	0·7	—	48·0	57·1	51·6	12·0	53·3	1·0(3)	1	1¼
15	TBA no.	3·5	68[b]	0·16	2·3	0·96	24·7	1·80	1·9	1	2

[a] Values as described under appropriate methods
[b] Assumed by Tarladgis.[12]
[c] The high C.V. for peroxide values is misleading when compared with other methods. A series of replicates between 0·0 and 0·8 may give a high C.V., but in interpretation such a range represents a satisfactory set of low figures, as values below 1·0 are considered in practice to be insignificant
[d] Excluding time of preparation of chloroform solution

Results and Discussion

The results obtained from FFA determinations showed steady increases as spoilage progressed with an almost complete absence of anomalous low values. Examination of the preliminary results in Table I indicates good agreement between replicates, good recoveries and high sensitivity ratio (2·53). The FFA figure was therefore applied to a large number of stored samples, together with the odour assessment (Method 6—Part I).[14] The correlation between the two results is shown graphically in Fig. 1. By considering these relationships, the approximate odour score can be calculated from the following formula:

$$\text{Odour score} = -1{\cdot}73 \log_e (\text{FFA}) + 7{\cdot}24$$
$$(r = -0{\cdot}69)$$

Using the results from the larger number of samples the sensitivity ratio becomes 2·32. Also from the correlation data, for acceptability (odour 7) the FFA of the extracted fat calculated as oleic acid should apparently not exceed 1·2%. The figure corresponding to odour 6 (just acceptable) however is much higher (2·1% FFA). As the range of the 90% confidence interval is considerably wider than with the TVN values, it is possible that a higher maximum figure than 1·2% could be considered as a practical acceptability limit.

In general the other fat spoilage values determined indicated an inconsistent pattern of rancidity formation. Firstly the iodine value must be rejected for quality assessment in view of the low sensitivity ratio. The three other fat values however would appear to be possible additional spoilage indicators. With the peroxide value, the high coefficient of variation between replicates is of less importance than with other methods. In most cases the fresh samples gave values of 1·0 mequiv. per kg or less, which is desirable, so that high coefficient of variation mean figures in some cases were derived from a small range of differing low values (e.g. between 0·0 and 0·8), which is insignificant from the viewpoint of practical interpretation. The disadvantage of employing peroxide and other values is brought out, however, by Fig. 2, which indicates the general trends during the spoilage period. In each case the values reach a maximum and then either remain constant or fall to zero. Consequently it may be possible to say that the peroxide value should not exceed say 5 mequiv./kg, but owing to the greater prominence of secondary reactions in the later stages of spoilage, very rancid fats sometimes produce misleading values. The incorporation of a peroxide value figure into a specification therefore would have to be associated with the rider: 'provided that the meat was organoleptically acceptable'.

A similar difficulty applies to the Kreis value, but this suffers from the additional difficulty of giving rather pronounced colours with comparatively fresh fats.

The TBA number may be more useful as a critical limit, although relatively high figures are given by some fresh samples, and again, in the later stages of spoilage the values may either fall away to zero (Fig. 2) or remain constant after a maximum is reached. The results reported in Table II, however, suggest that the TBA number assesses some forms of rancidity which are not necessarily measured in other methods. For its compilation the fat was cut off a piece of beef, and the lean and fatty portions were separately comminuted and stored at 5°. Except for the results obtained immediately after comminution, the lean portion gave considerably higher values than the fat stored in a similar way. This appears to bear out the claims of Tarladgis (personal communication) that the method possesses the advantage over other methods of rancidity assessment that, while solvents

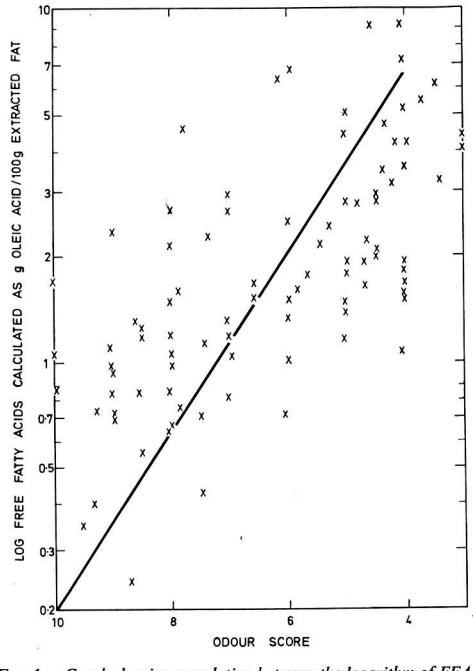

FIG. 1. *Graph showing correlation between the logarithm of FFA of the extracted fat and odour score for samples of beef assessed when fresh and after storage at 5°C.*
Odour = $-1{\cdot}73 \log_e$ (FFA) + 7·24

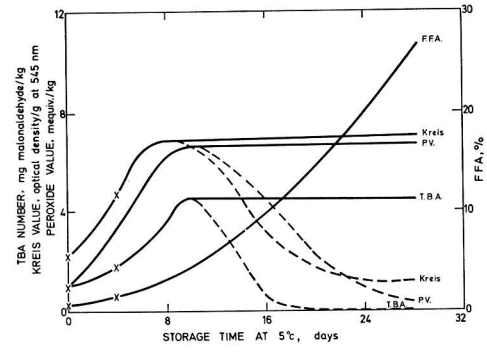

FIG. 2. *Graphs showing general trends in fat spoilage values of beef during storage at 5°C.*

only extract the neutral fat, TBA reacts with other lipids (including phospholipids), which play an important part in the oxidative deterioration. At the same time such findings make it more difficult to suggest a critical TBA limit of acceptability.

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APPLICATION OF CHEMICAL METHODS FOR THE ASSESSMENT OF BEEF QUALITY

IV.* Physicochemical and miscellaneous methods

By D. PEARSON

Methods which are used either to assess physicochemical changes during beef spoilage or to measure general effects not necessarily related to the breakdown of protein or fat directly are considered in relation to various criteria discussed in Part I. From the point of view of routine quality control, measurements of the extract-release volume and pH appear to be useful for spoilage assessment, provided that allowance is made for the differing results obtained during the *pre-rigor* period. From statistical correlations with odour scores most meats were considered acceptable provided that the ERV (extract-release volume, using the standardised method described) was at least 17 ml. The pH of the meat should preferably not exceed about 6·0. The joint use of total volatile nitrogen, free fatty acids, and ERV for deciding upon general acceptability in industrial control is considered.

Introduction

Several workers[1–3] have stressed the importance of pH in the study of changes which take place during meat spoilage. Immediately after slaughter the initial pH is about 7, but this falls to about 5·5 before rising in the *post-rigor* period to values above 6·0 and above as the meat deteriorates. The total acidity however does not show such marked alterations during storage. There is a marked increase in acidity in the immediate *post-mortem* period, but thereafter (as with the glycogen and reducing sugars) it appears to remain approximately constant[4,5]. Shank, Silliker & Goeser[6] have suggested that a study of the predominating volatile acids is important however with certain beef carcasses which have developed a sour non-microbial off-condition.

Barnes & Ingram[1] have shown that the oxidation–reduction potential in horse muscle falls after death, and they have described a standardised procedure for the determination. The greatest change however appeared to occur immediately after death. Colour changes produced in dyes such as methylene blue[7] and resazurin[8] due to alterations in the redox potential have also been recommended as spoilage indicators.

Working mainly with fish, Lang *et al.*[9] claimed that the determination of the volatile reducing substances (VRS), being a measure of the odoriferous components present, is superior for assessing odour. The figure obtained with one sample of ground round steak was also quoted. Truttwin[10] found that the direct titration with iodine of the total reducing substances in a cod macerate showed a steadily increasing value as the storage time progressed.

Hamm[11] discussed the various ways in which water may be held in muscle and showed that meat can, under certain conditions, imbibe large quantities. Grau & Hamm[12] and

* Part III: Preceding paper.

Wierbicki & Deatherage[13] measured the water-holding capacity (WHC) by calculating the area of water which diffused from meat into a filter paper under the influence of applied pressure. These workers showed that the WHC of freshly slaughtered meat is high, but it drops markedly within a few hours and then increases slowly during further storage. Hamm[14] attributed two-thirds of the *post-mortem* hydration drop to the breakdown of ATP and one-third to the fall in pH.

Jay[15] has developed a method for determining the extract-release volume (ERV) which is related to the water-holding capacity. The procedure is based on the volume of aqueous filtrate released from a slurry of meat in a fixed time. The ERV decreased as spoilage progressed and no filtrate at all was obtained with putrid meat.

Experimental

It was decided to apply the following methods to beef stored at 5° and consider them in relation to the criteria outlined in Part I: pH value; volatile acidity; direct titration with iodine; volatile reducing substances; extract-release volume.

Method 16 pH value

A dual spear glass and reference electrode was inserted directly into the prepared sample.

Method 17 Volatile acidity

The following procedure was adapted from the distillation method described by the A.O.A.C.[17]

50 g of prepared sample and 150 ml water were added to a weighed 500 ml conical flask, which was stoppered and shaken vigorously. Then 25 ml N sulphuric acid and about 40 ml 20% phosphotungstic acid (to precipitate protein) were added, and the contents were made up to 300 g with water. After vigorously shaking the mixture was filtered through a fluted filter paper (24 cm dia.). Then 150 ml filtrate were added to a 250 ml flask of a steam distillation apparatus. After being made acid to Congo red paper with sulphuric acid (1 vol. concentrated acid + 1 vol. water) the extract was steam-distilled so that exactly 200 ml distillate were collected in one hour (±5 minutes). The distillate was titrated with 0·01 or 0·05 N sodium hydroxide solution using phenolphthalein as indicator. After correction for the blank titration, the volatile acidity was calculated as 'ml 0·01 N alkali per 100 g sample'.

Method 18 Direct titration with iodine

10 g of the prepared sample were macerated in the 100 ml vortex beaker of an MSE Homogeniser with 50 ml water. The extract was then titrated, whilst being stirred magnetically, with 0·1 N iodine solution (using starch) until a permanent blue colour, stable for 15 min, was obtained. The result was calculated as 'ml 0·01 N iodine solution per 100 g sample' after correction for the blank.

Method 19 Volatile reducing substances

The closed-circuit apparatus used was essentially that described by Farber & Ferro,[18] but the procedure was applied to 10 g of a macerate of sample prepared by mechanically blending 25 g of prepared sample with 25 ml water. The flow-rate of 3 l/hour was checked by a Quickfit & Quartz air-flow meter inserted between the motor and aeration flask prior to addition of the sample.

Method 20 Extract-release volume

The procedure used was essentially that described by Jay,[15] but required modification because of the lack of British homogenisers of the required capacity:

Extraction reagent (0·05 M *phosphate buffer*)

50 ml 0·2 M-KH_2PO_4, 3·72 ml 0·2 M-NaOH, diluted to 200 ml. The pH (5·8) was checked before use.

Procedure

15 ± 0·1 g prepared sample was blended for 2 min with 60 ml extraction reagent in the 100 ml vortex beaker of a MSE Homogeniser. The homogenate was immediately poured into a filter paper (Whatman No. 1—18·5 cm dia.) folded thrice so as to make 8 sections, and the filtrate was collected at 21 ± 4°C in a measuring cylinder. The volume of filtrate collected 15 min. after the homogenate had been poured into the funnel was deemed the extract-release volume.

Results and Discussion

Fig. 1 shows the changes in pH values that occurred in stored samples of meat. A25 and B25 were comminuted from pieces of beef purchased from butchers and these showed a progressive increase in pH during storage. A26 was obtained from slaughterhouse meat and showed the normal *pre-rigor* drop to a minimum pH before the value rose as with other samples. Table I summarises the values obtained with 11 samples of beef stored at 5°. This indicates that for acceptability a maximum pH value of about 6·0 could be applied as a rapid test for supplies of *post-rigor* meat. In view of the simplicity of the determination of the pH value the method is obviously most useful for rapid factory checks. Further, the accuracy of the measurement (coeff. of variation of replicates 3·4%) tends to nullify the effect of the low sensitivity ratio, which is difficult to calculate in view of the differing values for fresh meat according to whether the *rigor* process has or has not been completed.

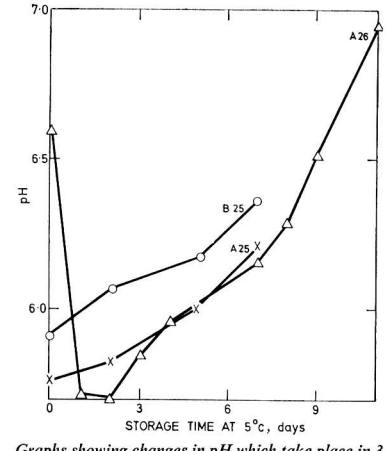

FIG. 1. *Graphs showing changes in pH which take place in 3 samples of beef during storage at 5°C.*

TABLE I

Data comparing the reliability of 5 miscellaneous methods employed for assessing the spoilage of beef (as applied to 11 samples stored at 5°C)

	Method	Coeff. of Variation between replicates (mean)	Values obtained with samples as received (fresh)				Values obtained at 'just spoiling' stage (mean)	Sensitivity 'just spoiling' value / 'fresh' value (mean)	Time of	
			min.	max.	mean	C.V.			1 determination	4 determinations
16	pH value	3·4	5·65	5·94[a]	5·79	5·4	6·1	1·05[a]	2 min	5 min
17	Volatile acidity	5·4	26	165	47	43·5	121	2·57	2 hours	3 hours
18	Titration with iodine	15·2	90	160	111	26·6	145	1·31	½ hour	1 hour
19	VRS	11·7	2·1	4·7	3·0	22·4	6·7	2·23	1½ hour	2 hours
20	ERV	3·8	14·1[a]	25·0	20·2	18·5	10·6	1·90[a,b]	½ hour	¾ hour

[a] Excludes one sample in which the *rigor* process was apparently incomplete
[b] Inverse ratio as ERV falls during spoilage

Also the ERV figures for A26 showed a rise, and, after rising to a maximum fell steadily as the meat spoiled during storage (Fig. 2). As all stored samples showed the steady fall in ERV values after the rigor process was completed (tentative 'inverse' sensitivity ratio 1·90), the method has considerable possibilities for use as a spoilage indicator. Subsequently the ERV was determined on a large number of stored samples and correlated with the odour assessment (Method 6[19]—Part I). The correlation is shown graphically in Fig. 3. By considering the relationships between the logarithms, the approximate odour score can be calculated from the following formula:

$$\text{Odour score} = 2{\cdot}07 \log_e (\text{ERV}) + 1{\cdot}23$$
$$(r = +0{\cdot}78)$$

Using the results from the larger number of samples the sensitivity ratio becomes 2·64. Because the ERV values fall during storage, however, such ratios are not really comparable with those from other methods. Also as with the pH value the ratio is more difficult to calculate because of the differing values for fresh meat according to whether the *rigor* process has or has not been completed. From the correlation it is apparent that, for acceptability, the ERV using the standardised method described should be at least 15·3 ml.

The volatile acidity and iodine titration values of fresh samples were found to differ too widely to be reliable for these methods to be used for spoilage assessments. A similar criticism applies to the VRS technique, but in addition, the final figures used are based on low back-titration differences of less than 1 ml 0·02 N sodium thiosulphate. Even with quite spoiled samples the back-titration differences are still quite small, so the accuracy of the method for meat is doubtful.

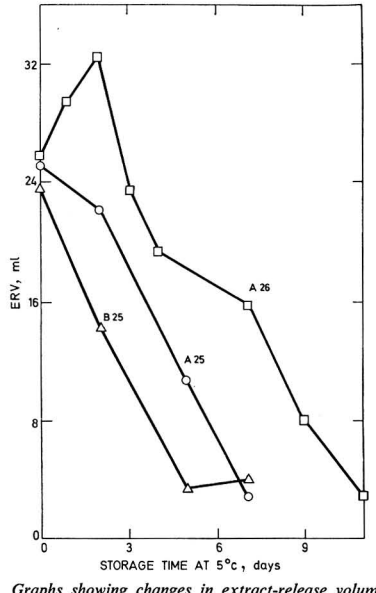

FIG. 2. *Graphs showing changes in extract-release volume which take place in 3 samples of beef during storage at 5°C.*

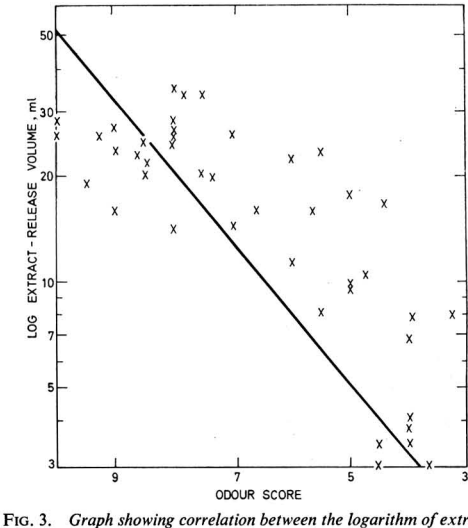

FIG. 3. *Graph showing correlation between the logarithm of extract-release volume and odour score for samples of beef assessed when fresh and after storage at 5°C.*
Odour = 2·07 $\log_e$ (ERV) + 1·23

TABLE II

Comparison of selected limiting values corresponding statistically to odours 6 and 7 with data obtained from acceptable beef examined on day of purchase

	Method	Limiting values corresponding to:		Range of values obtained for beef samples on day of purchase		
		Odour 6 (just acceptable)	Odour 7 (acceptable)	min.	max.	mean
7	TVN (mg N/100 g) (max.)	21·1	16·5	9·8	19·3	14·8
4,7	TVN/FF (mg N/100 g) (max.)	25·2	19·7	10·9	23·8	18·7
11	FFA of extracted fat (% oleic acid) (max.)	2·05	1·2	0·27	1·74	0·97
20	ERV (ml) (min.)	10·2	16·3	18·0	26·5	24·6

Conclusions

Table II quotes the figures for the selected values of total volatile nitrogen (TVN),[20] free fatty acids (FFA)[21] and ERV (above) corresponding statistically to 'just acceptable' odour 6 and 'acceptable' odour 7. Although some of the samples of freshly purchased beef (from ranges also quoted in Table II) did not 'comply' with values corresponding to odour 7, all meats were within the limits equivalent to odour 6. When apparently very fresh lean meat (with all outside fat cut off before comminution) was examined however, the FFA of the remaining intramuscular fat occasionally exceeded 2·05% (as oleic acid). As such samples contained only 4–8% total fat, the FFA present would not contribute markedly to the total acidity in the meat.

It would be preferable however if acceptability limits applied in practice corresponded to odour 7 rather than odour 6. Of 122 fresh and stored samples examined, all of the 73 meats with assessed odours below 7·0 gave one or more of the values of TVN, TVN/FF (fat-free) and FFA in excess of the odour 7 limiting figures quoted in Table II. On the other hand, 7 of the 49 apparently acceptable samples (with odour scores 7·0 or above) 'contravened' one of these limits corresponding to odour 7. Although in one instance there was higher TVN production with little spoilage of fat, the other 6 samples gave an FFA value above 1·2%. The lowest extract-release volume given however by these 7 apparently acceptable samples (19·5 ml) thus 'complied' with the minimum of 16·3 ml corresponding to odour 7 (Table II). Rapid increases in FFA with minimum protein breakdown occurred mainly in meats with a comparatively high fat content.

For critical acceptability limits to be applied in practice they must allow for each type of spoilage possibility:

(a) meats in which there is a concomitant increase in both TVN and FFA,
(b) meats in which protein breakdown predominates,
(c) meats in which fat hydrolysis predominates.

It is important therefore to have as basic criteria limits of TVN and FFA. To allow for meats in the (b) and (c) categories it should, from the above findings, be possible to pass samples which show values in excess of either the TVN or FFA limits (but not both) provided that the ERV is reasonably high. The author therefore considers that, for acceptability, beef should preferably not give figures in excess of the following:

TVN/FF .. .. 20 mg N/100 g
FFA of extracted fat .. .. 1·5% (as oleic acid)

Any meat which does not comply with one of these maxima can however, be considered to be acceptable provided that the ERV is at least 17 ml. If meats have a high TVN or FFA they are unlikely to be at the immediate post-slaughter stage, so difficulties in the interpretation of low values for *pre-rigor* meats are unlikely to arise. Similarly the pH would have advanced beyond the ultimate minimum value and a maximum such as 6·0 could be used as an additional or alternative criterion.

As different industrial organisations use beef for different purposes however the required standards may require some variation, and the actual values proposed here may be of less importance than the methods on which they are based. Having decided on which analytical methods to employ according to their high sensitivity ratio, reproducibility, etc., the most appropriate limits could then be decided upon after application to a large number of meats handled under industrial conditions.

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KINETIC STUDY OF THE RETROGRADATION OF GELATINISED STARCH

By RHONDA G. McIVER, D. W. E. AXFORD, K. H. COLWELL and G. A. H. ELTON

A method was developed for the study of starch retrogradation by differential thermal analysis. The kinetics of the crystallisation process were studied using the expression $\theta = \exp(-kt^n)$, where θ = the fraction of crystallisable starch remaining uncrystallised at time t. The values for the Avrami exponent (n) and the rate constant (k) were found to coincide with values previously obtained for the staling of bread, as followed by measurement of crumb elastic modulus. No difference between the time constants of gels cooled slowly and rapidly after gelatinisation was found, indicating that the nucleation process in starch crystallisation is instantaneous in the systems studied. The fact that the Avrami exponent is unity also suggests that the nucleation process is instantaneous, and that it is followed by rod-like growth of crystals.

Introduction

Because of its commercial importance, bread staling has been the subject of extensive research[1-3] and a considerable amount of evidence has accumulated to suggest that the major factor in such staling is increasing crystallisation of the starch component. Further evidence that crystallisation is responsible for staling has been obtained by Cornford *et al.*[4] who emphasised the importance of the negative temperature coefficient of the staling process and were able to show that the course of staling, as measured by changes in crumb elastic modulus, could be adequately represented by an equation of the Avrami type.[5-7] The Avrami equation has been widely applied to the crystallisation of high polymers and the fact that it can be applied to bread staling is strong evidence that bread staling depends on the crystallisation of some component of bread crumb. The analogy does depend, however, on the assumption that crumb elastic modulus is a linear measure of the extent of crystallisation.

Although there is thus considerable support for the idea that starch crystallisation is the major factor in bread staling, further evidence could clearly be obtained by extending the work of Cornford *et al.*[4] to determine the Avrami exponent and time constant for gels of starch and water in proportions approximating to those found in bread.

Changes in starch gels can be followed by the usual methods of measuring firmness.[8] However the firmness of a gel is an overall bulk property and assumptions would be involved in relating it to changes in the extent of crystallinity, as in the case of bread. A method of overcoming the problem is by the use of differential thermal analysis (d.t.a.). The crystallisation of starch is a thermal change which can be reversed, and d.t.a. will record the amount of heat required to 'melt' the retrograded starch and will indicate the temperature at which the phase change occurs. Very small samples are required, so replicates can be obtained from the same gel, and the parameter measured is proportional to the amount of crystallised starch in the sample.

Preliminary studies of d.t.a. of bread samples have been carried out by Axford & Colwell.[9] They found that, while an endothermic peak was not present in fresh bread, it developed during storage of the bread, the rate of increase in peak area being very similar to the increase in crumb firmness with time found by Cornford *et al.*[4] They also found that the endothermic peak was absent in a repeat run on the same sample of initially stale bread. This agrees well with the fact that bread can be re-freshened by being heated to above 70°, and it should be possible to demonstrate this with starch gels if the crystallisation of the starch fraction in bread is the major factor responsible for bread staling.

A great deal of information is available on the gelatinisation of starch, including some work on concentrated aqueous systems of wheat starch, although these latter systems have not been extensively studied. The subject of starch gelatinisation has been reviewed recently by Leach.[10] The roles of the amylose and amylopectin fractions of wheat starch during gelation have been examined by Schoch,[11] who concluded that the amylopectin fraction was chiefly responsible for the firming of bread during storage.

As has already been mentioned, since starch is a material of high molecular weight, it seems likely that its retrogradation kinetics can be studied by the theory of Avrami.[5-7] This theory has been shown to apply most accurately to the initial stages of the crystallisation when the nuclei are forming and growing.

If the area of the endothermic peak (A) obtained by d.t.a. of starch gels is a linear measure of the extent of crystallisation, then θ, the fraction of uncrystallised material, can be expressed by:

$$\theta = \frac{A_L - A_t}{A_L - A_0}$$

where A_L is the limiting peak area, A_t is the peak area at time t and A_0 is the peak area at zero time.

Substitution in the Avrami equation $\theta = \exp(-kt^n)$ gives:

$$\frac{A_L - A_t}{A_L - A_0} = \exp(-kt^n) \qquad (1)$$

where k is a rate constant.

$$\text{Hence } \log_e\left(\frac{A_L - A_t}{A_L - A_0}\right) = -kt^n \qquad (2)$$

$$\text{and } \log\left[-\log_e\left(\frac{A_L - A_t}{A_L - A_0}\right)\right] = \log k + n \log t \qquad (3)$$

When $\log\left[-\log_e\left(\frac{A_L - A_t}{A_L - A_0}\right)\right]$ is plotted against $\log t$, the Avrami exponent (n) is obtained from the gradient of the line, and the rate constant is obtained from the intercept, $\log k$. If n is found to have the value $n = 1$ within experimental error, then the best value for the rate constant can be determined from a graph of $\log_e (A_L - A_t)$ against t.

The Avrami exponent is a combined function of the number of dimensions in which growth takes place, and the order of the time dependence of the nucleation process (0 or 1). Table I shows the value of n for various types of nucleation and growth. The rate constant is a combined function of nucleation and growth-rate constants and its value can, in principle, be checked by independent determinations of the component constants by microscopy.[12] Although the Avrami exponent can be predicted when the modes of nucleation and growth are known, the usual procedure has been to use Avrami plots for determining mechanism.

The values of n do not have unambiguous meanings as many cases have been found of fractional n values which are significantly different from any whole number. Cases are also known where the Avrami analysis is misleading as checked microscopically. It is desirable to check that the data conform to the Avrami analysis over the full range of the readings. Within these limitations, the Avrami analysis is a guide to the mechanism of crystallisation.

TABLE I

Values for the Avrami exponent, n, for various types of nucleation and growth[12]

n	
$3 + 1 = 4$	Spherulitic growth from sporadic nuclei
$3 + 0 = 3$	Spherulitic growth from instantaneous nuclei
$2 + 1 = 3$	Disc-like growth from sporadic nuclei
$2 + 0 = 2$	Disc-like growth from instantaneous nuclei
$1 + 1 = 2$	Rod-like growth from sporadic nuclei
$1 + 0 = 1$	Rod-like growth from instantaneous nuclei

Information on the mode of nuclei formation can also be obtained by varying the rate of cooling during nucleation. If nucleation is sporadic, more rapid cooling will result in more nuclei being formed during the cooling period, and so crystallisation will proceed more rapidly, even though the gels are stored at the same temperature during the growth period. With instantaneous nucleation, however, the nuclei are generally impurities or more ordered regions in the system and their numbers may not alter appreciably with the rate of cooling. In this case, little or no difference in the rate of crystallisation would be observed.

Therefore, by measuring the rate of crystallisation after subjecting the freshly formed gels to slow and rapid rates of cooling, information on the nucleation process can be obtained.

Experimental

All the starch gels for the following experiments were prepared by mixing 500 g of wheat starch (moisture content 12·0%, as determined by heating at 130° for 1 h) with 380 ml water in a Hobart mixer until a homogeneous slurry was obtained. This slurry, which was of similar consistency to dough, was packed into plastic Petri dishes, care being taken to exclude all entrapped air. The starch was gelatinised by being heated in an 'Artica' microwave oven for 3·5 min.

Microwave heating was found to be quicker and easier than conventional oil-bath methods, and because of the rapidity of heating, it led to a lower moisture variation across the gel. The temperature of the gel immediately after heating was approximately 80°.

Because the Petri dish was completely filled with starch slurry, no moisture losses occurred during heating and so moisture transfer was kept to a minimum.

Crystallised starch in the gel was measured by d.t.a., the instrument used for all measurements being the Du Pont 900 Differential Thermal Analyser. The reference material in the heating block with the sample was a weighed quantity of ballotini (approx. 40 mg) and this was not altered during the course of the experiments. By altering the baseline slope adjustment, with each new thermocouple, an almost horizontal baseline was maintained.

The instrument was used at maximum sensitivity because of the small thermal changes being measured. At this sensitivity, according to the makers of the instrument, reproducibility of peak area from sample to sample is only expected to be within 5–30%, depending on the physical nature of the sample. By using a mains voltage stabiliser, early fluctuations were reduced, and to increase the accuracy of the experiment, 12–20 replicates were made of each reading.

Experimental factors affecting the d.t.a. of starch gels

To ensure that d.t.a. could be used to follow the ageing of starch gels as well as bread, the thermograms of fresh and aged starch gels were compared. An experiment was then performed to determine the effect on peak size of the weight of the sample and its moisture content and also to determine any significant differences between the Du Pont sample tubes.

A single starch gel prepared by the method described was stored at 21° in a sealed polythene bag to prevent moisture loss. After five days, thermograms were obtained by d.t.a. of 20 tared samples of the gel in five different sample tubes. The moisture content of each sample was determined after d.t.a. (in which the temperature did not rise above 80°) as the weight loss on heating at 130° for one h.

Since the shape of the endothermic peak was consistently sinusoidal, the area was defined by constructing a baseline connecting the highest points on either side of the inverted peak. Areas were measured to 0·1 cm² using a planimeter.

Ageing of starch gels

Starch gels prepared by the above method were stored at 21° and 4° in sealed polythene bags to prevent moisture loss. At 0, 1, 2, 3 and 7 days, gels from the 21° batch were examined by d.t.a., 20 replicate runs being used at each time interval. These were of sample weights ranging from 10 to 40 mg. The areas of the endothermic peaks were measured and graphed against sample weight. From this graph, the area for a 25 mg sample was determined and this was used as a measure of the crystallised starch present at that time. The limiting value of the peak area (A_L) was determined with a gel which had been stored at 4° for 14 days.

In this way, values for A_L, A_o and A_t were obtained and could be used to determine the Avrami exponent and time constant of the process.

Starch gels for the experiment involving different rates of cooling were cooled by two methods: in a refrigerator at 4° until the temperature of the gels had reached 21°, a period of 65 minutes; and in an insulated oven which initially was at 77° and was then allowed to cool naturally taking 6 h to reach 21°.

The experiment was repeated once.

Results

Experimental factors affecting the d.t.a. of starch gels

Method of measurement of peak size

The size of the peak is assumed to be a measure of the amount of crystallised starch. Thus size of peak would be expected to be directly correlated with sample weight, but it was decided to examine the correlation of various characteristics of the peak with the weight of sample used, in order to determine the best procedure for measuring this size.

The endothermic peaks obtained with various weights of an aged starch gel showed similarity in overall shape but differed in size. Measurements were made of several characteristics of the peaks and these were graphed against sample weight. A linear relationship was found in most cases, and the correlation coefficient and line of best fit were calculated from the data by the method of least squares. Those with the highest correlation coefficient based on 20 readings were area (0·97) and height (0·96) of peak. Because its use could more readily be justified on theoretical grounds, the former parameter was used in subsequent experiments.

Gel moisture content

To determine the effects of moisture variations within the gel and of drying out on the peak area, the relationship between peak area per unit sample weight and moisture content was examined. The moisture content of the samples varied between 48 and 54% with the majority falling in the range 50–52%. The graph was a scatter of points with a correlation coefficient of 0·09, based on 20 readings. It was concluded that moisture variations of this magnitude did not significantly affect the size of the endothermic peak and that any concentration effects or alterations in the rate of crystallisation caused by uneven moisture distribution, were outside the accuracy of the experiment.

Sample tube

Although sample tubes of different weights were used, no effect was found which was significant compared with the experimental error of the determinations.

Ageing of starch gels

The results of the peak area measurements on the ageing gels are shown in Fig. 1. It can be seen that they form an exponential curve similar to that obtained by Cornford *et al.*[4] for the staling of bread as measured by changes in elastic modulus of the crumb. The results for the two methods of cooling are shown on the same graph and it can be seen that the plots are very similar, any differences being less than the experimental error inherent in the method. Statistical analysis for significant difference between the two sets of data confirmed this conclusion.

Because of this, the four sets of data were treated as replicates of the same experiment and a mean of the four readings was used for the Avrami analysis. The value of A_0, the initial peak area, was found to be zero in all the gels studied and so Equation (3) becomes:

$$\log\left[-\log_e\left(\frac{A_L-A_t}{A_L}\right)\right] = \log k + n \log t$$

$\text{Log}\left[-\log_e\left(\frac{A_L-A_t}{A_L}\right)\right]$ was plotted against log t in Fig. 2, and $\log_e (A_L-A_t)$ against t in Fig. 3. In each case a linear relationship was obtained, and the equation of best fit was calculated by the method of least squares.

From Fig. 2, the Avrami exponent (n) was found to be 1·02. Thus within the experimental error, $n = 1$, and this value was used in plotting Fig. 3 as shown above.

From Fig. 3, the rate constant, k, was found to be 0·266 reciprocal days, giving a time constant ($1/k$) of 3·76 days.

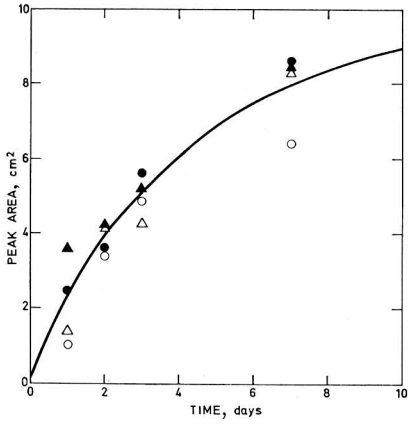

FIG. 1. *Ageing of 50% starch gels stored at 21°C*

	Run 1	Run 2
Cooled rapidly	▲	△
Cooled slowly	●	○

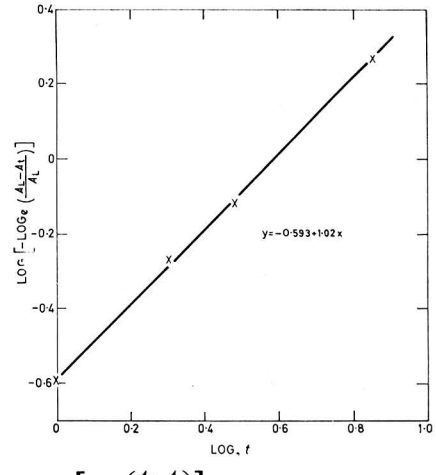

FIG. 2. Log $\left[-\log_e\left(\frac{A_L-A_t}{A_L}\right)\right]$ vs. log t for *50% starch gels stored at 21°C*

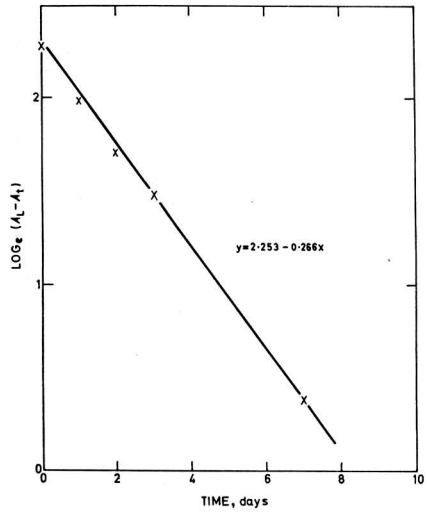

FIG. 3. $\text{Log}_e(A_L - A_t)$ vs. t for *50% starch gels stored at 21°C*

These values of the Avrami exponent and the time constant at 21° may be compared with those for bread at the same temperature. Cornford *et al.*[4] found that $n = 1$ in the case of bread while Axford *et al.*[13] found time constants of 3·28 and 3·68, depending on the breadmaking procedure used.

Discussion

During the ageing of starch gels of approximately 50% moisture content, the degree of crystallisation of the starch as measured by d.t.a. increases to a limiting value. The data collected for the area of the endothermic peaks can be fitted to a function in general agreement with Avrami's theory as applied to crystallisation. The exponent (n) and time constant ($1/k$) calculated by this method are in good agreement with the values found for bread. These results tend to confirm the role of starch retrogradation as the major factor in bread staling and the value of studies on starch gels as a model system for the staling of bread.

The value obtained for the Avrami exponent indicates that the mode of nucleation in starch crystallisation is instantaneous and that the growth of crystallites is in one dimension only. The occurrence of instantaneous nucleation is perhaps not unexpected, since when a concentrated starch slurry is heated to its gelation temperature, the resultant system is not homogeneous, and ordered regions probably remain. If the system is cooled, these ordered regions form nuclei for subsequent crystal growth and are effective as soon as the temperature of crystallisation is reached.

That no difference was found in the rates of staling of slowly- and rapidly-cooled gels indicates that the number of potential nuclei did not alter appreciably with the degree of supercooling as affected by the rate of cooling. The experimental results therefore support the suggestion that the nucleation process is instantaneous.

If the starch gels could be heated to a temperature where ordered regions are disrupted, and cooling led to sporadic nucleation, the rate of crystallisation would then depend on the rate of formation of nuclei as well as the growth rate of the crystals, and the rate of ageing would therefore be decreased.

The chemical species involved in the retrogradation of wheat starch have been considered by Schoch[11] who concluded that firming of bread was due to increasing order in the amylopectin remaining in the starch granules after the partial gelatinisation during baking, and not due to the amylose fraction which retrogrades immediately on cooling.

Kinetically, the retrogradation of the amylose fraction has a characteristic lag phase followed by a rapidly increasing reaction rate.[10] This is significantly different from the experimental results obtained here and suggests that the process observed was the retrogradation of the amylopectin fraction. In this laboratory, it has been found that starch gels of concentration 5–20% do not give an endothermic peak on d.t.a. after 14 days' storage at 4°, and this finding is in agreement with the results of Hellman, Fairchild & Senti.[14] In gels of this concentration it is possible that sufficient water is present to allow complete disorganisation of ordered regions of the starch capable of acting as nuclei. These ordered regions which were available in gels of higher starch concentration would not therefore be effective, and it seems likely that the nucleation and growth kinetics of the subsequent starch retrogradation would not be the same as in the gels studied in these experiments.

There is a great deal of work to be done on the effect of starch concentration on the mode of crystallisation, and this is at present being attempted in this laboratory together with investigations of the temperature-dependence of the retrogradation process.

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GLUCOSINOLATES IN SEED OF RAPE AND TURNIP RAPE AS AFFECTED BY VARIETY AND ENVIRONMENT

By E. JOSEFSSON and L.-Å. APPELQVIST

Seed meals of winter and summer types of varieties of *Brassica napus* and *B. campestris*, grown at different localities and in different years, have been analysed for content of glucosinolates which produce oxazolidinethiones and volatile isothiocyanates upon enzymic hydrolysis. Considerable differences existed in amounts of glucosinolates between species and between winter and summer types of the same species. Varietal differences were relatively small in most of the material studied. Samples of the Polish summer rape variety Bronowski and selections from this variety, however, exhibited very low glucosinolate contents; this is probably genetically determined.

Environmental variation generally amounted to ±15% of the average value. Under certain environments, however, 65% lower values than average were obtained.

Lack of sulphur in the growth medium of soil-free cultures resulted in seeds low in glucosinolates. In practical farming low glucosinolate content has been found in rape seed as a result of low sulphate content in the soil.

Introduction

Seed meals of rape and turnip rape contain 40–45% protein with a relatively well balanced amino acid composition.[1] Utilisation of such seed meals in animal nutrition, however, is limited by their high content of thioglucosides—now preferentially named glucosinolates. Major glucosinolates of rape and turnip rape seed are 3-butenyl glucosinolate[2–6] and 2-hydroxy-3-butenyl glucosinolate (refs. 6 & 7 and references cited therein) which upon enzymic hydrolysis give rise to the toxic products 3-butenyl isothiocyanate and 5-vinyl-2-oxazolidinethione, respectively.

Although the reduction of glucosinolate content in meal by industrial processes may be possible,[8] varieties with low glucosinolate content produced by plant breeding would be more economical in the long run. Plant breeding projects to develop such *Brassica* strains were therefore initiated.[9]

The present paper reports the glucosinolate content in seeds from genetic stocks of some European varieties of rape and turnip rape as well as the effect of polyploidisation, locality, soil type, levels of nutrients (nitrogen, phosphorus, potassium and sulphur), and of differences in growth conditions between different years on the levels of these compounds in selected seed materials. Some of the seed materials were also analysed for protein content.

Experimental

Materials

Seed samples were obtained from the following sources: Oil Crops Division and Cytogenetic Division, Swedish Seed Association, Svalöf; General Swedish Seed Company, Svalöf; Norddeutsche Pflanzenzucht, Germany; Ets. Ringot, La Chapelle d'Armentiéres, France; Agricultural Research Centre, Tikkurila, Finland and Instytut Hodowli i Aklimatyzacji Roślin, Poland.

Reagents were of analytical reagent grade. Diethyl ether was stored over potassium hydroxide and redistilled before use. Iso-octane was of reagent grade.

Myrosinase was prepared essentially by the method of Wrede.[10,11]

Analytical methods

All samples were analysed for glucosinolates according to the method of Appelqvist & Josefsson.[11] Glucosinolates producing volatile isothiocyanates were calculated as gluconapin (potassium 3-butenylglucosinolate) and those producing oxazolidinethiones as progoitrin (potassium 2-hydroxy-3-butenylglucosinolate).

In certain cases glucosinolates were also analysed by estimation of enzymically released sulphate. The sulphate was determined gravimetrically by precipitation as barium sulphate.

Crude protein (N × 6·25) was determined by the Kjeldahl method. Sulphur content of rape seed meal was estimated by the 'Magnesium nitrate method' of the A.O.A.C.[12] Content of soluble sulphate in soil was assayed by KCl (2%, w/v) extraction, barium sulphate precipitation, and gravimetric determination. The sulphur analyses were performed at Lantbrukskemiska Kontrollstationen, Kristianstad, Sweden.

Fertiliser experiments

Eighteen different combinations of nitrogen (at two levels), phosphorus (at three levels) and potassium (at three levels) were added, together with a common supply of other essential minerals, to soil-free cultures of summer rape cv. Regina II. Details of the experiment—hereafter referred to as the NPK experiment—are given in a paper presenting fatty acid data from the same seeds.[13]

Experiments in which all nutrients were kept constant except for sulphur were carried out in 1965 and 1966. Summer rape cv. Regina II was sown in the spring in 25 litre plastic boxes containing 2 kg Perlite (Deutsche Perlite A.G., Dortmund). The following chemicals were added to each box:

$Mg(NO_3)_2 . 6 H_2O$	16 g
$Ca(NO_3)_2$	37 g
K_2HPO_4	17 g
$Ca(H_2PO_4)_2$	17 g
$CaCO_3$	15 g
$Fe\text{-}Na_2\text{-}EDTA$	375 mg
$MnCl_2 . 4 H_2O$	10 mg
H_3BO_3	7·5 mg
$Cu(NO_3)_2 . 3 H_2O$	0·75 mg
$(NH_4)_6Mo_7O_{24} . 4 H_2O$	0·5 mg
Zn acetate	0·5 mg

Half the amount of each chemical was added at sowing, one fourth 35 days after sowing, and one fourth 45 days after sowing.

The only nutrient varied was sulphur, which was added as sodium sulphate. Five different quantities of sulphate were tested: 0, 0·5, 2, 8, and 32 g SO_4^{2-} per box in 1965 and 0, 0·5, 1, 2, and 8 g SO_4^{2-} per box in 1966. Four replications of each treatment were made. The boxes were kept in a glasshouse and the plants were watered with de-ionised water whenever it was considered necessary.

Results and Discussion

Svalöf varieties and breeding lines of *B. napus* and *B. campestris* grown under various conditions

Table I shows the glucosinolate content in seed meals of Svalöf varieties of rape and turnip rape. Since at the present time only one variety of summer turnip rape is marketed in Sweden, one strain of this type from the breeding material (Sv 58/4) was included in the study. The samples were taken from isolated multiplications of these varieties in different years and localities.

The results in Table I confirm the quantitative glucosinolate differences between species and types previously indicated.[11] Total quantities of glucosinolates were lower in turnip rape than in rape. Summer rape had less of both glucosinolate groups than winter rape, whereas summer turnip rape had less gluconapin but more progoitrin than the winter turnip rape. An approximately tenfold difference in progoitrin content was found between winter rape and winter turnip rape, the two dominating *Brassica* oil crops in Europe. Meal from winter turnip rape should thus be more valuable than meal from winter rape. At present the two crops are not distinguished in the trade.

As was expected, because the varieties within the same type of *B. napus* were closely related genetically, no significant inter-varietal differences were observed for the winter or summer rape. Similarly, only small inter-varietal differences were observed within the winter and the summer turnip rapes, with the possible exception of the progoitrin content of the two winter turnip rape varieties.

Results from studies of differences in glucosinolate content between different species and varieties have been reported by Wetter & Craig,[14] Appelqvist,[9] and Trzebny.[15,16] Wetter & Craig[14] and Trzebny[15] also studied the effects of environmental factors on glucosinolate contents. In these investigations the analytical methods of Wetter[17,18] were used, which are known to give figures for oxazolidinethiones that are about 60% of the true value for rape and turnip rape samples[11] and only about 40% of the true value for *Crambe abyssinica*.[19,11] For this reason a comparison of these earlier results with those of the present study is meaningful only with regard to gluconapin content.

Wetter & Craig[14] reported values similar to the present ones for glucosinolates producing volatile isothiocyanates in varieties of summer rape and summer turnip rape. Differences between varieties within species were not significant for either gluconapin or progoitrin. In a study of four winter rape varieties Trzebny[15] reported significant varietal differences in contents of both gluconapin and progoitrin.

The intra-varietal differences caused by environmental factors generally amounted to ± 15%, calculated on a variety mean (Table I). A single sample of the Victor variety of winter rape, however, contained considerably less of both glucosinolate groups compared with the other ten samples of that variety investigated (Table II). The phosphate fertiliser added for the last 15 years to the field from which this sample was harvested contained only 0·2% sulphur, whereas phosphate fertilisers with 10 or 13% sulphur are most commonly used in Swedish farming. A soil sample obtained from the same field in 1967 (actual seed sample in Table II being grown in 1963–1964) contained only 1·6 mg soluble sulphate per 100 g air-dried soil compared with an average of 3–6 mg for Swedish soils.[20] Two samples of Victor rape harvested in 1965 and 1967 on the same farm in fields which had received the same type of phosphate fertiliser low in sulphate contained 1·21 and 0·47% gluconapin respectively while their contents of progoitrin were 3·50 and 1·63%. The sample with the lowest glucosinolate content was obtained from the 'lightest' soil.

Earle *et al.*[21] found that two of the 30 samples of *Crambe abyssinica* investigated had only about half the 'normal' amount of *epi*-progoitrin. This difference was caused by environmental factors. Wetter & Craig[14] found significant differences between localities in Western Canada with

TABLE I

Content of glucosinolates in seed meals of Svalöf varieties of rape and turnip rape, grown at various localities in Sweden
% in dry matter

Species and variety or strain	Number of samples	Glucosinolate content					
		gluconapin		progoitrin		gluconapin + progoitrin	
		mean	range	mean	range	mean	range
Brassica napus, winter type							
Matador	3	1·71	1·56–1·89	4·69	4·62–4·73	6·40	6·29–6·58
Heimer	7	1·71	1·49–2·00	4·51	4·08–5·48	6·24	5·57–7·48
Victor	11	1·79	0·83–2·15	4·31	2·56–5·19	6·11	3·39–7·34
Brassica napus, summer type							
Regina II	8	1·27	0·87–1·60	3·04	2·60–3·68	4·31	3·72–5·06
Rigo	3	1·31	1·16–1·49	3·72	3·35–4·26	5·03	4·51–5·75
Brassica campestris, winter type							
Duro	3	3·10	2·95–3·20	0·22	0·14–0·29	3·32	3·09–3·49
Rapido II	3	2·95	2·84–3·09	0·50	0·25–0·65	3·44	3·09–3·71
Brassica campestris, summer type							
Bele	2	1·78	1·71–1·85	1·26	1·19–1·30	3·03	3·01–3·04
Sv 58/4	5	2·15	1·96–2·47	1·15	1·08–1·23	3·31	3·11–3·59

TABLE II

Content of glucosinolates and crude protein in seed meals from isolated propagations of Victor winter rape
% in dry matter

Cultivation conditions				Glucosinolate content			Protein content
Locality	Soil type	Year of harvest	Latitude °N	gluconapin	progoitrin	gluconapin + progoitrin	
Svalöf	Sandy clay	1963	56	1·78	4·44	6·22	45·6
,,	,, ,,	1964	56	1·82	4·26	6·08	43·2
Landskrona	,, ,,	1963	56	1·85	4·44	6·29	44·6
,,	,, ,,	1963	56	1·78	4·15	5·93	46·7
,,	,, ,,	1964	56	1·89	4·29	6·18	43·5
Mariestad	Light intermediate clay	1964	58	1·75	4·18	5·93	39·7
Norsholm	,, ,, ,,	1964	58	2·04	4·54	6·58	43·6
Björkvik	,, ,, ,,	1964	58	1·85	4·36	6·21	43·3
Kisa	Fine Mo-clay	1964	58	0·83	2·56	3·39	45·0
Linköping	Heavy (very heavy) clay	1964	58	2·00	5·01	7·01	43·2
Ekerö	Light intermediate clay	1964	59	2·15	5·19	7·34	44·1
	Mean	1963–1964	56–59	1·79	4·31	6·11	43·9
	Range			0·83–2·15	2·56–5·19	3·39–7·34	39·7–46·7

regard to progoitrin but not to gluconapin content. No significant tendency for different glucosinolate content between years could be observed in the present study, which is in accordance with the results of Trzebny.[15]

The correlation between gluconapin and progoitrin content in the winter rape and summer rape material was calculated. A strong positive correlation ($r = +0{\cdot}94$, $P < 0{\cdot}0005$) was found for the 21 samples of winter rape. A positive correlation for the content of the two glucosinolate types in the 11 samples of summer rape ($r = +0{\cdot}43$), however, was not significant. Environmental factors probably affected the amounts of the two major glucosinolate groups in the same way, but further calculations on similar seed materials are necessary before any definite conclusion can be drawn.

Miscellaneous European varieties of *B. napus* and *B. campestris* grown under various conditions

Analyses of some samples of the German winter rape variety Lembke, grown in West Germany in different years and localities, are presented in Table III. Variation in the content of total glucosinolates in this material was about ± 10% for both years and localities.

In the collection of important European winter rape varieties studied, samples of cv. Sarepta and Nain de Hamburg showed lower glucosinolate contents than the others (Table IV). Although this reduction may have been caused by varietal differences, environmental effects, as exemplified by cv. Victor (Table II), may have been important. The varied results for Sarepta might be explained in this way.

A large collection of *Brassica napus* samples compiled from various sources, was analysed in efforts to obtain low glucosinolate types. The lowest glucosinolate content in any rape seed material investigated was found in the Polish summer rape variety Bronowski. To determine if the low content was characteristic of the variety or caused by environmental factors, further samples of this variety were analysed. The results are summarised in Table V. Two of the samples showed considerably higher values than the others, but even so the values were lower than for other summer rape material. Whether these two exceptions resulted from out-crossings with high glucosinolate types or from special environmental conditions has not yet been definitely established.

TABLE III

Content of glucosinolates in seed meals of winter rape, var. Lembke, cultivated under different conditions in Germany
% in dry matter

Growing conditions	Glucosinolate content		
	gluconapin	progoitrin	gluconapin + progoitrin
a) Cultivated at the same locality in different years			
1962	2·04	4·10	6·14
1963	1·49	3·81	5·30
1964	1·56	3·81	5·37
1965	1·89	4·50	6·39
Mean	1·74	4·06	5·80
b) Cultivated at different localities in 1965			
Schleswig-Holstein, Plön	1·64	4·14	5·78
Schleswig-Holstein, Segeberg	2·11	4·50	6·61
Westfalen	2·15	4·90	7·05
Mean	1·97	4·51	6·48

TABLE IV

Content of glucosinolates in seed meals of some European varieties of rape and turnip rape, grown at various localities

% in dry matter

Species and variety	Locality	Year	Glucosinolate content		
			gluconapin	progoitrin	gluconapin + progoitrin
Brassica napus, winter type					
Rapol	Germany	1965	1·66	4·92	6·58
Diamant	,,	,,	1·45	4·98	6·43
Tonus	Nord, France	,,	1·73	4·16	5·89
Nain de Hamburg	,, ,,	,,	1·21	2·64	3·85
Sarepta	,, ,,	,,	1·10	2·81	3·91
,,	Versailles, France	unknown	2·30	3·28	5·58
,,	Svalöf, Sweden	1961	1·38	2·94	4·32
Valois	Versailles, France	unknown	2·22	5·55	7·77
,,	Svalöf, Sweden	1961	1·43	5·45	6·88
Dublański	,, ,,	1965	1·81	4·12	5·93
Niemierczański	,, ,,	,,	2·00	4·73	6·73
Skrzeszowicki	,, ,,	,,	1·77	4·04	5·81
Warszawski	,, ,,	,,	1·87	4·46	6·33
Poświcki	,, ,,	,,	2·06	4·68	6·74
Górczański	,, ,,	,,	2·03	4·30	6·33
Brassica napus, summer type					
Zollerngold	France	1965	1·76	3·18	4·94
Czyżowskich	Czechoslovakia	,,	1·50	4·05	5·55
,,	Svalöf, Sweden	,,	1·27	3·78	5·05
Mtochowski	Czechoslovakia	,,	1·80	3·59	5·39
Brassica campestris, winter type					
Gruber	Tikkurila, Finland	1965	3·84	0·46	4·30
Rapido I	Hahbiala, Finland	,,	3·07	0·57	3·64

TABLE V

Content of glucosinolates in seed meal samples of Bronowski summer rape and offspring from that variety

% in dry matter

Sample	Growing conditions	Glucosinolate content		
		gluconapin	progoitrin	gluconapin + progoitrin
I.L. 1997	Unknown (received from Poland)	0·15	0·79	0·94
I.L. 2243	Unknown (received from Czechoslovakia)	0·87	2·78	3·65
Bronowski IHAR	Grown in Poland 1967	0·11	0·53	0·64
Sv 64–1055	Grown in field at Svalöf in 1964	1·29	2·35	3·64
Sv 67–9018$_{1-11}$	Individual plants, grown in greenhouse at Svalöf	0·07–0·36	0·10–0·36	0·17–0·58
Bronowski IHAR	Grown in France	0·06	0·12	0·18
Bronowski 2178 L 5	,, ,, ,,	0·05	0·15	0·20

Detailed studies on samples low in glucosinolates

Soil analysis showed that the low glucosinolate content in cv. Bronowski did not result from a low sulphate concentration in the soil, as was the case of the Kisa sample of cv. Victor. Analysis of a soil sample from the greenhouse chamber where the plants Sv 67–9018$_{1-11}$ (see Table V) were grown showed a content of 23·0 mg soluble sulphate per 100 g air-dried soil, which is a higher value than that which is common in Swedish soils (3–6 mg).[20] Furthermore, five strains of other summer rape material grown in glasshouse compartments adjacent to the one containing Sv 67–9018$_{1-11}$ had 0·84–1·49% gluconapin and 4·33–5·23% progoitrin calculated on a dry matter basis. The sulphate content of the soil cannot have been limiting to the glucosinolate production which is more likely to be caused by a genetic difference.

To determine if the content of glucosinolates producing isothiocyanates carrying the CH_3SO- group on the side chain—not measured in the present analysis[11]—were greater in Bronowski summer rape and Victor winter rape (at Kisa, Table II) than in the high glucosinolate samples, enzymically released sulphate was determined. A comparison between the two methods shows good agreement (Table VI). Thus there cannot have been a large increase in content of glucosinolates with a CH_3SO-group in this material. Further studies with the aid of paper chromatography (see ref. 6 for details) revealed that the glucosinolate pattern of a sample of Bronowski was the same as that of the summer rape variety Regina II.

In the meal of the 1967 Polish grown Bronowski seed sample the total sulphur content was only 0·72% of the dry matter compared with 1·55% for a Regina II sample containing average amounts of glucosinolates. Bronowski summer rape, therefore, might have a genetically determined reduced uptake or inter-plant transport of sulphur. Presently, studies are being undertaken at this laboratory to throw some light on this interesting problem.

TABLE VI

Determination of enzymatically released aglucones and sulphate in two seed samples low in glucosinolates
μ moles/g dry matter

Sample	Aglucone determination			Sulphate determination
	gluconapin	progoitrin	gluconapin and progoitrin	SO_4^{2-}
Bronowski IHAR summer rape, grown in Poland 1967	2·7	12·5	15·2	15·9
Sv Victor winter rape, grown at Kisa, Sweden, 1964	21·1	60·0	81·1	81·6

Effect of polyploidisation and large climatic differences on glucosinolate content

To compare glucosinolate content of the same varieties cultivated at localities with extremely different climatic conditions, seed of some varieties of *Brassica campestris* which had been grown in Sweden and Turkey were analysed. Both diploid and tetraploid types of the actual varieties were included in the investigation (see Appelqvist[22] for details). The results of this study are shown in Table VII. Although no clear difference between localities was found, a trend to higher glucosinolate content in tetraploids than diploids was observed. Statistically this difference could be attributed to progoitrin content ($P < 0{\cdot}05$) and not to gluconapin content ($P > 0{\cdot}2$).

No significant difference between localities in protein content of the seed meal was observed. In most cases the tetraploids showed higher protein contents than the corresponding diploids, which is in accordance with results obtained in other studies of turnip rape, black mustard, white mustard,[23] and other species.[24,25]

Fertiliser experiments

To elucidate the significance of fertilising as an environmental factor, a constant amount of sulphate was added and the amounts of N, P, and K fertilisers were varied, or the amounts of N, P, and K were kept constant and the amount of sulphate was varied.

The results of the fertiliser experiment on summer rape, variety Regina II, with variation of N, P, and K showed some variation in glucosinolate content with different nutrient combinations, the range for gluconapin content being 1·64–2·26% and for progoitrin 3·32–4·80% of the dry matter. The differences, however, were very irregular and no clear trend could be observed, which is contrary to the results of Trzebny,[15] who found in a field experiment with winter rape that a high rate of nitrogenous fertilisers caused a decrease of content of volatile isothiocyanates and oxazolidinethiones. It should be observed that in the present experiments two nitrogen levels in soil-free culture were used, while Trzebny[15] compared seeds from field experiments with and without added nitrogen fertiliser. Eaton[26,27] reported that 'minus

TABLE VII

Content of glucosinolates and crude protein in seed meals of diploid and tetraploid strains of *Brassica campestris* cultivated in 1960 at Izmir in Turkey and at Svalöf in Sweden
% in dry matter

Type and variety	Level of ploidy	Locality	Glucosinolate content			Protein content
			gluconapin	progoitrin	gluconapin + progoitrin	
Winter turnip rape						
Lembke	2n	Svalöf	3·42	0·18	3·60	37·3
		Izmir	3·13	0·43	3·56	39·2
	4n	Svalöf	4·36	0·43	4·79	41·8
		Izmir	4·07	0·43	4·50	41·0
Vilnensis	2n	Svalöf	2·69	0·61	3·30	40·0
		Izmir	2·58	0·61	3·19	40·8
	4n	Svalöf	3·49	0·90	4·39	41·3
		Izmir	2·87	0·79	3·66	38·8
Mean, 2 n			2·96	0·46	3·42	39·3
Mean, 4 n			3·70	0·64	4·34	40·7
Summer turnip rape						
Bele*	2n	Svalöf	1·89	1·19	3·08	36·8
		Izmir, sown in spring	2·18	1·23	3·41	40·4
		Izmir, sown in autumn	2·22	0·94	3·16	37·4
Strubes*	4n	Svalöf	1·93	1·59	3·52	42·1
		Izmir, sown in spring	1·96	1·88	3·84	43·1
		Izmir, sown in autumn	1·67	2·02	3·69	37·1

* Derived from Strubes diploid

N' leaves of *Brassica nigra* plants were more pungent than 'plus-N' ones, the former leaves being much smaller than the latter. Because the larger leaves had much less sulphur available per unit of tissue, the lower isothiocyanate content in these 'plus-N' leaves could result from 'dilution'. Allcroft & Salt[28] found an increase in the goitrogenic effects of thousandheaded kale grown in the field when sodium nitrate was added to the soil, while no such increase occurred when ammonium sulphate was used as fertiliser. Similar results were obtained by Sedlák *et al.* in a study on cabbage goitrogenicity.[29] Pryor[30] reported that deficiency of sulphur lowered the content of volatile isothiocyanates greatly in some cruciferous species while nitrogen starvation did not. The data discussed above might indicate that the relation between amounts of available sulphur and nitrogen is more important in determining glucosinolate content than the quantities of available sulphur and nitrogen *per se*. Further experiments are needed to elucidate the effect of various fertilisers on content of glucosinolates.

In the sulphur nutrition experiment of 1965 the plants which received no sulphate fertiliser developed very weakly and yielded no seed. The various amounts of sulphate given to the boxes gave no significant differences in development or seed yield of the plants. In 1966 the plants which had been raised on sulphate-free nutrient solution grew better than corresponding plants in 1965 and yielded seed, but the harvest was lower than from the plants which had been given sulphate (Fig 1).

The results of the 1965 experiment indicated that the glucosinolate content was constant when the quantity of sulphur exceeded 8 g SO_4^{2-} per box. The differences between the various sulphate concentrations were reduced and the strongest concentration was excluded in the 1966 experiment (Fig. 1).

As may be seen in the figure, the seed harvested from those boxes to which none or a very low amount of sulphate fertiliser had been given, contained very small quantities of glucosinolates. The glucosinolate contents increased successively with larger quantities of sulphate until a constant level was reached at 2·0 g SO_4^{2-} per box. The values at the constant level were about the same as those obtained from the same variety grown in the field.

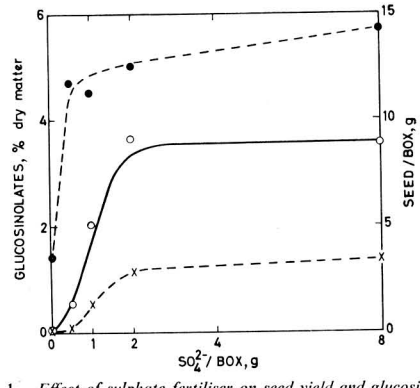

FIG. 1. *Effect of sulphate fertiliser on seed yield and glucosinolate content of summer rape*
●———● Seed
○———○ Progoitrin
×———× Gluconapin

It should be remarked that sulphur-containing fertilisers are not the only sources of sulphur for plants. In the present experiments for example sulphur may have been obtained from minor impurities in other chemical used. Furthermore the sulphur-rich seeds sown contribute to the supply of sulphur for the growing plant. The sulphur dioxide in the air is a further source from which the plant can obtain this element.[20,31]

In their investigation on effect of sulphate fertilising on content of thiocyanate-producing glucosinolates in fresh cabbage, Sedlák *et al.*[29] obtained results similar to those of the present study. An indication of increasing glucosinolate content was observed, however, at a sulphate level where in the present study the amounts of glucosinolates were constant.

In a study of *Brassica nigra* plants Eaton[26] found that leaves of plants grown in a 'minus sulphur' nutrient lost most of their ability to yield volatile sulphur compounds.

According to Johansson[20] the soils in Sweden usually contain 1–2 mg of easily accessible sulphur per 100 g soil. This is less than the sulphur content in the boxes given 0·5 g SO_4^{2-} in the present experiment, which yielded very small quantities of glucosinolates. Much larger quantities of sulphur are bound to the organic substances in the soil and are continually transformed to inorganic sulphate. Even if the sulphur dioxide in the air is taken into account it is possible that in certain cases some of the variance in the material in Tables I–IV may be caused by low contents of sulphate available in the soil. In selection experiments for plants with low content of glucosinolates, therefore, it seems important to add sulphate fertilisers in order to keep accessible sulphate at optimum levels.

Seed yield and protein content in all boxes given sulphate was about the same (Table VIII), which indicates that the plants utilise the sulphur for protein synthesis when only small quantities of sulphur are available. Another indication of a directed sulphate utilisation is that the proportion of progoitrin to gluconapin was about 3 : 1 in seed obtained from the boxes with 1–8 g SO_4^{2-} but 8 : 1 in the boxes with 0·5 g SO_4^{2-}. This agrees with the results of the experiment in 1965. In the box with no sulphate fertiliser the proportion was 2·2 : 1; however, the values were so low for both substances that the relative analytical errors were considerable. The high quotient at the low sulphate level may indicate that the two groups of substances are synthesised from common sulphur-containing precursors and that the introduction of the OH group is a relatively late step in the biosynthetic chain. Lack of sulphur would depress the biosynthesis of that part of the molecule which is common to the two groups of substances, but not the hydroxylating process. This is in accordance with the pathway for the biosynthesis of glucosinolates proposed by Underhill & Wetter.[32]

TABLE VIII

Content and yield of crude protein (% in dry matter) in seed meal from Regina II summer rape, grown in soil-free culture and fertilised with various amounts of sulphate

g SO_4^{2-}/box	Crude protein content	Yield of crude protein g/box
0	37·1	0·91
0·5	45·6	3·05
1·0	48·1	3·41
2·0	49·6	3·81
8·0	48·0	4·26

Since the 'crude protein' values in Tables II and VIII were obtained by using the Kjeldahl method, which is a measure of the total nitrogen content that could be converted to ammonia by treatment with sulphuric acid, other analyses have to be performed to evaluate any differences in content of true protein. Eaton[27] reported that sulphur-deficient plants accumulated ammonia, amino acids, amides and nitrates. Work is in progress to evaluate the quality of the protein of seed meals from Bronowski rape as well as from rape grown in sulphate-deficient medium.

Conclusions

Many problems remain to be solved in connexion with hereditary and environmental influences on glucosinolate content. Proof has been obtained that environmental factors occasionally exercise a strong influence. One factor of great importance in soil-free cultures is the amount of available sulphate in the growth medium. Indications that sulphate availability may be of significance in practical cultivation also exist. Whether it is possible to lower the glucosinolate content of oil crops in practical farming through cultural methods remains to be established.

There is some evidence that varieties of oil crops with low, genetically controlled glucosinolate content exist. Successful selection for low content of glucosinolates producing volatile isothiocyanates has been reported by Troll[33] for rape seed and by Schröck[34] and Lamberts[35] for turnip. Studies on content of thiocyanate-producing glucosinolates in fodder rape revealed that there was a correlation in content between individual plants and their inbred offspring.[36] If genetically controlled low-glucosinolate types can be isolated, they may be used in a plant breeding programme to combine this character with a desirable oil quality[37] and good agronomic properties.

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DISTRIBUTION OF FATTY ACIDS IN LIPIDS AS AN AID TO THE IDENTIFICATION OF ANIMAL TISSUES

I.—Bovine, porcine, ovine and some avian species

By A. W. HUBBARD and W. D. POCKLINGTON

Fat was extracted from various cuts of meat, from domestic and foreign sources. The majority of the samples were of pork, beef and lamb, but specimens from rabbit, various species of poultry, and three African ruminants, were also prepared.

The fatty acid compositions of the samples were determined by gas-chromatography of their methyl esters, on polar and non-polar stationary phases.

The use of such results both for the identification of the animal source of meat, and of the purity of lard samples, is discussed. It is suggested that the presence of lard in certain branched-chain fatty acids, characteristic of ruminant fat, provides evidence of adulteration with beef or mutton tallow.

Introduction

The Food and Drugs Act, 1955, the Merchandise Marks Act, 1887–1953, and classification for Customs tariff purposes, all require analytical means of detecting infringements and irregularities, particularly the substitution or adulteration of one meat with another visually identical meat in processed products.

Serological methods have long been used to distinguish between the tissues of various animal species. Oswald[1] recently used serological methods for the detection of horseflesh in beef products, duck egg albumin in hen egg albumin, and the substitution of one fish species for another. The wider application of serological techniques is limited by the restricted range of anti-sera available, and the denaturing of the serum due to processing.

The present investigations aim at establishing whether the distribution of fatty acids in the glyceridic fraction of lipids (simply extracted or rendered) from raw and cooked animal tissues could provide a means of establishing the identity of the meat; and in addition, whether there were sufficient differences in the distribution to detect adulteration in commercial animal fats, since confirmation of the authenticity of such fats, based on conventional chemical and physical methods, seldom permits an unequivocal opinion to be given.

The second objective was to develop a simple rapid procedure for the quantitative estimation of fatty acids which could be used by the enforcing authorities.

Experimental

Sampling

Samples of fatty tissue were cut from various locations on the carcasses of animals, home produced and imported, as detailed later in Tables III–VI. The fatty tissues were dissected from the muscle tissue and rendered at 100° and the fat carefully separated from water and insoluble matter. Processed meats, dehydrated with anhydrous calcium sulphate, were extracted with petroleum ether (b.p. 40–60°).

Conversion to methyl esters

The choice of method was largely dictated by the nature of the fat. At the start of the investigation of the methods available for the conversion of fatty acids to methyl esters four were in general use, viz. employing diazo methane,[2] methanol and hydrochloric acid with micro sublimation,[3] methanol and hydrochloric acid on ion exchange resin,[4] and boron trifluoride–methanol.[5] The methods are comparable for the higher molecular weight fatty acids, but as a routine method for the preparation of methyl esters from animal fats (excluding milk) the method of Stoffel, Chu and Ahrens,[3] slightly modified, was found to provide a speedy and quantitative procedure and was employed throughout these investigations.

Approximately 100 mg of fat were dissolved in 1 ml benzene, and refluxed with 10 ml 0·2 N hydrochloric acid in methanol for 30–40 minutes. The methyl esters were recovered by extraction of the residue remaining after evaporation of the solvents. Recoveries of methyl esters from the esterification of standard mixtures of pure fatty acids containing more than 10 carbon atoms were close to 100%. For caproic acid recovery was 95%, but for caprylic acid recoveries as low as 65% were obtained.

Apparatus

The majority of the analyses were carried out on a Pye Argon Chromatograph (β-ionisation detector) and the methyl esters injected directly on the column, using glass micropipettes. For the later work, a Perkin-Elmer F11 instrument with a flame ionisation detector was employed. In this case the methyl esters, in ether, were injected by means of a Hamilton syringe into an injection block maintained some 30–40° above the temperature of the column.

Stationary phases, packing of columns and operating conditions

The 1·2 metre columns of the Pye Argon chromatograph were packed using a PIFCO massager as vibrator in the

conventional manner. The non-polar packing was 5% w/w Apiezon L grease on 100/120 mesh acid/alkali washed Celite with an Argon flow rate between 50–60 ml/min at an operating temperature range of 185°–200°. This column resolved all the mono-unsaturated esters from the analagous saturated esters, but failed to separate 18 : 2* from 18 : 3, and 20 : 4 from 20 : 5. For acids in the C_{18} region a polar column packed with 20% w/w 1,4-butane-diol succinate on 100/120 mesh acid/alkali washed Celite, operating at 185°, was employed.

The Perkin Elmer F11 instrument was employed with a 2·3 mm o.d. 2 metre stainless steel column packed in a similar manner with 8% w/w 1,4-butane-diol succinate polar stationary phase. This column was operated at 160° with a nitrogen inlet flow rate between 20–25 ml/min. The Perkin Elmer F11 chromatograph was used to determine the C_{14} and C_{16} branched chain fatty acids, the Pye Argon chromatograph for the determination of the C_{15} branched chain fatty acids. Elution times for the F11 instrument were much shorter and while the resolution was equal to or better than those obtained with the Pye Argon Chromatograph, there was a tendency for the esters with prolonged retention times to give tailing peaks. On the Pye Argon Chromatograph symmetrical peaks were obtained even after retention times of up to 6 hours. 1,4-butane-diol succinate was preferred to polyethylene glycol adipate and diethylene glycol succinate on account of its better stability and the marked reduction in conditioning time. Columns were conditioned before use in a separate heating jacket run at normal operating temperatures with a slow flow of nitrogen.

To prolong the useful life of the columns the heating units were switched off by means of a time switch immediately after the last analysis of the day, and switched on 2–3 hours before commencing work the following day.

Quantitative assessment of chromatograms

Standard mixtures of pure fatty acids were prepared and methylated, and peak areas were measured from the product of (peak width at half height) × (height).

Table I outlines typical results obtained from chromatographing standard mixtures of four and five component fatty acids, each present in differing concentrations. Since the composition calculated from the peak areas was found to be in close agreement with the actual proportions taken area response factors were not applied and the figures given later in Tables III–VI represent the peak area percentages of the component fatty acids (as methyl acids).

Although some authors[7] have claimed that the argon ionisation detector may not be linear over several orders of magnitude for certain organic compounds others[8,9] have claimed that a linear response to fatty acid methyl esters can be obtained with this detector. In the present experiments the argon ionisation detector was found to be linear for all practical purposes and in Table II the means and standard deviations of nine replicate determinations of the various fatty acids in a specimen of lard adulterated with tallow are shown.

TABLE I

Typical responses of the β-ionisation detector to standard mixtures of fatty acid methyl esters

Fatty acid methyl ester	% by weight	Peak area, %	% difference	% error
12 : 0	0·10	0·10	—	—
	0·50	0·50	—	—
14 : 0	0·050	0·047	−0·003	−6·0
	2·00	2·13	+0·13	+6·5
	5·00	5·17	+0·17	+3·4
15 : 0	1·00	1·01	+0·01	+1·0
16 : 0	20·0	21·4	+1·4	+7·0
	32·0	32·6	+0·6	+1·9
17 : 0	1·50	1·42	−0·08	−5·3
18 : 0	10·0	10·0	—	—
	30·0	29·1	−0·9	−3·0
18 : 1	20·0	19·2	−0·8	−4·0
	52·0	51·0	−1·0	−1·9

Detector voltage: 1000/1250V; amplifier attenuation X3/10;
Sample volume: 0·025–0·1μl; column conditions as in text.

TABLE II

Means, standard deviations and coefficients of variation of nine replicate determinations* of various fatty acids in a sample of 'lard'

Fatty acid	Mean m%	Standard deviation s	Coefficient of variation, 100 s/m
14 : 0br	0·014	0·002	14
16 : 0br	0·049	0·012	24
10 : 0	0·065	0·012	18
14 : 1	0·10	0·01	10
12 : 0	0·12	0·03	25
15 : 0br	0·13	0·02	15
15 : 0	0·14	0·02	14
20 : 2	0·39	0·06	15
17 : 0	0·41	0·05	12
18 : 3	0·43	0·04	9
17 : 1br	0·56	0·08	14
20 : 1	0·94	0·13	13
14 : 0	1·9	0·2	11
16 : 1	2·7	0·3	11
18 : 2	6·9	0·3	4
18 : 0	18·4	0·5	3
16 : 0	27·0	0·5	2
18 : 1	39·7	0·5	1

* The nine replicates consisted of three determinations by each of three operators and in most cases the 'between operator' variance was significantly greater than the 'within operator' variance. These components are combined in the standard deviations quoted.

Figs 1–4 illustrate typical chromatograms of pork and beef fat obtained with high orders of detector sensitivity or amplifier gain. These provide measurable peak areas for accurate assessment of the minor component fatty acids. For complete analysis a further chromatogram with all peaks on scale was run on the polar column and the on-scale peak areas from each of the related chromatograms intercalated. The major source of error was in peak width measurement. Narrow peak widths were generally calculated from a similar peak run with a greater chart speed. Where samples were run consecutively, it was found that there was very little change in peak widths even over several days. This enabled a standard set of peak widths to be adopted for a limited period, and resulted in a considerable saving of

* Notation: Acids or isomeric mixtures are represented by numbers indicating chain length and the number of double bonds without specific reference to the position of double bonds. Branched chains are indicated by 'br'.

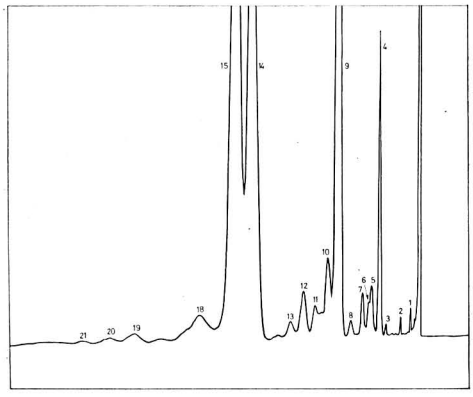

FIG. 1. *Fatty acids of beef fat*
8% 1,4-Butane-diol succinate at 160°C

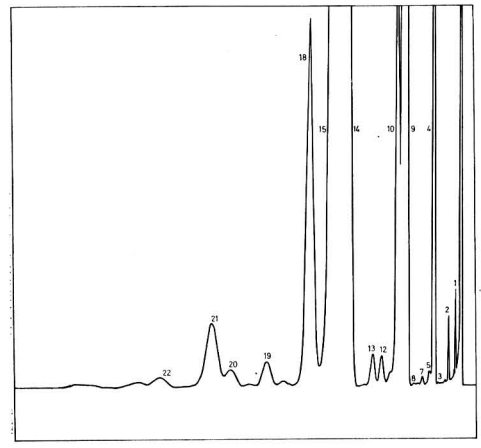

FIG. 2. *Fatty acids of pork fat*
8% 1,4-Butane-diol succinate at 160°C

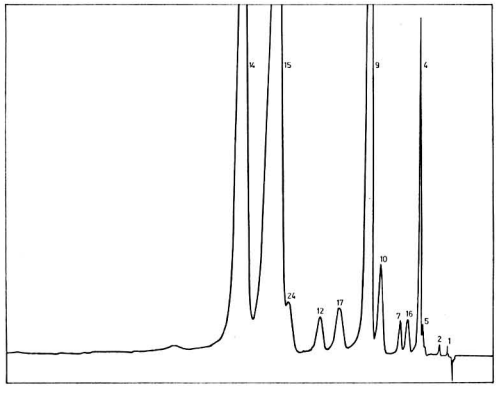

FIG. 3. *Fatty acids of beef fat*
5% 'Apiezon L' at 185°C

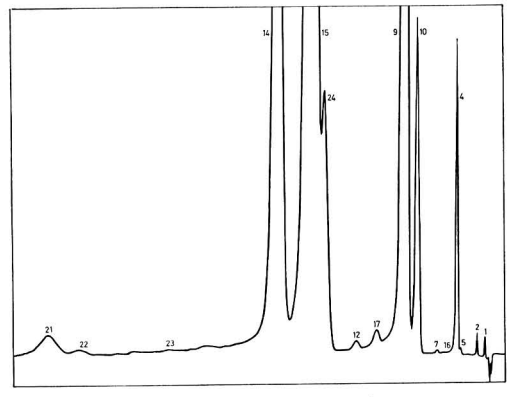

FIG. 4. *Fatty acids of pork fat*
5% 'Apiezon L' at 185°C

FIGS. 1–4
Chromatogram peak identification

1 10 : 0	7 15 : 0	13 17 : 1	19 18 : 3
2 12 : 0	8 16 : 0 br	14 18 : 0	20 20 : 0
3 14 : 0 br	9 16 : 0	15 18 : 1	21 20 : 1
4 14 : 0	10 16 : 1	16 15 : 0 br	22 20 : 2
5 14 : 1 + 15 : 0 br	11 17 : 0 br	17 17 : 1 + 17 : 0 br	23 20 : 4 + 20 : 5
6 15 : 0 br	12 17 : 0	18 18 : 2	24 18 : 2 + 18 : 3

time spent in measurement. The validity of the use of standard peak widths was confirmed by chromatographing a standard fatty acid mixture under varying operating conditions arranged so that peak heights varied considerably. Peak widths of some minor components were calculated by interpolation, any error incurred will not effect the overall percentages.

Identification of Fatty Acids

A knowledge of the nature of the lipids under examination will readily afford an identification of such major components as palmitic, stearic and oleic acids. Logarithmic plots of retention times against number of carbon atoms in the acid will then provide identification of all the commonly occurring straight chain saturated and mono-unsaturated acids. To

identify the poly-unsaturated acids, the procedure outlined by James[6] was followed whereby a graphical relationship of the acids between the $\log_{10}$ (retention volume) in 'Apiezon L' grease and in 1,4-butane-diol succinate is plotted.

The presence of acids containing up to 6 double bonds can thus be confirmed. In the absence of reference standards of iso and ante-iso acids, the C_{15} branched-chain fatty acids present in ruminant fat were tentatively identified from graphs constructed from the published retention volumes of iso and ante-iso branched acids in 'Apiezon L' at 197°.[6] The Pye Argon Chromatograph operating conditions were adjusted until identical relative retention volumes were obtained for C_{14}, C_{16}, C_{18} saturated acids, and the peak assigned to the C_{15} branched chain acids identified from graphs taking into

TABLE III

Percentage component fatty acids of beef fat

Country	Depot	14 : 0	14 : 1	15 : 0	15 : 0^{br}	16 : 0	16 : 1	17 : 0	17 : 1 17 : 0^{br}	18 : 0	18 : 1	18 : 2	18 : 3
Yugoslavia	L	4·4	2·3	0·4	0·3	29·0	7·4	0·7	1·8	11·1	39·3	2·1	1·2
,,	R	2·9	0·2	0·5	0·4	27·0	1·5	1·2	1·2	28·3	33·4	2·4	0·8
,,	K	2·9	0·1	0·4	0·4	26·2	1·3	1·2	1·2	32·1	30·9	2·5	0·5
,,	LG	3·4	0·7	0·5	0·3	28·9	2·7	1·0	1·3	16·6	41·9	2·0	0·7
Argentine	L	4·2	2·4	0·5	0·4	28·0	7·7	0·7	1·8	7·2	43·3	1·5	2·1
,,	R	2·9	0·2	0·5	0·4	26·5	1·5	1·2	1·1	24·6	38·3	1·6	1·0
,,	K	3·1	0·3	0·4	0·3	25·0	1·5	1·1	1·1	25·6	39·2	1·2	1·3
,,	LG	4·0	1·3	0·5	0·3	26·4	4·6	1·0	1·8	11·0	46·1	1·4	1·6
,,	LG	4·2	1·5	0·5	0·4	28·4	4·1	1·0	1·7	10·9	43·6	1·5	1·9
,,	LG	3·0	1·7	0·4	0·3	26·0	5·3	0·8	2·0	8·3	49·3	1·2	1·6
,,	K	3·7	0·3	0·8	0·6	30·5	1·7	1·4	1·4	23·2	33·8	1·4	1·1
,,	R	4·1	1·5	0·5	0·4	30·6	4·3	1·0	1·5	12·2	41·2	1·3	1·4
Sweden	R	2·4	0·2	1·0	0·9	22·8	1·4	1·7	1·8	31·7	32·0	1·7	2·3
,,	LG	3·6	0·7	0·9	0·6	28·6	2·7	1·5	1·9	17·5	39·6	0·9	1·4
,,	K	3·9	0·2	1·1	0·9	27·8	1·5	1·8	1·5	29·8	29·0	1·4	1·0
,,	C	3·9	0·6	0·7	0·6	24·8	2·4	1·5	1·7	21·4	39·6	1·1	1·7
,,	BK	2·2	1·1	0·3	0·2	23·2	5·3	0·7	2·1	8·0	54·2	1·0	1·9
England	T	3·9	0·6	0·4	0·4	28·6	2·4	1·1	1·4	20·2	39·2	0·7	1·2
,,	K	2·9	0·2	0·4	0·4	27·6	1·2	1·2	1·0	29·6	34·0	0·6	0·9
,,	R	2·8	0·2	0·4	0·4	27·3	1·6	1·1	1·3	25·4	37·7	0·5	1·3
,,	LG	2·4	1·2	0·3	0·3	24·0	5·6	0·6	1·9	7·5	53·1	1·0	2·0
,,	R	2·9	0·4	0·5	0·4	24·9	1·7	1·3	1·5	22·6	42·0	0·7	1·0
,,	R	2·9	0·3	0·5	0·6	27·4	1·4	1·3	1·4	27·8	35·5	0·9	0·6
Uruguay	K	2·4	0·2	0·7	0·8	26·0	1·1	1·8	1·4	36·0	28·0	0·8	0·7
,,	R	3·1	0·2	0·7	0·8	27·7	1·4	1·5	1·4	30·4	30·9	0·8	0·7
,,	LG	3·7	0·7	0·6	0·6	29·2	3·6	1·2	1·8	15·1	41·1	1·0	1·1
,,	L	4·0	0·7	0·7	0·5	32·2	2·8	1·3	1·1	18·9	34·7	1·4	1·3
Eire	R	2·6	0·1	0·8	0·9	23·3	1·1	1·5	1·3	33·0	32·6	1·4	1·3
,,	C	3·4	0·5	0·7	0·7	24·3	2·7	1·3	1·8	20·7	41·2	1·2	1·4
N. Ireland	R	2·4	0·1	1·0	0·9	24·0	0·9	1·4	1·5	42·7	22·8	0·9	1·1
,,	LG	4·3	0·5	0·6	0·6	32·2	3·7	1·1	1·6	16·4	36·8	1·0	1·2
Scotland	BK	2·6	0·3	0·7	0·6	27·6	2·0	1·2	1·4	23·5	37·4	1·2	1·5
,,	T	3·2	0·5	0·5	0·5	25·7	3·2	1·1	1·6	19·0	42·2	1·1	1·3
,,	K	3·3	0·2	0·6	0·6	28·8	1·1	1·5	1·1	30·4	30·5	1·1	1·0
,,	LG	3·4	1·2	0·4	0·4	25·7	4·2	1·0	1·7	12·0	46·9	0·7	2·1
,,	R	4·3	0·3	0·8	0·6	34·1	1·7	1·4	1·4	23·0	29·6	1·3	1·0
Rhodesia	R	4·3	1·4	0·4	0·2	31·2	3·8	0·9	1·2	11·4	42·6	1·7	0·6
,,	K	3·2	0·1	0·4	0·5	28·6	1·4	1·4	1·0	32·0	30·1	0·6	0·4
,,	LG	2·9	0·7	0·5	0·6	28·0	3·1	1·0	1·8	13·3	45·3	0·8	1·7
,,	R	3·6	1·2	0·6	0·7	25·5	4·1	1·0	2·0	13·9	43·8	0·9	0·2
Australia	R	3·7	0·4	0·6	0·6	31·1	2·3	1·4	1·4	21·6	35·4	0·6	0·8
,,	L	3·3	2·0	0·4	0·3	28·6	7·1	0·7	1·7	7·9	46·2	0·7	1·1
,,	R	3·7	1·8	0·5	0·4	30·8	6·0	1·6	1·9	9·3	42·1	0·3	1·5
,,	L	3·5	0·4	0·6	0·3	33·6	2·1	1·3	1·2	21·7	33·9	0·5	0·8
S. Africa	FQ	4·7	1·1	0·9	1·0	28·5	4·4	1·4	2·2	15·6	37·6	0·8	1·2
,,	FQ	4·1	0·5	0·8	1·0	28·1	2·7	1·7	2·0	21·7	34·9	1·0	1·0
Bechuanaland	FQ	3·6	0·2	0·5	0·5	26·5	6·1	0·9	2·2	9·7	47·1	0·9	1·6
,,	FQ	3·6	1·5	0·6	0·5	27·1	6·2	0·8	2·1	8·4	46·4	0·9	1·8
,,	HQ	4·1	0·2	1·1	0·9	28·6	2·0	1·8	1·6	26·2	30·8	1·0	1·2
,,	HQ	3·7	0·3	0·7	0·8	27·4	1·9	1·7	1·6	25·5	35·0	0·6	0·4
Corned Beef		3·4	0·8	0·6	0·5	28·8	3·9	1·2	1·6	16·5	40·6	1·0	0·9
Stewed Steak		3·3	0·5	0·6	0·4	32·7	3·4	1·2	1·5	17·8	37·7	0·8	0·2
,, ,,		3·4	0·5	0·6	0·7	27·0	2·3	1·6	1·8	22·3	38·4	0·7	0·5

Other acids detected: 10 : 0 max 0·1; 12 : 0 max 0·2; 14 : 0^{br} 0·07–0·12; 16 : 0^{br} 0·16–0·34; 20 : 0 less than 0·1; 20 : 1 less than 0·1

Location of Fat: BK : Brisket; C : Cod; LG : Leg; FQ : Forequarter; HQ : Hindquarter; R : Rump; K : Kidney; L : Loin; T : Throat

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

(Supplementary Issue)

A supplementary issue of the "Journal of the Science of Food and Agriculture" now being published contains papers (with discussions) read at a symposium on Pesticidal Carbamates. The symposium was organised by the Pesticides group of the Society of Chemical Industry and held on 23rd April, 1968 at the School of Pharmacy, London, W.C.2.

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account its relative position with regard to the known saturated and mono-unsaturated acids. Relative retention volume data plotted from reference branched chain acids which have recently become available[10] has also enabled us tentatively to identify both the C_{14} and C_{16} branched-chain fatty acids as the iso isomers. These acids are completely resolved on a 8% 1,4-butane-diol succinate column, but incompletely resolved from the corresponding mono-unsaturated fatty acids on an 'Apiezon L' column.

Further work by Walker *et al.*[11] employing hydrogenation to produce the alkyl fragment of the molecule as a saturated hydrocarbon has shown peak 16 of Fig. 3, to consist of unresolved pentadecanoic branched chain acids, of which two have been tentatively identified as iso and ante-iso pentadecanoic acids.

Results

Tables III–VI show the component fatty acids found in samples of beef, pork and lamb fats. The fatty acid distribution of some avian species, together with examples of game animals which are currently proposed as possible sources of meat for human consumption, are given in Table VI. In the illustrations of typical chromatograms of beef and pork fats, peak 3, Figs 1 and 2, is a branched chain 14 : 0

TABLE IV

Percentage component fatty acids of pork fat

Country	Depot	14 : 0	16 : 0	16 : 1	17 : 0	17 : 1 17 : 0^{br}	18 : 0	18 : 1	18 : 2	18 : 3	20 : 1	20 : 2
Sweden	K	1·5	27·2	1·5	0·2	0·2	20·6	37·5	9·3	0·9	0·7	0·2
,,	FL	1·6	25·9	2·0	0·2	0·2	17·7	40·2	9·6	0·8	0·9	0·3
,,	K	1·4	24·6	1·2	0·2	0·2	20·0	40·0	10·2	0·9	0·9	0·3
,,	FL	1·5	24·7	1·3	0·2	0·2	16·6	41·3	11·5	0·1	1·6	0·8
,,	K	1·7	29·4	2·4	0·3	0·5	20·4	39·5	4·5	0·6	0·6	0·1
,,	FL	1·5	26·1	2·9	0·3	0·6	14·0	46·8	5·4	1·0	0·7	0·2
,,	K	2·1	32·1	2·0	0·2	0·3	19·8	48·7	9·3	1·4	1·2	0·4
,,	FL	1·5	24·8	2·2	0·2	0·3	13·6	45·8	8·9	1·4	0·9	0·2
Canada	HL	1·3	23·2	1·6	0·1	0·2	13·4	42·7	15·7	0·8	0·6	0·2
,,	F	1·5	25·4	1·4	0·2	0·1	15·2	39·4	14·8	0·9	0·5	0·3
,,	FL	1·4	22·3	1·5	0·2	0·2	13·4	42·1	15·9	1·0	0·5	0·5
,,	F	1·9	28·7	1·4	0·3	0·2	18·5	34·4	13·9	0·1	0·5	0·5
,,	FL	1·4	28·3	1·2	0·2	0·2	16·6	41·0	10·0	0·1	0·8	0·3
,,	HL	1·4	26·9	1·0	0·2	0·2	18·1	40·0	10·6	0·1	1·0	0·3
Yugoslavia	F	1·7	24·8	1·4	0·2	0·2	16·3	43·7	9·4	0·1	1·9	0·1
,,	FL	1·2	23·4	1·3	0·3	0·3	16·2	45·0	10·3	0·3	1·0	0·1
,,	HL	1·4	23·0	1·2	0·3	0·3	15·4	45·9	10·1	0·6	1·0	0·5
,,	F	1·9	27·9	2·2	0·2	0·2	14·4	42·6	9·3	0·5	0·4	0·2
,,	FL	1·7	26·1	2·3	0·4	0·4	13·7	44·9	10·2	0·1	0·6	0·1
,,	HL	1·6	25·1	2·4	0·3	0·3	12·5	44·9	11·2	0·6	0·6	0·2
N. Ireland	F	1·5	30·4	1·7	0·2	0·3	22·5	34·1	7·3	1·0	0·6	0·2
,,	HD	1·4	25·5	3·0	0·3	0·4	13·9	44·0	9·6	1·2	0·8	0·5
,,	HL	1·6	29·0	2·7	0·2	0·2	17·0	39·2	7·6	1·1	0·7	0·3
,,	L	1·7	28·1	2·2	0·3	0·3	19·4	37·3	8·5	1·0	0·6	0·3
,,	K	1·6	26·0	2·2	0·3	0·3	17·7	40·6	9·3	1·3	0·3	0·1
,,	L	1·5	29·2	2·3	0·2	0·3	17·7	38·8	8·9	1·8	0·9	0·4
England	U*	1·7	26·3	3·3	0·2	0·3	11·0	47·5	7·4	1·2	0·7	0·3
,,	F*	1·6	26·9	2·2	0·3	0·3	16·7	41·1	8·6	1·2	0·7	0·2
,,	L*	1·4	23·6	3·2	0·2	0·3	12·7	49·2	7·3	0·9	0·7	0·3
,,	FL*	1·4	26·2	2·9	0·1	0·2	10·8	52·0	4·5	1·1	0·2	0·1
,,	LG*	1·4	26·4	1·9	0·2	0·3	11·0	52·0	3·9	0·3	1·0	0·5
,,	L	1·7	28·0	2·5	0·1	0·2	18·3	40·2	7·4	0·9	0·4	0·1
,,	HL	1·7	25·2	3·2	0·2	0·3	13·5	45·2	8·3	1·0	0·6	0·3
,,	HD	1·4	24·6	3·3	0·2	0·3	12·1	46·3	9·5	1·2	0·3	0·3
,,	F	1·6	30·8	2·3	0·2	0·3	18·6	37·2	6·4	1·0	0·5	0·2
,,	HL	1·5	29·2	3·4	0·2	0·3	13·8	43·2	6·4	0·7	0·8	0·2
,,	HD	1·9	26·3	4·5	0·3	0·5	10·4	47·3	6·8	0·4	1·1	0·3
,,	F	1·6	27·4	3·3	0·5	0·5	14·9	42·3	7·0	0·8	0·8	0·1
,,	HD	1·6	25·6	3·9	0·2	0·4	10·5	47·1	8·5	1·0	0·7	0·1
,,	HL	1·8	29·1	4·3	0·3	0·6	12·0	45·6	6·7	1·0	0·8	0·2
Denmark	TM	1·6	27·4	3·6	0 5	0·5	13·6	41·6	8·0	1·1	0·8	0·5
,,	TM	1·5	27·8	2·8	0·4	0·5	17·3	39·2	7·9	1·0	0·7	0·3
,,	TM	1·5	27·3	3·7	0·4	0·5	13·3	44·2	7·8	1·0	0·5	0·1
,,	CL	1·5	27·4	2·5	0·4	0·4	18·7	41·2	5·9	0·8	0·7	0·1
,,	CL	1·5	28·3	2·3	0·3	0·3	18·0	40·1	7·0	0·8	0·8	0·3
,,	CL	1·9	31·0	2·5	0·3	0·3	20·0	37·6	4·5	0·6	0·7	0·1
Luncheon Meat		2·1	26·4	5·3	0·3	0·6	10·7	44·2	7·9	0·8	1·5	0·6

Other acids detected: 10 : 0 0·03–0·1; 12 : 0 0·05–0·1; 14 : 0^{br} max 0·01; 14 : 1 max 0·06; 15 : 0 max 0·05; 15 : 0^{br} max 0·03; 16 : 0^{br} max 0·05; 20 : 0 max 0·2; 20 : 4, 20 : 5 max 0·3

F : Flare
FL : Foreloin
HD : Head
HL : Hindloin
K : Kidney
L : Loin
LG : Leg
U : Udder
TM : Trimmings
CL : Caul Fat
* From sow carcass

acid (iso isomer). Peak 6, Fig. 1 represents only part of the 15 : 0 branched chain isomers, others are included in the 14 : 1 peak 5. For this reason an 'Apiezon L' column was used to measure the 15 : 0 branched chain acids which form the composite peak 16, Fig. 3. Peak 8, Figs 1 and 2 represents a 16 : 0 acid (iso isomer). It will be noted from Fig. 3 that peak 16, although asymmetric nevertheless, does permit a reasonable estimation of peak area to be made for comparative purposes.

Discussion

Much has been published[12] on the even numbered straight chain fatty acids occurring in animal fats, particularly the influence of dietary fats, and the location of the fat depot. The ranges given in Tables III–V indicate the extremes likely to be found in beef, lamb and pork fat without regard to location, and include the variation likely to be introduced by the various diets employed in the extensive and intensive rearing of animals.

The major fatty acid of the glyceride components i.e. those present in excess of 10%, are of little diagnostic value. The major differences are between fats from various locations on the carcass and show that the external fats are considerably more unsaturated than the internal fats. Minor components i.e. between 0·5–10%, are of use in distinguishing between pork, beef and lamb tissue fats. In pork, the 14 : 0, 17 : 0 and 17 : 1 levels are less than the corresponding levels in beef and lamb fats. The 18 : 2 content of pork fat is greater than either beef or lamb fat. There are sufficient differences in the 10 : 0, 12 : 0 and 15 : 0 contents of mutton and beef fat to enable these fats to be identified.

TABLE V

Percentage component fatty acids of lamb fat

Country	Depot	10 : 0	12 : 0	14 : 0	15 : 0	15 : 0^{br}	16 : 0	16 : 1	17 : 0	17 : 1 17 : 0^{br}	18 : 0	18 : 1	18 : 2	18 : 3
New Zealand	FL	0·1	0·1	2·8	0·6	0·4	24·4	0·9	1·3	1·0	26·5	36·8	2·0	2·8
,, ,,	K	0·1	0·2	2·3	0·3	0·3	20·3	0·7	0·8	0·7	35·2	35·0	1·7	2·4
,, ,,	LG	0·1	0·2	2·5	0·4	0·3	23·2	0·9	1·0	0·8	28·4	35·2	2·9	4·2
,, ,,	CP	0·3	1·2	9·7	0·4	0·3	24·3	2·0	0·5	0·7	15·3	35·4	5·9	3·3
Argentine	K	0·2	0·5	7·3	0·5	0·3	22·9	0·9	0·9	0·7	26·3	35·2	1·6	2·7
,,	LG	0·3	0·8	8·3	0·6	0·3	25·1	1·3	0·8	0·8	19·4	37·6	1·5	3·4
Australia	LG	0·5	1·3	7·6	0·6	0·3	25·3	1·5	0·7	0·8	18·8	37·9	1·1	3·5
,,	K	0·4	1·0	6·3	0·6	0·4	23·6	1·0	0·8	0·7	27·8	33·9	1·2	2·4
England	BT	0·06	0·2	3·5	0·6	0·2	22·8	1·5	1·3	1·5	15·4	46·2	2·0	4·4
,,	K	0·1	0·2	3·1	0·6	0·3	22·1	0·8	1·2	0·9	32·1	32·0	2·5	4·0
,,	LG	0·1	0·3	3·7	0·7	0·3	24·0	1·0	1·4	1·3	22·7	38·6	2·0	3·7

Other acids detected:
14 : 0 max 0·1 — 20 : 0 less than 0·1
14 : 0^{br} 0·05* — 20 : 1 less than 0·1
16 : 0^{br} 0·09* — * typical values

BT : Brisket
FL : Foreloin
K : Kidney
LG : Leg
CP : Chop (Shop Purchase)

TABLE VI

Percentage component fatty acids of some meats of commercial value

	12 : 0	14 : 0	14 : 1	15 : 0	15 : 0^{br}	16 : 0	16 : 1	17 : 0	17 : 1 17 : 0^{br}	18 : 0	18 : 1	18 : 2	18 : 3	20 : 1	20 : 4 20 : 5	22 : 6
Chicken	0·1	0·4	0·1	0·1	0·1	18·9	5·5	0·1	0·1	4·6	52·2	17·2	0·5	0·3		
Turkey	0·1	0·9	0·3	0·1	0·1	21·8	8·3	0·1	0·1	4·3	35·6	26·6	1·5	0·1		
Grouse (*Lagopus scoticus*)	0·1	0·6	0·3	0·1	0·1	16·7	1·9	1·5	0·3	5·7	10·7	31·9	30·3	0·1		
Partridge (*Perdix cinerea*)	0·1	0·9	0·2	0·1	0·1	21·0	7·6	1·8	1·8	3·8	39·8	15·5	9·6	0·1		
Duck	0·1	0·6	0·1	0·1	0·1	23·2	5·7	0·1	0·1	4·2	52·9	12·6	0·9	0·1		
Pheasant (*Phasianus colchicus*)	0·1	1·0	0·4	0·1	0·1	28·1	11·4	0·1	0·1	5·9	40·4	6·1	6·7	0·1		
Rabbit	0·8	3·9	0·8	0·3	0·1	34·6	3·9	1·8	0·4	5·4	27·0	18·1	3·1	0·1		
Hartebeest (*Alcelaphus caama*)	0·1	2·0	0·1	0·4	0·4	20·0	1·2	1·3	1·5	32·8	32·0	4·9	2·1	0·1	1·3	
Wart Hog (*Phacochoerus aethiopicus*)	0·1	1·1	0·1	0·4	1·1	25·0	2·7	1·0	1·1	10·9	18·9	28·1	6·5	0·1	2·8	
Cape Buffalo (*Syncerus caffer*)	0·1	1·5	0·1	0·6	0·8	20·1	2·1	0·9	1·4	23·5	20·4	11·4	4·3	0·1	9·8	3·3*
Cape Buffalo (*Syncerus caffer*)	0·1	2·7	0·3	0·9	0·9	30·2	4·8	1·1	1·9	17·7	32·1	3·9	2·0	0·1	1·4	

Other acids detected:
10 : 0 less than 0·1
20 : 0 less than 0·1

* Structure unknown

Trace quantities of odd numbered carbon and branched chain fatty acids in ruminant milk and body fats have been reported by Hansen *et al.*[13–15]; by Holman & Hofstetter[16] in bovine reproductive tissue; and by Keeney *et al.*[17] in lipids from bovine serum, rumen bacteria and protozoa. More recently Hansen[18] suggested that the enzymatic reactions of particular rumen micro-organisms are responsible for the conversion of phytol into branched chain fatty acids. This accords with the present observations that branched chain fatty acids are present at significant levels in ruminant fats, but not in non-ruminant fats. Comparison of the four chromatograms, (Figs 1–4), shows that at peaks 3, 6, 8 and 16 there are significant amounts of these branched-chain acids in the case of beef fat while in pork fat they occur only at very low levels. The presence of significant amounts of these acids may be taken as evidence of ruminant fat; and if present in 'lard' they indicate adulteration with tallow. The presence of elaidic acid in ruminant fats has been observed when the methyl esters are separated on 'Apiezon L' columns. These observations have been confirmed by infra-red examination and in the absence of hydrogenated fat, provide a further indication of the presence of tallow.

Wolff[19,20] has proposed that the ratios $\frac{C_{14}}{C_{16}} \times 100$, $100\,\frac{(C_{14}\text{unsaturated} \times C_{15}\text{branched})}{C_{14}}$ and $100\,\frac{(C_{14}\text{total} \times C_{15}\text{total})}{C_{16}}$ could be used to detect the adulteration of lard. Grieco[21] questioned the limiting figures calculated from the Wolff ratios and reported the occurrence of C_{14} and C_{16} complex chain acids in lipids obtained from omentum of sheep and cows and their possible use in the detection of tallow in lard. Doro & Remoli[22] working with Italian fats have used these trace components to detect the presence of tallow and horse fat in 'lard'.

From Tables III–V it will be seen that in fifty samples of beef fat examined, the combined values for 15 : 0 iso and ante-iso isomers range between 0·2 and 1·0%; and in lamb fats between 0·2 and 1·4%. This value does not exceed 0·03% in the 46 samples of pork fat examined, and in the large majority of samples it was less than 0·02%. The levels of 14 : 0 and 16 : 0 branched-chain fatty acids in beef fat is 0·10% and 0·25% respectively, and in lamb fat 0·05% and 0·10% respectively. It seems probable that the very small amounts of branched chain fatty acids found in pork tissues normally or legally used in the production of lard are of dietary origin. These 14 : 0 and 16 : 0 branched-chain fatty acids are barely detectable in pork fat but occasional samples have been found with levels as high as 0·01% and 0·04% respectively. In intestinal fats the 14 : 0, 15 : 0 and 16 : 0 branched-chain acids did not exceed 0·01%, 0·03%, and 0·05% respectively. The minimum level of tallow which can be detected in experimental mixtures of lard and beef is about 2%; but in practice it is probable that 5% is the minimum level that can be detected with certainty. In addition to the various fatty tissues described in Tables III–V, of some two hundred samples of commercial lard imported into United Kingdom during the period 1964–1967, from the U.S.A., Sweden, France, Belgium, Italy, Denmark, Rumania, Eire, Germany and Poland, only eleven consignments were found to contain 15 : 0 branched chain acids greater than 0·05%, and these were associated with significant levels of 14 : 0 and 16 : 0 branched chain acids. The examination of a wide range of canned pork luncheon meats, pork and beef mixtures, and offals has confirmed that the fatty acid pattern of the lipids, particularly the absence of significant amounts of branched chain fatty acids, is a reliable means of establishing the authenticity of the pork product.

The patterns of the fatty acid compositions in avian species given in Table VI indicate that they may be of considerable help in establishing the identity of the meat, but further detailed work is required to establish the normal range of constituent fatty acids. Where sufficient authentic samples have been examined to establish the normal distribution of fatty acids, the results can be used to establish the identity of various meats used for human consumption. Commercial processing does not markedly affect the fatty acid distribution.

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ENZYME METHOD FOR DETERMINATION OF α-LINKED GLUCOSE POLYMERS IN BIOLOGICAL MATERIALS

By J. C. MACRAE* and D. G. ARMSTRONG

A review of methods available for the determination of starch suggested, for reasons given, that none was satisfactory for the determination of α-linked glucose polymers in biological materials. Therefore, a method was devised in which the specific hydrolysis of starch completely to glucose is achieved by means of an amyloglucosidase. Glucose is estimated using glucose oxidase. Contents of α-linked glucose polymers in a number of biological samples were determined, and compared with those obtained using 0·36 N-H_2SO_4 to effect hydrolysis.

Introduction

In connexion with the research programme being carried out in this Department on certain aspects of digestion in the ruminant animal, a method suitable for the determination of α-linked glucose polymers in samples of digesta taken from various parts of the alimentary tract was required. It was recognised that such samples may contain only small amounts of α-linked glucose polymer comprising not only starches of feed origin—their nature depending upon the constituents of the feed—but also starch-like materials of protozoal and bacterial origin, which arise from microbial fermentation of the food in the rumen. It is known that on high-starch diets protozoa can synthesise and store starch granules,[1–3] and certain amylolytic bacteria do likewise.[4,5] Unlike many of the plant starches which have an amylose content of 20–30%[6] some at least of the protozoa[7] and bacteria[8] store starches which contain very little, if any, amylose.

Methods for the estimation of starch

For convenience, methods can be divided into two categories, direct and indirect. In those classed as direct the starch is first separated from other materials by solvation with minimun degradation, and then estimated by methods utilising physical or physico-chemical properties of the polymer. In those classed as indirect the starch is degraded to products which can be determined simply and accurately, and which bear a constant proportionality to the weight of starch.

Direct methods

A method that has been widely used for the estimation of starch in single plant materials[9–12] and which has been recommended for use in determining low starch concentrations[13] utilises the physico-chemical property of amylose to form a blue complex with iodine. The colour intensity developed in any one solvent is proportional to the weight concentration of amylose.[14] Thus the method is unsuitable for the determination of total starch content in material containing mixed starches of differing amylose content.

The polarimetric method for starch estimation is based upon the optical activity of starch when in solution. The specific rotations of a number of starches are the same,[15] and optical rotation is not affected by the ratio of α 1 : 4 to α 1 : 6 linkages.[16] Although the method has been criticised on the grounds that different starches have different gelatinisation properties and this affects optical activity,[17] it has been widely used for routine cereal analysis.[18–20] Both Hoffpauir[21] and Hadorn[13] considered that the method is unsuitable for the analysis of materials low in starch content (less than 10%); the angles of rotation for very dilute solutions of starch are too small to be read accurately. For this reason the method was considered to be unsuitable for the present study.

A third direct method utilises the colour reaction of starch with anthrone reagent under acid conditions.[22,23] The starch is gelatinised with perchloric acid at room temperature prior to colour development. However several workers have reported interference by other carbohydrates. Pectic acid, arabinose and xylose form coloured complexes with anthrone[22] and the presence of cellulose also results in overestimation of starch.[24] Hexose sugars other than glucose are also reported to give colour reactions with anthrone although to a lesser extent.[25]

Indirect methods

Although most of the indirect methods involve hydrolysis of the starch by dilute mineral acid, and/or enzymes, to glucose or maltose, with subsequent estimation of the sugar produced, one method[26] involves oxidation of the starch to carbon dioxide and water, and measurement of the carbon dioxide produced. Because of the lengthy nature of this last mentioned procedure, the possibility of carbon dioxide arising from non-starch materials, and the large number of samples to be analysed this method was not considered for the present work.

Acid hydrolysis to glucose has been achieved for microbial polysaccharides by the use of 1N-HCl in sealed tubes at 100° for 3 hours,[27] for the starch in bandages by refluxing it with 0·3N-HCl at 100° for 1½ hours,[28] and for pure starches by refluxing them in 1·5N-H_2SO_4 for 2 hours.[29] A correction factor was applied in this last study to allow for the loss of glucose occurring during hydrolysis. The loss was reported

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to be appreciably greater if HCl was used instead of H_2SO_4. Since glucose can be accurately measured in low concentrations it was felt that a technique based upon initial acid hydrolysis warranted further consideration.

A feature of a number of methods for the determination of starch has been the use of enzymes to effect hydrolysis. Such hydrolysis is specific, but complete hydrolysis of the starch to glucose is difficult to achieve. Thus Hanes[30] used malt β-amylase to hydrolyse starch, and applied a conversion factor to the product as measured by its reducing capacity. Since the β-amylase would only attack α 1 : 4– glucose linkages and not α 1 : 6– linkages the product would include limit dextrins (amylopectin residues) in addition to maltose. The method is thus unsuitable for the estimation of mixed starches. A human salivary amylase has been used to hydrolyse starch in wheat, maize, potato and rice,[31] and in the microbial fractions of abomasal contents,[32] with the estimation of the 'maltose' produced by the method of Hanes.[30] Since this enzyme cannot attack α 1 : 6 linkages the product formed will contain quantities of limit dextrins, the amounts present depending upon the proportion of amylopectin present in the starch. More recently salivary amylase has been shown[33] to contain slight maltose activity, and therefore the products yielded could also contain small amounts of glucose. Since amylase enzymes are not capable of effecting complete hydrolysis to glucose their use for the determination of mixed starches is not satisfactory.

The incompleteness of enzyme hydrolysis has been recognised in the development of methods based upon combined enzyme and acid hydrolysis. Thus methods involving takadiastase[11,34] or diastase,[35] to achieve initial breakdown, followed by acid hydrolysis have been developed.

Experimental

Development of a method for estimating α-linked glucose polymers

Dr. W. F. J. Cuthbertson, Glaxo Laboratories, Greenford, Middlesex, drew the attention of the authors to the existence of an amyloglucosidase capable of degrading starch completely to glucose. The enzyme, prepared from *Aspergillus niger* fermentation broths, is available commercially in a highly purified form under the trade name Agidex (Glaxo Laboratories, Greenford, Middlesex). A high-grade kieselguhr is used as a diluent. The characteristics of the enzyme have been extensively studied,[36–38] and it has been shown to attack α 1 : 4–, α 1 : 6–, and α 1 : 3– linkages, progressively splitting off the glucose units from the non-reducing ends of the starch chains. It does not attack raw starch, which has first to be gelatinised. This has been achieved in the present study by refluxing it with water at 100° for 4 hours; in other laboratories gelatinisation of starch is achieved by autoclaving at 130°.[39,40] The gelatinised product was then incubated with the enzyme at 60° and at pH 4·5 for at least 24 hours. It has been found convenient in using the method for routine analysis of materials containing a wide range of starch contents to keep the amount of enzyme constant, and at a level sufficient to ensure complete hydrolysis of all starch present. Glucose was then determined enzymically using glucose oxidase.[41] Since the oxidase method gives a linear response to glucose concentrations from 0–20 mg/100 ml, the weight of material taken for analysis is that which yields a final concentration of glucose within this range. When allowance was made for the dilutions incurred in the procedure, 0·15–0·20 g air-dry material was suitable for cereals. For digesta from ruminant animals experience showed that 0·50 g of air-dry material (dried under reduced pressure at 50°) was a convenient amount to take for analysis. Details of the analytical procedure are given in the Appendix.

In the studies detailed below samples were also subjected to mild acid hydrolysis (0·36 N-H_2SO_4 at 100° for 8 hours), glucose and total reducing sugar being determined in the resulting hydrolysates by the use of glucose oxidase[41] and copper reduction[42] respectively.

Results and Discussion

The recoveries of glucose obtained when the enzyme method was used to hydrolyse wheat, maize, potato, and rice starches are shown in Table I. These starches were all British Drug Houses, A.R. grade. The value found for wheat starch, 1·103 ± 0·013 g glucose per g starch, is not significantly different statistically from that obtained using acid hydrolysis, viz. 1·097 ± 0·051 g glucose per g starch. All the values shown in Table I, with the possible exception of rice starch which is slightly lower than the other three, are in good agreement with some conversion factors reported in the literature for procedures involving acid hydrolysis. Thus Pirt & Whelan[29] reported 1·104 g glucose per g starch, and Clegg[23] reported 1·11 g glucose per g starch. The present results are also in agreement with the theoretically calculated recovery shown in Table II. It can be seen from Table II that as the value of n (the number of glucose units in the starch chains) increases, the yield of glucose per g starch hydrolysed approaches 1·111 g.

Table I also shows that when the method is applied to β-linked glucose polymers, viz. powdered cellulose, filter paper, and cotton wool, there is no glucose present in the hydrolysates. These results indicate that the presence of β-linked glucose polymers such as cellulose in the biological material to be analysed will not interfere with the determination.

TABLE I

Hydrolysis of pure starches and cellulose products by 'Agidex' hydrolysis

Substrate	Number of determinations	Recovery of glucose, g per g substrate
Wheat starch	10	1·103±0·013
Maize starch	6	1·100±0·012
Potato starch	5	1·103±0·011
Rice starch	5	1·073±0·024
Powdered cellulose	4	nil
Shredded filter paper	4	nil
Cotton wool	4	nil

TABLE II

Theoretical glucose recovery per g glucan for various chain lengths

Chain length (n)	Recovery of glucose, g per g glucan
2	1·052
5	1·085
10	1·099
20	1·105
50	1·109
100	1·110
500	1·1107

The decision to use a 4 hour period of reflux for gelatinising the starch was taken on the basis of the results presented in Table III. Ground barley was boiled under reflux with 50 ml distilled water for progressively lengthening periods of time before being subjected to 'Agidex' hydrolysis, and subsequent glucose determination. The recoveries of glucose per g dry matter increased as the pre-hydrolysis boiling time increased up to 3 hours, and then remained constant up to and including a reflux period of 6 hours. The reason for the fall in the values obtained after an 8 hour period of reflux is not known.

Table III also shows comparisons of glucose in the hydrolysate prepared enzymically and measured both by the glucose oxidase method, which is specific for glucose, and the non-specific copper reduction method which measures all reducing substances present. It can be seen that for any one hydrolysate the glucose values are very similar by both methods; statistical examination of the data showed no significant difference between the two sets of results. These results confirm that the 'Agidex' enzyme only hydrolyses glucose polymers, and will not hydrolyse non-glucose sugar polymers such as are present in biological material such as hay and barley.

TABLE III

Recoveries of glucose from ground barley after it had been refluxed with water for varying lengths of time before enzymic hydrolysis

Period of reflux with water	Number of samples analysed	mg glucose per g dry matter determined by	
		oxidase method	Cu reduction method
1 minute	2	669 (±2·0)*	647 (±2·5)
30 minutes	2	684 (±1·5)	675 (±2·5)
1 hour	2	703 (±1·5)	700 (±1·5)
3 hours	2	710 (±0·0)	707 (±1·0)
4 hours	4	710 ±1·5	705 ±7·0
6 hours	4	710 ±2·0	706 ±5·5
8 hours	2	694 (±1·5)	685 (±2·0)

* The values in parentheses indicate the spread of duplicates; those not enclosed in brackets are standard deviations.

Table IV shows the results obtained for a number of biological materials using the two hydrolysis procedures. The samples of protozoa and bacteria were obtained from the rumen of a sheep by the method of McNaught, Owen, Henry & Kon.[43] The duodenal, ileal and faecal samples were obtained from trials with sheep fitted with duodenal and ileal re-entrant cannulae. For each hydrolysis procedure, with the exception of those from the sample of protozoa, glucose and total reducing sugars were determined by the oxidase and copper reduction methods respectively.

The data in Table IV relating to enzyme hydrolysis confirm that the amyloglucosidase used for hydrolysis is specific to glucose polymers. Thus the values obtained by the oxidase method and by that involving copper reduction are very similar. Acid hydrolysis on the other hand resulted in appreciable quantities of non-glucose reducing substances being released; the values obtained by the Cu-reduction method are higher than those found using the oxidase method. These differences were particularly marked in all samples other than the barley, and can be largely attributed to the presence of non-glucose reducing sugars resulting from the hydrolysis of hemicellulosic material. Examination of the acid hydrolysates from hay, duodenal and faecal samples by paper chromatography showed that considerable quantities of xylose and arabinose were present, together with traces of rhamnose, ribose, and galactose.

It can also be seen from Table IV that the glucose contents determined enzymically in the acid hydrolysates are in reasonable agreement with those determined using Agidex to effect hydrolysis.

It will be noted that in Table IV the term α-linked glucose polymers is given to values determined using enzymes to hydrolyse the polymers and to determine the glucose yielded. From the foregoing it would appear that the value can be obtained if a non-specific method for the determination of glucose, such as copper reduction, is used on the enzymically prepared hydrolysate. The results would also suggest that mild acid hydrolysis followed by measurement of the glucose yielded by a method specific for that sugar would give a fairly accurate measure of the fraction designated α-linked glucose polymer.

TABLE IV

Analysis of biological samples for their content of α-linked glucose polymer determined by a method involving enzymes for the hydrolysis and subsequent estimation of glucose yielded

Comparable data are also given for glucose and total reducing substances yielded after acid hydrolysis

Sample	Enzyme hydrolysis		Acid hydrolysis		
	Oxidase method, mg glucose/g DM	Cu reduction method, mg reducing sugar*/g DM	Oxidase method, mg glucose/g DM (1)	Cu reduction method, mg reducing sugar*/g DM (2)	mg non-glucose reducing sugar*/g DM (2–1)
Hay	16·8	16·9	17·3	213·5	196·2
Barley	710	707	705	756	51
Rumen protozoa	729	n.d.**	709	n.d.	—
Rumen bacteria	219	213	211	326	115
Duodenal sample	8·4	8·4	9·3	121·9	112·6
Ileal sample	3·4	3·6	8·4	156·3	147·9
Faeces	nil	nil	7·0	179·2	172·2

* expressed as glucose
** not determined

The use of the amyloglucosidase to achieve hydrolysis was preferred since it specifically attacks α-linked glucose polymers. Acid hydrolysis is non-specific and in addition necessitates a number of laborious manipulations of the acid hydrolysate before subsequent determination of glucose enzymically. These are not required if the enzymically prepared hydrolysate is used. A further advantage of using amyloglucosidase to achieve hydrolysis is that a non-specific method can be used for subsequent measurement of the glucose yielded.

In this study the term α-linked glucose polymer is preferred to that of starch in designating the fraction measured by the 'Agidex' procedure. This preference is in part due to uncertainty as to the exact nature of possible glucose polymers in microbial products, and in part because no attempt was made to remove dextrins, and other short-chain glucose polymers prior to the estimation. For plant materials where the α-linked glucose polymers are known to be present as starch, a true starch value could easily be obtained if the dextrins, other short-chain glucose polymers and sugars were removed prior to gelatinisation, using one of the conventional methods, e.g. extraction with alcohol.[23]

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APPENDIX

Analytical procedure for the determination of α-linked glucose polymer

A suitable quantity W of the dried sample (0·20 g for cereals; 0·50 g for ruminant digesta) is transferred into a 150 ml flat-bottomed Soxhlet flask, a few glass beads are added to prevent 'bumping', and the flask and contents are weighed.

After the addition of 50 ml glass-distilled water, the contents are refluxed for 4 hours at 100° to gelatinise the α-linked glucose polymers present.

Allow to cool and add 50 ml buffer solution (0·2 M NaAc/HAc, pH 4·5), and 0·40 g of the enzyme ('Agidex'). After it has been rinsed down with a small quantity of glass-distilled water, the flask and its contents are re-weighed. Assuming unit density the volume of liquid in the flask V can be calculated.

A thin layer of liquid paraffin is placed over the liquid to inhibit microbial growth, and the contents are incubated at 60° for a minimum period of 24 hours.

After incubation the flask and contents are cooled to room temperature, and duplicate 1 ml aliquots are withdrawn from beneath the liquid paraffin by means of a pipette. These are de-proteinised by the method of Somogyi,[42] and the concentration of glucose in the filtrate is determined by the method of Marks.[41]

The concentration of α-linked glucose polymers in the material (mg/g) is calculated as:

$$\text{glucose concentration determined (mg/100 ml)} \times \frac{V}{100} \times \frac{1}{W}$$

To express the result on a dry matter basis residual moisture must be determined.

Duplicate distilled water blanks are taken through the complete procedure to obtain the very small blank reading given with 'Agidex' alone. A glucose blank is also necessary.

EFFECT OF PASTEURISATION ON THE CHEMICAL COMPOSITION OF LIQUID WHOLE EGG

II.*—Comparison of the protein fractionation patterns of raw and pasteurised liquid whole egg

By T. L. PARKINSON

Samples of commercial raw and pasteurised whole egg were re-examined after 41 weeks' storage under frozen conditions. As well as differences between the two samples, the protein fractionation patterns revealed marked differences from the original unstored material. A second comparison of commercial raw and pasteurised egg indicated that considerable differences could occur in the protein fractionation patterns of raw egg. This was confirmed in subsequent experiments.

Two experiments on egg white showed that pasteurisation for 2½ min at 135°F caused no change in the fractionation pattern of the proteins, and provided sound evidence for the reproducibility of the fractionation procedure.

The separate influences of time and temperature of heating on liquid whole egg were studied in a series of laboratory-scale pasteurisation experiments. It appeared that time was a more important factor than temperature and that a heating time of 5 minutes, which is approximately the time that the bulk of the egg is held at the pasteurising temperature in commercial plants, caused significant changes in the fractionation patterns of both soluble and insoluble proteins.

When pasteurisation was carried out for 2½ min at 148–152°F, and also when time was varied at 150°F, the thiol and disulphide concentrations of the soluble proteins were not altered by any of the pasteurisation conditions studied.

A short study was made of the distribution of α-amylase activity during the fractionation of the soluble proteins of raw whole egg.

Preliminary experiments on gel filtration of the insoluble protein portions indicated that this technique is capable of revealing changes in molecular weight distribution resulting from pasteurisation and gave support to the view that heat treatment of whole egg results in aggregation or association of some of the proteins.

Introduction

Part I of this series[1] described a scheme, for the fractionation of the proteins of liquid whole egg, that appeared to be capable of revealing differences between raw and pasteurised egg. Further experiments showed that such differences can vary considerably in different batches of commercially pasteurised egg, and it was considered that the most satisfactory method of studying the effect of pasteurisation was to undertake a batch-wise process on a small scale in the laboratory, so that accurate control of time and temperature could be maintained and all the sample would be subjected to the same conditions. This does not occur in the commercial process, in which egg flows continuously through the pasteuriser, and different particles of the egg may be held there for times varying from 2½ min upwards. Winbolt[2] has stated that the average holding time may be in the region of 4 to 6 min, and it may be assumed that a high proportion of the egg will have been held for this time, and a lower proportion for even longer. Thus, commercial pasteurised egg can be regarded as a mixture of volumes of egg that have received different heat treatments. Winbolt[2] also stated that, owing to considerations of plant design, the egg must actually be heated to 149°F to be certain of meeting the legal requirement of a minimum of 148°F. It is conceivable that on occasions the temperature may rise higher than this, and so it was decided that times of 2½, 5 and 10 minutes and temperatures of 148°, 150° and 152°F would be suitable for the small-scale study.

The possibility that these heat treatments may cause aggregation of some of the proteins, with a consequent increase in molecular weight, has been examined by gel filtration on an agarose gel.

Experimental

Pasteurisation

This was carried out by a method described by Shrimpton *et al.*[3] Stainless steel tubing of internal diameter 3 mm, wall thickness 0·5 mm and length 280 cm, giving a holding volume of approximately 10 ml, was wound into a convenient rectangular shape. The tube was filled with egg, each end was closed with a steel screw-cap and washer, it was placed in a thermostatically controlled water-bath at the selected temperature for the required time and was then immediately immersed in cold water. The resulting pasteurised egg was forced out of the tube by a stream of nitrogen.

Protein fractionation

Dialysis, ultra-centrifuging and ion-exchange chromatography were carried out as previously described.[1]

Gel filtration

Sufficient Sepharose 4B (Pharmacia Fine Chemicals) was suspended in 0·2 M phosphate buffer (pH 6·7), poured into a

* Part I: *J. Sci. Fd Agric.*, 1967, **18**, 208

column of internal diameter 2·2 cm and allowed to settle. Excess buffer was drained off, the sample was placed on the column and elution was carried out with 0·2 M phosphate buffer (pH 6·7). Void volume and total volume were measured by passing Blue Dextran and potassium dichromate through the column.

α-Amylase activity

The method of Briggs[4] was adapted as follows: 0·8 g soluble starch was boiled in a solution containing 132 ml 0·1 M disodium hydrogen phosphate, 1·4 ml 1M citric acid, 0·08 g 'Shirlan' and 667 ml distilled water. Eight g agar was added gradually, with continual stirring, and when it was completely dissolved the solution was cooled, poured into 6 in. diameter Petri dishes and stored in a refrigerator overnight. Holes were cut in the gel with a cork-borer and 0·25 ml volumes of the appropriate fractions were pipetted into the holes. The dishes were covered and stored in an incubator at 37°C for 24 h. The gels were then flooded with a solution containing 2·5 g potassium iodide, 0·635 g iodine and 10 ml dilute nitric acid in 500 ml, and allowed to stand a few minutes. Excess solution was then poured off, and the α-amylase activity was assessed by the size of the clear zone surrounding each hole.

Analytical methods

Nitrogen was determined by the Kjeldahl method. Thiol and disulphide determinations were carried out by the amperometric titration method of Leach,[5] using the apparatus described by Stevens.[6] Starch-gel electrophoresis was carried out as previously described.[1]

Results

Commercial raw and pasteurised whole egg after frozen storage

Fractionation of the proteins of samples of commercially pasteurised egg and of the corresponding raw egg taken before it entered the pasteuriser was described in Part I,[1] the fractionation patterns being recorded in Figs 5 and 7 of that paper. These samples were re-examined after storage in the frozen condition for 41 weeks, when it was found that the raw egg still gave a satisfactory baking performance, but the pasteurised egg was unsatisfactory in this respect.

The fractionation patterns for the soluble proteins are shown in Fig. 1, and it is apparent that the proteins eluted by the buffer solutions containing 0·05, 0·08 and 0·10 M salt are present in much lower concentrations in the pasteurised egg than in the raw egg; these concentrations are also significantly lower than those of the corresponding fractions in the original unfrozen pasteurised sample (Fig. 5 in Part I), even when allowance is made for the smaller amount of egg (50 ml, compared with 70 ml) used in the fractionation of the frozen samples.

A feature of the separations shown in Fig. 1 is the absence, in both raw and pasteurised samples, of the larger fraction that normally comes through with the first eluant; this probably results from adsorption of natural carotenoid pigments, and possibly some protein as well, on the insoluble fractions of the frozen samples.

A further distinction between the two raw samples is that, in the frozen sample, there was no protein eluted by the 0·03 M salt buffer but a greatly increased concentration of protein was eluted by 0·10 M salt buffer.

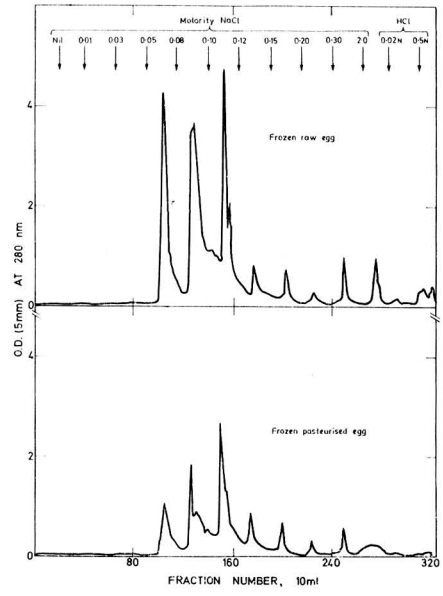

FIG. 1. *Fractionation of soluble proteins of frozen raw and pasteurised whole egg on diethylaminoethyl-cellulose, using 0·002 M glycine-phosphate buffer solutions containing increasing concentrations of sodium chloride*

The insoluble portion from the frozen raw egg was very gelatinous and clogged the ion-exchange column at an early stage, so that no fractionation could be achieved. The corresponding precipitate from the pasteurised sample was satisfactorily fractionated and showed a pattern similar to that given by the unstored unfrozen pasteurised egg (Fig. 7 in Part I[1]).

Second comparison of commercial raw and pasteurised whole egg

The experiment described in Part I[1] was repeated. Both raw and pasteurised samples gave satisfactory results in baking tests, the raw sample being marginally superior.

The fractionation patterns for the soluble proteins are shown in Fig. 2. It will be seen that both samples differed considerably from the corresponding samples of the previous experiment (Fig. 5 in Part I[1]) in two major respects. The big yellow-coloured fraction eluted after the void volume by the first eluant, was either absent or very much reduced and a big, very turbid fraction was eluted by 0·02 N hydrochloric acid. In the case of the raw sample, the large fraction eluted by the 0·03 M salt buffer in the first experiment was very much reduced in the second experiment.

Comparison of the patterns for raw and pasteurised egg in Fig. 2 shows that three of the major fractions were unaffected,

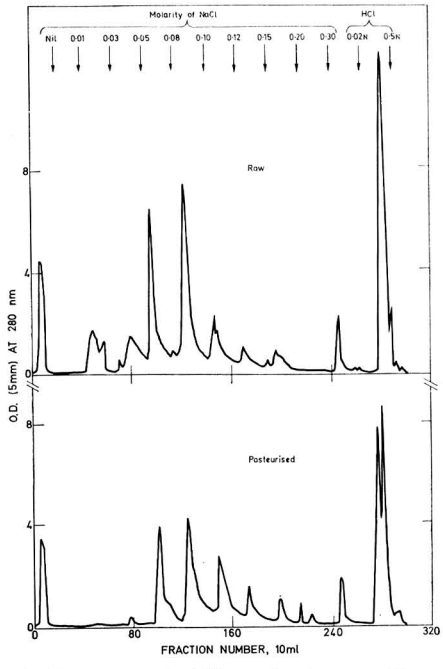

FIG. 2. *Fractionation of soluble proteins of commercial raw and pasteurised whole egg (second experiment)*
Details as in Fig. 1

namely those eluted by 0·05 and 0·08 M salt buffers and 0·02 N hydrochloric acid, but pasteurisation caused marked decreases in the void volume fraction and in the fractions eluted by 0·01, 0·03 and 0·30 M salt buffers and increases in those eluted by 0·10 and 0·15 M salt buffers. It can be concluded, therefore, that the changes resulting from commercial pasteurisation were different in the two cases.

In this instance it was not possible to fractionate the insoluble proteins as they were so gelatinous that they clogged the column.

Comparison of raw and pasteurised egg white

Opportunity was taken of the availability of two samples from the same batch of egg white, one untreated and the other pasteurised in a commercial plant for 2½ min at 135°F, to assess the effect on egg white proteins of this relatively mild heat treatment.

These samples had been kept in frozen storage and, after being defrosted, 50 ml portions were dialysed against 0·02 M glycine solution at 0–3°C for six days and were then centrifuged at 21,000 rev/min at 0–5°C for three hours. The small precipitates were discarded, and the supernatant liquors were placed on columns (31×2·2 cm) of re-cycled DE 52. On this occasion the cake of DE 52 after re-cycling was divided into two equal portions, and these were separately equilibrated with glycine solution, poured into tubes and allowed to settle under gravity, so that the two columns were identical in materials and dimensions.

The two fractionation patterns were so similar that they were virtually superimposable, indicating not only that little or no change in the proteins occurs during pasteurisation of egg white for 2½ min at 135°F, but also that the fractionation method is reproducible for identical samples.

A similar finding was obtained with another pair of commercial samples, taken before and after pasteurisation under the same conditions.

Laboratory-scale pasteurisation experiments

The apparatus used was similar to that described by Shrimpton *et al.*[3] and only allowed about 10 ml of egg to be pasteurised at a time. In general four separate pasteurisations were carried out for each set of conditions and these were then bulked for subsequent dialysis and fractionation. In such cases, a 40 ml volume of the unpasteurised egg was also dialysed and fractionated.

Different temperatures for 2½ minutes

For the first experiment the time was kept constant at 2½ min, the minimum time set by the regulations,[7] and temperatures of 148°, 151° and 154°F were used.

It was difficult to draw definite conclusions from the fractionation patterns obtained for the soluble proteins, partly because the pattern for the unpasteurised egg differed considerably from those obtained on previous samples of raw egg. Furthermore, a second sample of the unpasteurised egg that was given a much longer dialysis time produced a somewhat different pattern from the first one, suggesting that these patterns might be affected by the length of time of dialysis.

By the time this experiment was completed a second fractionation apparatus had been obtained, making it possible to compare directly pairs of samples that had been given identical dialysis and centrifugation treatments and had been fractionated on identical columns, prepared as described above for the test on egg white. The experiment was repeated at temperatures of 148°, 150° and 152° F, the heating time being kept constant at 2½ min in all cases. The 'raw' and '150°' samples were the first pair to be examined at the same time after 6 days' dialysis, and the '148°' and '152°' pair were examined together after a total of 19 days' dialysis.

The weights of the insoluble portions, i.e. the washed precipitates remaining in the tubes after decanting and draining off the supernatant liquid from the second centrifugation were:

	g
Raw (unpasteurised) sample	4·4
Sample heated to 148°F	6·0
150°F	3·9
152°F	5·4

The fractionation patterns of the soluble proteins did not reveal any noteworthy differences. It is valid to compare the patterns of each pair of samples, and the 'raw' and '150°' samples showed a broad similarity, especially in the fractions eluted by 0·05 M and 0·08 M salt buffers. A fairly small fraction eluted by 0·03 M salt buffer was even smaller in the '150°' sample, and a small fraction eluted by 0·01 M salt buffer was present in the '150°' but absent from the 'raw' sample. The fractions eluted by 0·10 M and 0·15 M salt buffers were quantitatively greater, and those eluted by 0·30 M and 2·0 M salt buffers were less, in the '150°' sample than in the 'raw' one. Comparison of the other pair showed that

quantitatively smaller fractions were eluted by 0·05 M, 0·08 M, 0·10 M, 0·12 M and 0·15 M salt buffers and by hydrochloric acid from the '152°' sample than the '148°' sample. Comparison of the '150°' and '148°' patterns showed a general quantitative similarity, with the higher temperature having a larger fraction eluted by 0·15 M salt buffer and smaller ones by 0·12 M and 2·0 M salt buffers and by hydrochloric acid. The sample given the most severe treatment, namely 152°F, showed smaller fractions eluted by 0·03 M, 0·05 M, 0·08 M and 0·15 M salt buffers and larger ones by 0·01 M and 0·03 M salt buffers than did the 'raw' sample.

The main feature observed in the patterns from the insoluble proteins was a progressive increase in the concentration of the fraction eluted immediately after the void volume as the temperature of pasteurisation increased. In addition, two fractions eluted by 0·05 M salt buffer from the '152°' precipitate were not present to an appreciable extent in the patterns from the raw and lower-temperature samples.

A similar pasteurisation experiment was conducted to assess the distribution of protein between 'soluble' and 'insoluble' portions, i.e. between supernatants and precipitates. Two portions of liquid whole egg were pasteurised at each temperature, yielding about 20 ml. The products and a 20 ml portion of untreated raw egg were dialysed for five days and then centrifuged as before. Nitrogen determinations were made on the supernatants and precipitates, and thiol and disulphide determinations were made on the supernatants. Protein was calculated by multiplying the nitrogen figure by 6·68. The results are quoted in Table I, and show no clear pattern of change in protein distribution. The thiol and disulphide groups, when calculated in terms of the soluble protein content, are identical (within experimental error) in the raw egg and all three samples of pasteurised egg.

TABLE I

Distribution of protein and thiol and disulphide groups in egg pasteurised for 2½ min at different temperatures

	Raw egg	Pasteurised egg		
Temperature of pasteurisation, °F	—	148	150	152
Wt. of precipitate, g	2·42	2·34	3·19	3·00
Wt. of protein in precipitate, g	0·71	0·54	0·72	0·62
Wt. of protein in supernatant, g	1·42	1·12	1·33	1·05
Thiol groups in supernatant (mequiv/g soluble protein)	42	42	42	44
Disulphide groups in supernatant (mequiv/g soluble protein)	74	78	77	73

Different times at 150°F

On this occasion the temperature of the water-bath was kept constant at 150°F and times of 2½, 5 and 10 minutes were used, four separate pasteurisations being carried out for each time. The weights of insoluble, washed precipitates after dialysis and centrifugation were:

	g
Raw (unpasteurised) sample	5·75
Sample heated for 2½ min	5·34
5 min	3·57
10 min	10·50

In this particular case, the pasteurised samples all showed considerable amounts of floating material (approximate weights for samples treated for 2½ min, 2·9 g; 5 min, 4 g; and 10 min, 1·8 g). In all of the other experiments described, the amounts of any floating material were insignificant.

The fractionation patterns for the soluble proteins are shown in Figs 3 and 4. Those from the raw egg and the egg heated for 5 min are shown together in Fig. 3, as they were given identical dialysis and centrifugation treatments and were fractionated on identical columns. This also applies to the other pair of samples, the fractionation patterns of which are shown in Fig. 4.

Fig. 3 shows that pasteurisation of this sample of egg for 5 min caused marked quantitative reductions in the fractions eluted after the void volume and by 0·05 M, 0·08 M, 0·10 M, 0·12 M, 0·15 M and 0·20 M salt buffers, slight increases in those eluted by 0·01 M and 0·03 M salt buffers and large increases in those eluted by 2·0 M salt buffer and hydrochloric acid. The major differences between the '2½ min' and '10 min' fractionation patterns are that the longer heating period showed considerable reductions in the fractions eluted by 0·08 M, 0·10 M, 0·12 M and 0·15 M salt buffers.

Figs 5 and 6 show the fractionation patterns for the insoluble proteins, comparing 'raw' and '5 min' samples in Fig. 5, and '2½ min' and '10 min' samples in Fig. 6. Except in one minor instance (a small fraction eluted by 2·0 M salt buffer) the '5 min' sample showed considerably smaller fractions than the raw sample. On the other hand, the '10 min' sample showed more and considerably bigger fractions than the '2½ min' sample.

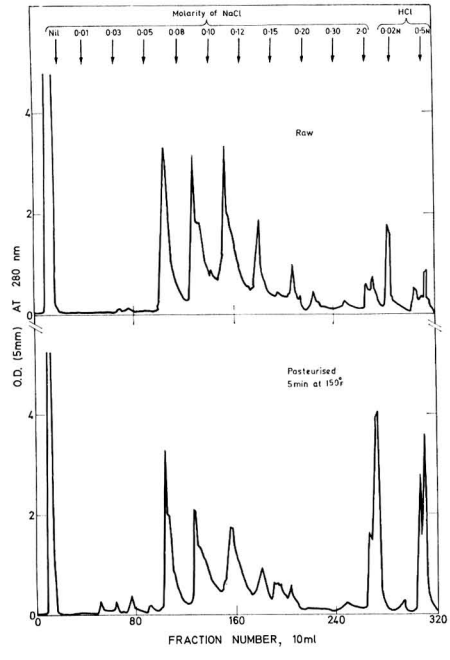

FIG. 3. *Fractionation of soluble proteins of raw egg and egg pasteurised for 5 min at 150°F*
Details as in Fig. 1

Results of similar pasteurisations carried out to assess the protein distribution are given in Table II, from which it appears that the protein content of the egg falls as the time of heating at 150° F increases, and that this fall occurs in both soluble and insoluble portions.

It was thought possible that this might result from thermal or enzymic degradation, and the experiment was repeated with the pasteurised egg being dialysed against distilled water instead of glycine solution. After dialysis the total nitrogen content of each diffusate was determined. These were very small (0·004–0·006 g nitrogen in the entire diffusate) and did not show any progressive increase with increase in time of heating. The total protein content again fell progressively as the time of heating increased and this obviously cannot result from breakdown of proteins to dialysable nitrogenous compounds.

TABLE II

Distribution of protein and thiol and disulphide groups in egg pasteurised at 150°F for different times

	Raw egg	Pasteurised egg		
Time of pasteurisation, min	—	2½	5	10
Wt. of precipitate, g	3·68	3·20	3·25	2·81
Wt. of protein in precipitate, g	1·08	0·88	0·78	0·59
Wt. of protein in supernatant, g	1·46	1·15	1·08	1·01
Thiol groups of supernatant (mequiv/g soluble protein)	39	37	39	42
Disulphide groups in supernatant (mequiv/g soluble protein)	81	71	80	75

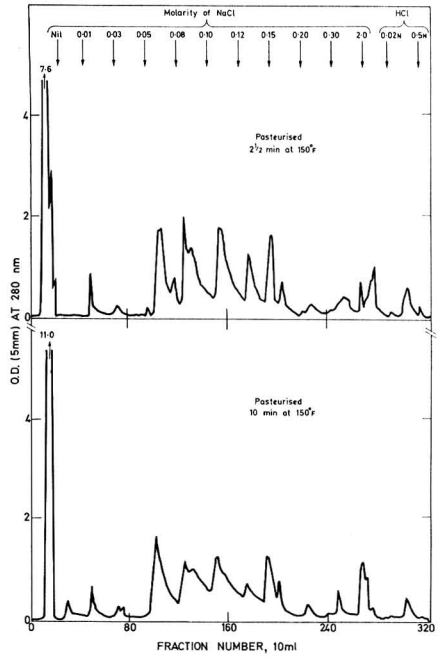

FIG. 4. *Fractionation of soluble proteins of egg pasteurised for 2½ min and 10 min at 150°F*
Details as in Fig. 1

A more detailed investigation, in which the residues from the stainless steel and Visking tubing and from beakers and funnels used for transferring the egg were all examined, showed that there was no loss of nitrogen either during pasteurisation or during dialysis and, in particular, that the total nitrogen content did not decrease when a sample of egg was pasteurised for 10 min at 150°F and then dialysed and centrifuged. This would suggest that the apparent progressive decrease in protein shown in Table II is probably an artefact resulting from an increasing difficulty in transferring egg from one container to another. As in the constant-time experiment the thiol and disulphide concentrations in the soluble proteins were not significantly altered by any of the pasteurisation treatments used.

Different temperatures for 5 minutes

Having established in the previous experiments that time of heating is probably a more important factor than temperature, it was decided to repeat the variable-temperature experiment with a longer heating time. The time chosen was 5 minutes, this being in the middle of the time range taken by the bulk of liquid egg to pass through a commercial plant.[2] The temperatures selected were again 148°, 150° and 152° F, and the weights of the insoluble, washed precipitates after dialysis and centrifugation were:

	g
Raw (unpasteurised) sample	3·85
Sample heated at 148°F	3·25
152°F	3·28

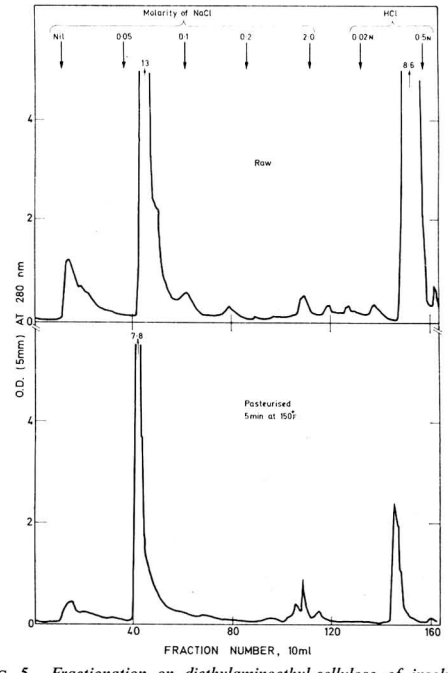

FIG. 5. *Fractionation on diethylaminoethyl-cellulose of insoluble proteins of raw egg and egg pasteurised for 5 min at 150°F, using 0·2 M phosphate buffer, pH 6·6, containing increasing concentrations of sodium chloride*

Fractionation patterns for the soluble proteins are shown in Figs 7 ('raw' and '150°' samples) and 8 ('148°' and '152°' samples). On this occasion quantitative differences between the patterns from the raw sample and from the sample heated at 150°F for 5 min were less marked than previously (cf. Fig. 3), but nevertheless they existed. The most noteworthy difference was that the '150°' pattern shows a considerable reduction in the size of the fractions eluted after the void volume and the presence of a large and a smaller fraction eluted respectively by buffer containing no salt and by the 0·01 M salt buffer; also, fractions eluted by 0·03 M, 0·20 M and 0·30 M salt buffers were larger and those eluted by 0·05 M and 2·0 M salt buffers were smaller in the '150°' pattern than in the 'raw' pattern. Owing to differences in shape, it is difficult to tell if there was any reduction in the size of the fractions eluted by 0·08 M and 0·10 M salt buffers. It may be noted that the pattern of the 'raw' sample in this experiment differed markedly from those of raw samples in previous experiments (see Figs 3 and 5, and also Figs 1, 4 and 5 in Part I), especially regarding the sizes of fractions eluted by 0·05 M, 0·08 M and 0·10 M salt buffers, and it is possible that minor differences in the structure of some proteins are more significant than their relative concentrations. Fig. 8 reveals marked differences between the patterns given by the '148°' and '152°' samples. In particular, the higher temperature caused considerable reductions in the fractions eluted by 0·05 M, 0·08 M, 0·15 M and 2·0 M salt buffers and significant increases in those eluted by 0·10 M salt buffer and hydrochloric acid.

Fractionation patterns for the insoluble proteins from the raw egg and from the egg treated at 150°F for 5 min are shown in Fig. 9. It will be seen that the pasteurised sample produced more and bigger fractions, especially in the earlier stages of elution, and the difference between the two patterns bears a strong similarity to the difference between the corresponding patterns for the commercial raw and pasteurised samples shown in Fig. 7 of Part 1.[1] The fractionation patterns of the insoluble proteins from the '148°' and '152°' treatments were more like that of the raw sample shown in Fig. 9 than that of the '150°' sample. This may have resulted from differences in dialysis times of the two pairs of samples or, in the case of the '152°' sample, from the possible formation of an insoluble portion which remained on the DE 52 column and was not eluted.

α-Amylase distribution

It is known that one particular protein, namely the enzyme α-amylase, is affected by pasteurisation and it was considered that it would be interesting to find out which fractions of egg contained this enzyme.

Preliminary tests showed that α-amylase activity was present in the soluble portion and absent in the insoluble portion of raw egg that had been dialysed and centrifuged, and that the strongest activity appeared in the soluble protein fraction eluted by 0·30 M salt buffer. When fractions taken from the soluble proteins of the raw egg used in the experiment on pasteurising for 2½ min at different temperatures were examined, the strongest activity appeared in the fraction eluted by 2·0 M salt buffer and there was fairly strong activity in

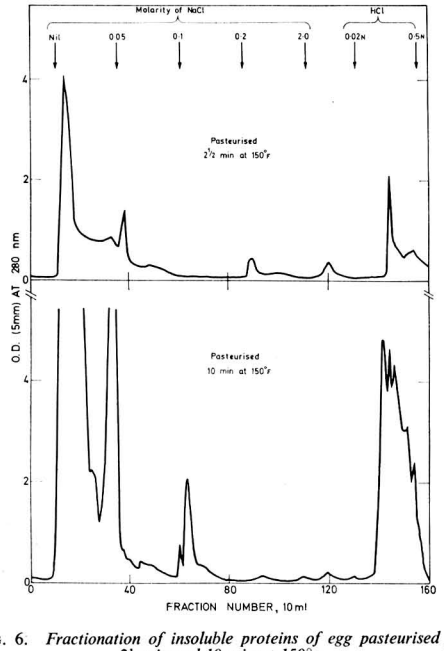

FIG. 6. *Fractionation of insoluble proteins of egg pasteurised for 2½ min and 10 min at 150°F*
Tall peaks eluted by Nil and 0·05M salt buffer rose to O.D. values of 22 and 10 respectively.
Details as in Fig. 5

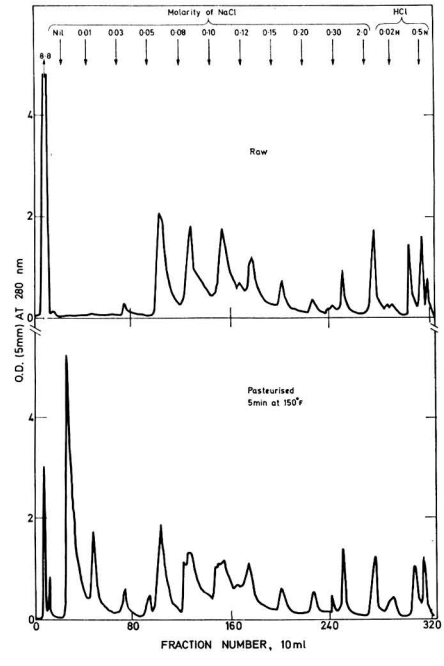

FIG. 7. *Fractionation of soluble proteins of raw egg and egg pasteurised at 150°F for 5 min*
Details as in Fig. 1

those eluted by 0·20 M and 0·30 M salt buffers and in that emerging at the beginning of the elution with 0·02 N hydrochloric acid; other fractions examined were negative. These samples were taken from the 'peaks' of the fractions, where protein concentration was highest; and in the next experiment (on raw egg soluble proteins shown in Fig. 7) fractions from 'troughs' (i.e. regions of lowest optical density) as well as peaks were examined. Peaks and troughs from fractions eluted by 0·30 M and 2·0 M salt buffers all gave positive results, but the hydrochloric acid eluates examined were all negative.

Gel filtration of insoluble proteins

It is a reasonable hypothesis that pasteurisation will lead to association and aggregation of proteins, and if this were so it would lead to a change in molecular weight distribution. It should be possible to detect such a change by subjecting all or part of the sample to gel filtration. Since very high molecular weights are likely to be involved, it was considered that an agarose gel, such as Sepharose, would be more suitable than Sephadex for this particular application.

0·5 g amounts of the precipitates from the experiment in which egg was heated at 150°F for different times, as recorded in Table II, were dissolved in 5 ml volumes of 0·2 M phosphate buffer (pH 6·7), placed on a column (40 × 2·2 cm) of Sepharose 4B and eluted with the same phosphate buffer, the course of the elution being followed by monitoring the optical density at 254 nm with a 'Uvicord' recorder.

Fig. 10 compares the elution patterns of the raw egg insoluble proteins with those of the precipitates from the samples heated for 2½ and 10 minutes, and it is clear that the proportion of compounds of higher molecular weight increases as the time of heating increases. The pattern for the 5 minute precipitate is not shown in Fig. 10, as it was similar to that of the 10 minute precipitate but showed somewhat lower optical density values throughout.

Discussion

Surveying the results as a direct comparison of pasteurised with unpasteurised samples, it seems that the only consistent quantitative changes were those concerning the soluble protein fractions eluted by 0·05 M and 0·08 M salt buffers. These appear to be either unchanged or considerably reduced by pasteurisation, and when the pasteurisation is carried out under known conditions, more severe treatments generally lead to greater quantitative reductions in these fractions. Changes in other fractions, though occasionally quite marked, varied in direction, being sometimes positive and sometimes negative. Examination by starch-gel electrophoresis has failed to show that the quantitative reductions in the 0·05 M and 0·08 M salt buffer fractions are due to destruction or change of any particular protein. Both fractions have been shown to contain a number of separate proteins, and it will need further fractionation of each to provide information on which individual proteins are most affected. It is also worth noting that different batches of egg show not only different

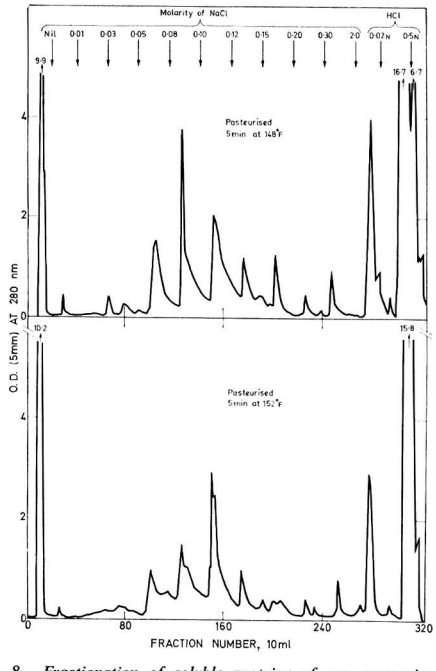

FIG. 8. *Fractionation of soluble proteins of egg pasteurised at 148°F and 152°F for 5 min*
Details as in Fig. 1

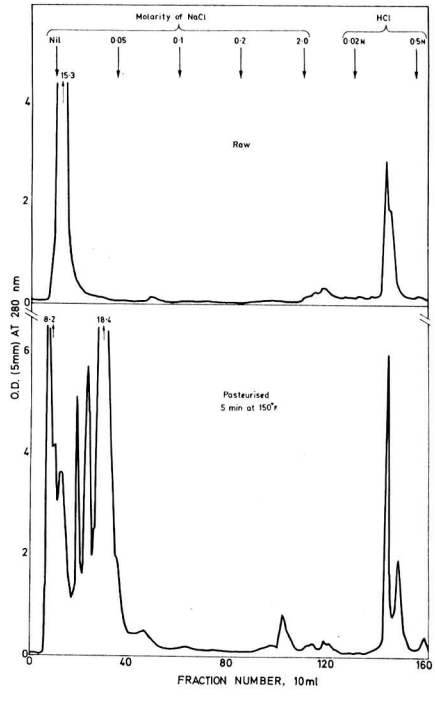

FIG. 9. *Fractionation of insoluble proteins of raw egg and egg pasteurised at 150°F for 5 min*
Details as in Fig. 5

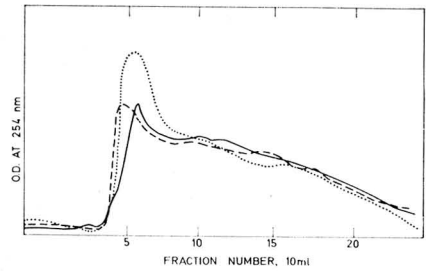

FIG. 10. *Gel filtration of insoluble proteins of raw egg and egg pasteurised for 2½ min and 10 min at 150°F. Eluant: 0·2 M phosphate buffer, pH 6·6*

—— Raw
- - - 2½ min
..... 10 min

fractionation patterns of soluble proteins before pasteurisation but also different changes in the patterns as a result of pasteurisation (cf. Figs 3 and 7).

The fractionation patterns of insoluble proteins have so far given inconsistent results. For example, when one batch of egg was pasteurised at 150°F for 5 min the pasteurised egg showed smaller fractions than did the unpasteurised egg (Fig. 5), but in another experiment with a different batch of egg, the insoluble proteins from egg heated under exactly the same conditions showed more and much larger peaks than did those from the corresponding raw egg (Fig. 9). There does, however, appear to be a tendency for more severe treatments to result in the appearance of more and bigger peaks in the fractionation patterns of the insoluble proteins. Gel filtration of the insoluble proteins on Sepharose 4B showed that there is an increase in the proportion of higher molecular weight material when whole egg is pasteurised at 150°F and that this increase is greater when the heating times are longer; this finding supports the view that pasteurisation results in association or aggregation of some of the proteins in whole egg.

The progressive fall in protein content that was observed when whole egg was heated at 150°F for increasing lengths of time was apparently caused by increasing difficulties of transference at the different stages of the method and not as a result of enzymic or thermal degradation. It may be noted that the thiol and disulphide concentrations of the soluble proteins were not altered by any of the treatments used in the laboratory-scale experiments, and that the disulphide concentration is approximately twice the thiol concentration.

Acknowledgments

This work was done under the sponsorship of the British Egg Marketing Board, to whom thanks are due. The author also thanks Dr. G. A. H. Elton and colleagues for helpful discussion, Miss L. J. Dyer and Mrs P. J. Walker for skilled experimental assistance, the Analytical Section for nitrogen determinations and Mr. D. J. Stevens for thiol and disulphide determinations.

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EFFECT OF PASTEURISATION ON THE CHEMICAL COMPOSITION OF LIQUID WHOLE EGG

III.*—Effect of staling and freezing on the protein fractionation patterns of raw and pasteurised whole egg

By T. L. PARKINSON

Two particular fractions of the soluble proteins were consistently reduced by heating liquid egg for 5 min at 150°F. These fractions were quantitatively greater in the soluble proteins from frozen egg. The soluble proteins appeared to be more stable to heat in eggs that had been stored for 15 days at 70°F than in new-laid eggs. Unfrozen new-laid and stale egg and frozen new-laid egg all showed an increase in insoluble material after pasteurisation, and frozen egg contained more insoluble materials than unfrozen egg. Gel filtration showed that the proportion of compounds of high molecular weight in the insoluble proteins was markedly increased by pasteurisation and even more markedly increased by freezing.

Introduction

In a previous paper in this series[1] it was noted that changes in the fractionation patterns of soluble egg proteins resulting from pasteurisation differed for different batches from commercial plants. Such variability may be due to a number of factors, and one of these is the age of the egg. It is known that storage of eggs in shell at ambient temperature leads to changes in some of the protein fractions. Smith & Back[2] (quoted by Vickery[3]) showed that during storage of shell eggs at 68°F (20°C) most of the ovalbumin undergoes a spontaneous intramolecular rearrangement to a stable form, S-ovalbumin, having a greater resistance to denaturation by heat. Yashika & Kosin[4] showed that the immunodiffusion patterns from whites of eggs stored 21–25 days at 13·5°C were different from those of corresponding eggs examined within 9 hours of being laid and attributed these results to a de-polymerisation of the gamma-globulin fraction. Baker & Manwell[5] noted that the resolution of certain egg white proteins by starch-gel electrophoresis deteriorated with age; although little change occurred in the first two weeks of storage, after this time it became progressively more difficult to differentiate the mucin and globulin bands, until after five weeks it was impossible to resolve them at all. In view of the above findings it was considered necessary to investigate the effect of staling on the patterns obtained by the protein fractionation scheme that is being used in the present study and to examine whether the changes occurring on pasteurisation showed any differences in stability between new-laid and stale eggs.

It has been known for some time that freezing and subsequent thawing of eggs results in a marked increase in the viscosity of egg yolk[6–8] and of whole egg[9, 10] and it was considered that the effect of this treatment on the protein fractionation patterns should also be studied. A logical extension of this would be an examination of the effect of pasteurisation on thawed frozen egg, both new-laid and stale, and clearly the most satisfactory results would be obtained by using a single batch of egg for the whole series of experiments. A heating time of 5 min at 150°F was used in all experiments, this being considered to be typical of the average conditions experienced by egg in commercial plants (see Part II of this series[1]).

* Part II: Preceding paper

Experimental

A batch of eggs was obtained from the same flock within a few hours of laying. Two dozen were broken out, mixed thoroughly in a Hobart mixer for 3 minutes and strained through a nylon domestic tea-strainer. One 40 ml portion was dialysed against 0·02 M glycine solution, and four 10 ml portions were pasteurised, as previously described,[1] for 5 minutes at 150°F, and then bulked and dialysed. The remainder of the melange was frozen and stored for seven weeks at −5°F in a deep-freeze unit and was then thawed and treated as above.

A further two dozen eggs were stored for 15 days in a room maintained at 70°F and then broken out, mixed and treated in the same way as the new-laid eggs. In this case the residual mixed egg was kept in frozen storage for nine weeks.

The methods of examination used have been described in previous papers in this series.[1, 11]

Results

New-laid eggs

The fractionation patterns of the soluble proteins obtained by centrifuging dialysed raw and pasteurised new-laid egg are compared in Fig. 1. One feature of these patterns is that some of the eluting buffers bring out more than one overlapping fraction, a finding which was not observed in most of the patterns already published.[1] Pasteurisation for 5 minutes at 150°F resulted in a marked quantitative reduction of the proteins eluted by 0·05 M and 0·08 M salt buffers and increases in those eluted by 0·30 M and 2·0 M salt buffers and 0·02 N hydrochloric acid.

In this experiment the weights of insoluble portions obtained by centrifuging the dialysed material from approximately 40 g of egg and decanting off the top layer and weighing the residue were:

Raw	4·95 g
Pasteurised	7·60 g

The fractionation patterns of the insoluble proteins are compared in Fig. 2, which shows more and larger peaks for the pasteurised than the unpasteurised egg. The first fraction from each separation, called the 'inert fraction' and comprising those substances not adsorbed on the DE52 column and so eluted immediately after the void volume of eluant had passed through, was examined by gel filtration. 5 ml portions were

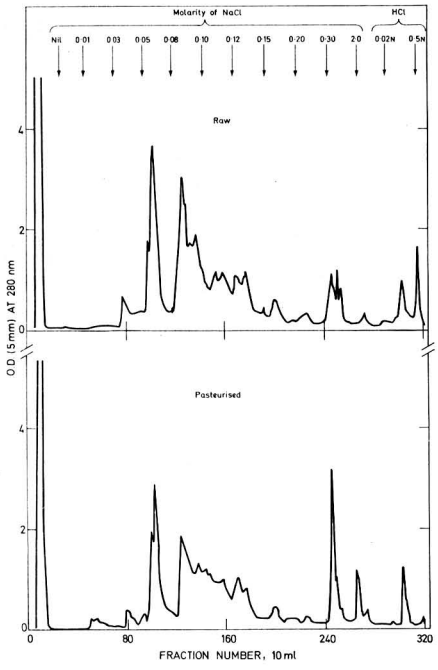

FIG. 1. *Fractionation of soluble proteins of raw and pasteurised new-laid egg on diethylaminoethyl-cellulose, using 0·002 M glycine-phosphate buffer solutions, pH 7·0, containing increasing concentrations of sodium chloride*

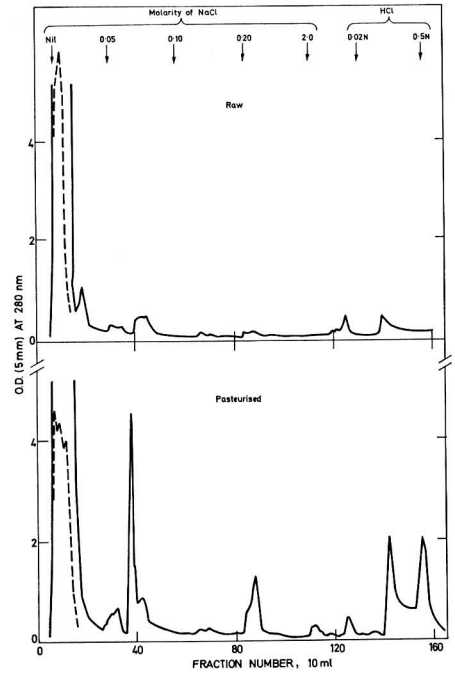

FIG. 2. *Fractionation of insoluble proteins of raw and pasteurised new-laid egg on diethylaminoethyl-cellulose using 0·2 M phosphate buffer solutions, pH 6·7, containing increasing concentrations of sodium chloride*

Dotted line represents O.D. scale reading × 10

placed on the top of a column (96 × 2·2 cm) of Sepharose 4B and eluted with 0·2 M phosphate buffer (pH 6·7). Fig. 3 compares the two samples and shows that the inert fraction from the pasteurised egg contains a much higher proportion of compounds of higher molecular weight and lower proportions of lower molecular weight than the corresponding inert fraction derived from raw egg.

Stale eggs

Corresponding fractionation patterns of soluble and insoluble proteins from the eggs stored for 15 days at 70°F before mixing are shown in Figs 4 and 5 respectively. The weights of the insoluble portions obtained after dialysis of approximately 40 g of egg were:

Raw	4·95 g
Pasteurised	5·35 g

The changes in soluble-protein patterns resulting from heat treatment (Fig. 4) mainly concern the fractions eluted by 0·05 M, 0·08 M and 0·10 M salt buffers. The first of these did not appear to show any marked quantitative reduction, but there was a change in the shape of the fraction, that from the pasteurised egg showing two peaks whereas the fraction from

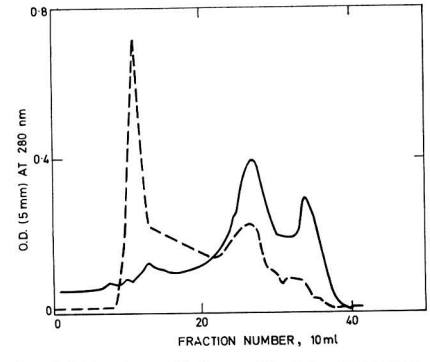

FIG. 3. *Gel filtration on Sepharose 4B of 'inert' fractions of insoluble proteins of raw and pasteurised new-laid egg. 5 ml portions of fractions used*

Solid line—raw egg; dotted line—pasteurised egg

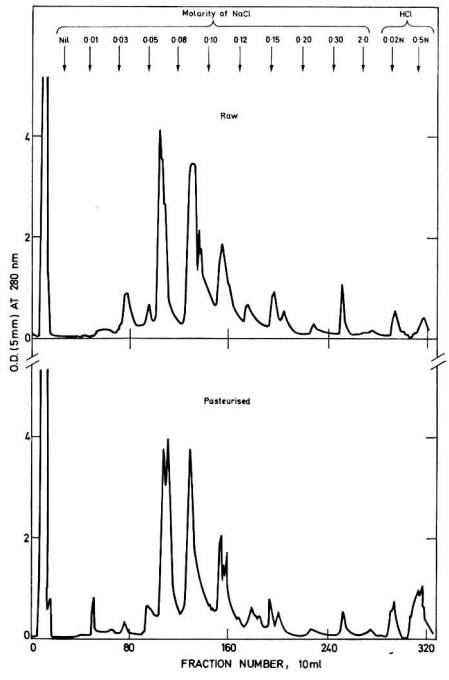

FIG. 4. *Fractionation of soluble proteins of raw and pasteurised stale egg on diethylaminoethyl-cellulose*
Eluants as in Fig. 1

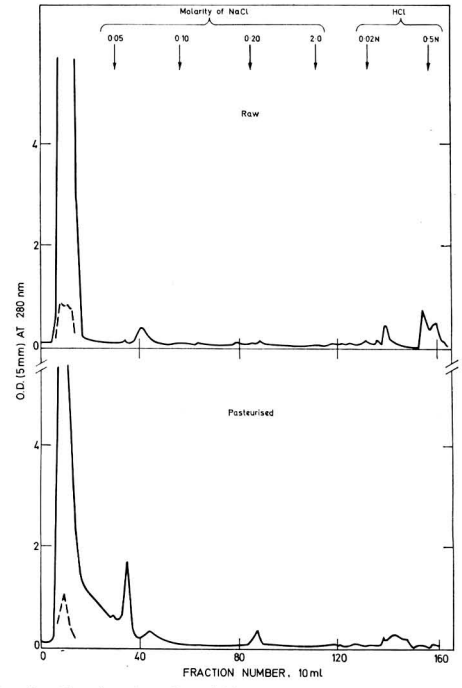

FIG. 5. *Fractionation of insoluble proteins of raw and pasteurised stale egg on diethylaminoethyl-cellulose*
Eluants as in Fig. 2. Dotted line represents O.D. scale reading × 10

the raw egg had one peak with a shoulder. The 0·08 M salt buffer fraction from pasteurised egg had less peaks and was significantly smaller than the corresponding fraction from the raw egg; the reverse was the case with the 0·10 M salt buffer fractions. Minor changes were apparent in some of the other fractions.

These patterns should also be compared with those from the new-laid egg, i.e. Fig. 4 should be compared with Fig. 1. Considering the raw egg samples first, the effect of staling appeared to be mainly a simplification of some of the fractions by reduction in the number of peaks in each. The different shapes of the fractions, particularly that eluted by 0·08 M salt buffer, make it difficult to draw any definite conclusions about quantitative changes, but it is clear that staling resulted in a general decrease in those proteins eluted by salt buffers of 0·30 M and above and by hydrochloric acid. The fractionation patterns of the soluble proteins of the pasteurised samples, on the other hand, show considerable differences between the new-laid and stale eggs. The fractions eluted from stale egg by 0·05 M, 0·08 M and 0·10 M salt buffers were all considerably greater, and that eluted by 0·30 M salt buffer much less, than the corresponding fractions from the pasteurised new-laid egg; there were also some minor differences among the other fractions. It is reasonable to infer that the proteins in the stale eggs were more stable towards the heat treatment used than those in the fresh egg.

Fig. 5 shows that there was little difference between the insoluble protein fractions of raw and pasteurised stale egg; the latter showed a fraction eluted by 0·05 M salt buffer that was absent from the pattern for raw egg. Comparing Fig. 5 with Fig. 2, there was little difference between the insoluble protein fractions of the two raw egg samples, but the pasteurised new-laid sample showed more peaks than the pasteurised stale egg. For both raw and pasteurised samples the first ('inert') fractions from the stale egg insoluble proteins were much smaller than the corresponding fresh egg fractions, as shown by the dotted lines in Figs 2 and 5.

Fig. 6 records the results of submitting the 'inert' fraction from each ion-exchange separation of insoluble proteins to gel filtration on Sepharose 4B. In this case the fractions had been concentrated approximately five times by the method of Kohn,[12] and a smaller volume was used. As was the case with new-laid eggs, the pasteurised sample had a much higher proportion of compounds of high molecular weight and lower proportions of compounds of lower molecular weight than the unpasteurised fraction.

Frozen new-laid egg

This sample was removed from deep-freeze storage and allowed to thaw overnight. Four 10 ml portions were pasteurised for 5 minutes at 150°F and the bulked pasteurised egg was dialysed against 0·02 M glycine at 0—5°C for five days. A 40 ml portion of unpasteurised defrosted egg was also dialysed under the same conditions.

Weights of the insoluble portions from approximately 40 g of egg after dialysis and centrifugation were:

Raw 7·72 g
Pasteurised 9·97 g

It will be noted that these were considerably higher than the corresponding insoluble portions from the unfrozen egg samples.

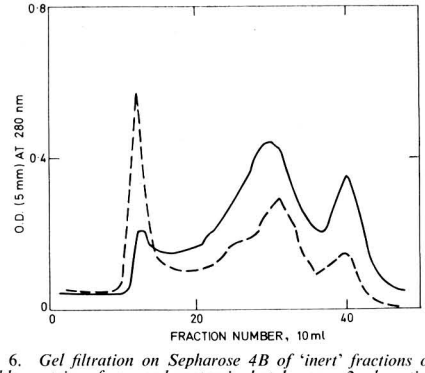

FIG. 6. *Gel filtration on Sepharose 4B of 'inert' fractions of insoluble proteins of raw and pasteurised stale egg. 2 ml portions of concentrated fractions used*
Solid line—raw egg; dotted line—pasteurised egg

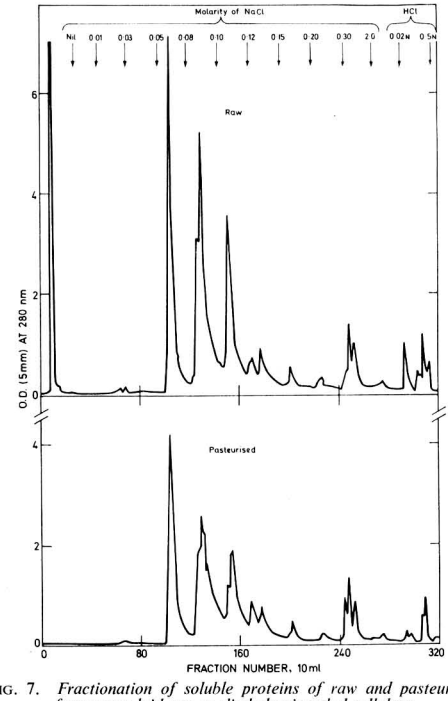

FIG. 7. *Fractionation of soluble proteins of raw and pasteurised frozen new-laid egg on diethylaminoethyl-cellulose*
Eluants as in Fig. 1

The fractionation patterns of the soluble and insoluble proteins are shown in Figs 7 and 8 respectively. Once again, pasteurisation for 5 minutes at 150° F resulted in significant decreases in the fractions of soluble proteins eluted by 0·05 M and 0·08 M salt buffers; in this case there was also a marked decrease in the fraction eluted by 0·10 M salt buffer.

Another feature in Fig. 7 is that the strong yellow fraction emerging after the void volume of the first eluant occurred in the raw but not in the pasteurised soluble proteins; this suggests that the carotenoid pigments became adsorbed on the insoluble proteins of the pasteurised egg but not on those of the raw egg.

Comparing the fractionation pattern of the soluble proteins from the frozen raw egg in Fig. 7 with that of those from the original new-laid egg in Fig. 1, it will be seen that the fractions eluted by 0·05 M, 0·08 M and 0·10 M salt buffers were all decidedly larger for the frozen egg than for the fresh egg. This was also the case for the corresponding pasteurised samples.

The differences between the insoluble protein patterns from raw and pasteurised egg, as shown in Fig. 8, were similar to those observed with the unfrozen fresh egg, as shown in Fig. 2. It will be noted that the first ('inert') fractions eluted in each case were very much larger for the frozen than the unfrozen samples. Further fractionation of these 'inert' fractions from the frozen egg by gel filtration is shown in Fig. 9. In both cases the proportion of material of high molecular

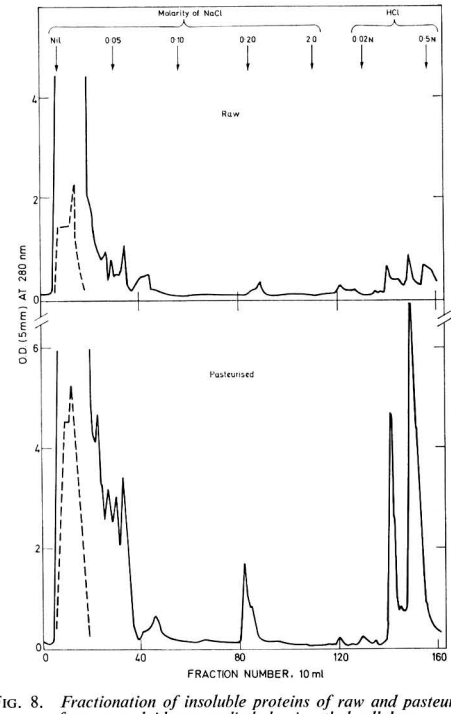

FIG. 8. *Fractionation of insoluble proteins of raw and pasteurised frozen new-laid egg on diethylaminoethyl-cellulose*
Eluants as in Fig. 2. Dotted line represents O.D. Scale Reading × 20

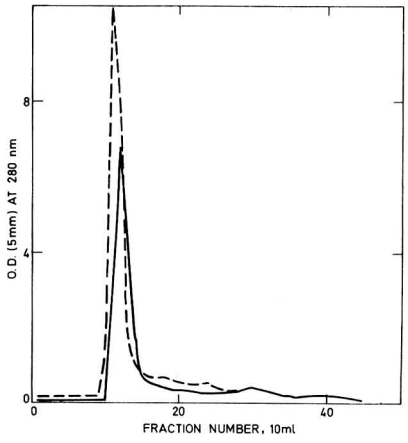

FIG. 9. *Gel filtration on Sepharose 4B of 'inert' fractions of insoluble proteins of raw and pasteurised frozen new-laid egg. 5 ml portions of fractions used*

Solid line—raw egg; dotted line—pasteurised egg

weight was much greater than in the corresponding fractions from fresh egg, as shown in Fig. 3, indicating that freezing caused a very considerable aggregation of proteins and that this was further increased by subsequent pasteurisation.

Frozen stale egg

This sample was treated in the same manner as the frozen new-laid egg. Weights of insoluble portions from approximately 40 g of egg after dialysis and centrifugation were:

Raw	14·3 g
Pasteurised	11·5 g

In addition, the pasteurised sample had an oily floating layer, weighing 3·9 g.

The fractionation patterns of the soluble and insoluble proteins are shown in Figs 10 and 11 respectively. The former shows that pasteurisation again caused a quantitative reduction in the fractions eluted by 0·05 M and 0·08 M salt buffers; there was also a reduction in the 0·10 M salt buffer fraction, but otherwise little or no change. It is significant that both raw and pasteurised samples lacked the large initial fraction following the void volume of eluant, suggesting that the carotenoid pigments had been adsorbed on the insoluble proteins.

Comparing Fig. 10 with Fig. 7, there is an indication that the fraction eluted from frozen stale egg by 0·08 M salt buffer showed a greater stability towards freezing, thawing and

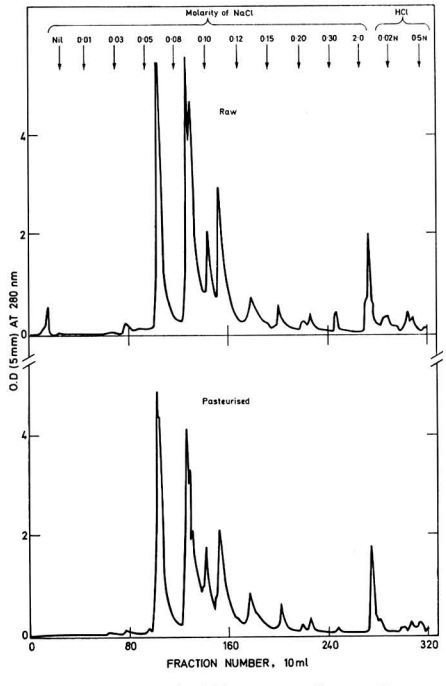

FIG. 10. *Fractionation of soluble proteins of raw and pasteurised frozen stale egg on diethylaminoethyl-cellulose*

Tall peak eluted by 0·05M salt buffer rose to an O.D. value of 8·7

Eluants as in Fig. 1

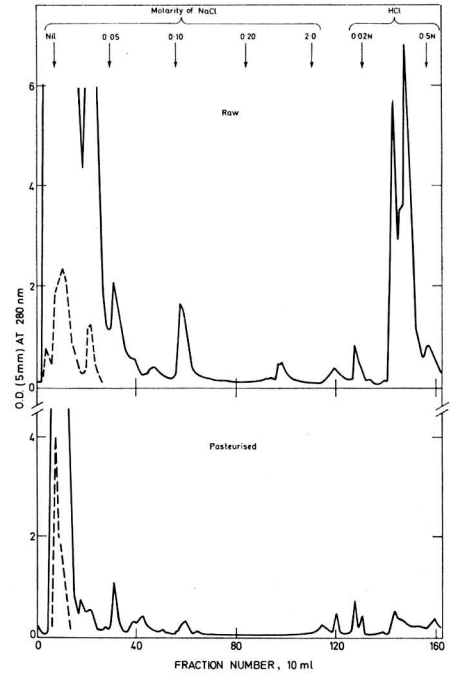

FIG. 11. *Fractionation of insoluble proteins of raw and pasteurised frozen stale egg on diethylaminoethyl-cellulose*

Eluants as in Fig. 2. Dotted line represents O.D. scale reading × 20

pasteurisation than the corresponding fraction from frozen new-laid egg. Other fractions showed differences similar to those already noted between the unfrozen samples of stale and new-laid egg.

A more significant comparison is that between the soluble protein patterns of the frozen (Fig. 10) and unfrozen (Fig. 4) stale egg. Considering the unpasteurised samples first, freezing and defrosting resulted in significant quantitative increases in the fractions eluted by 0·05 M, 0·08 M, 0·10 M and 2·0 M salt buffers, a marked decrease in the first fraction eluted (probably caused by adsorption of pigments on the insoluble proteins) and minor decreases in some other fractions. On the other hand, differences between the patterns of unfrozen and frozen samples after pasteurisation were only small.

The patterns of the insoluble proteins (Fig. 11) showed an unexpected result, as the changes were of an opposite nature to those recorded (in Fig. 8) for the frozen new-laid samples, namely, the unpasteurised sample had more and larger peaks. It was observed that the insoluble proteins from the unpasteurised sample contained a wax-like material that tended to run down the outside surface of the DE 52 column, and also that elution from this column was slower and more difficult than from the column carrying the insoluble proteins from the pasteurised egg.

On this occasion the 'inert' fractions were each diluted to 100 ml with 0·2 M phosphate buffer (pH 6·7), and 5 ml portions of each solution were submitted to gel filtration on Sepharose 4B, the results being displayed in Fig. 12. This time the change resulting from pasteurisation was not a straightforward increase in the proportion of material of high molecular weight but a combination of formation of a small proportion of material with an even higher molecular weight than was found previously and a general spreading out towards lower molecular weight ranges, suggesting that both association and dissociation had taken place. This is markedly different from the other experiments and may reflect some change that resulted in the 'inert' fraction changing its nature before fractionation of the insoluble proteins, e.g. part of it becoming insoluble in 0·2 M phosphate buffer and so not passing through the column. On account of the dilution factor involved, these gel filtration patterns cannot be compared directly with those in Fig. 9 but they provide further evidence that the freezing-defrosting cycle causes protein aggregation.

The opportunity was taken of determining the fat and protein contents of these diluted inert fractions, and the following results were obtained:

	Inert fraction from insoluble proteins	
	Raw egg	Pasteurised egg
Fat (acid-hydrolysis), % w/v	0·82	0·95
Nitrogen, % w/v	0·106	0·093
Protein (N × 6·68), % w/v	0·71	0·62
Ratio fat: nitrogen	7·7	10·2

By analogy with the findings of Radomski & Cook[13] on fractionation of egg yolk granules on triethylaminoethyl-cellulose, it would be expected that β-lipovitellin would be one constituent of the inert fraction. As this substance has a fat:nitrogen ratio of approximately unity, these inert fractions must contain a high proportion of compounds of higher lipid content than β-lipovitellin. Lipovitellenin has a fat:nitrogen ratio of approximately 50:1, but this is usually associated with the low-density (floating) material and one would not expect to find it in the high-density precipitate.

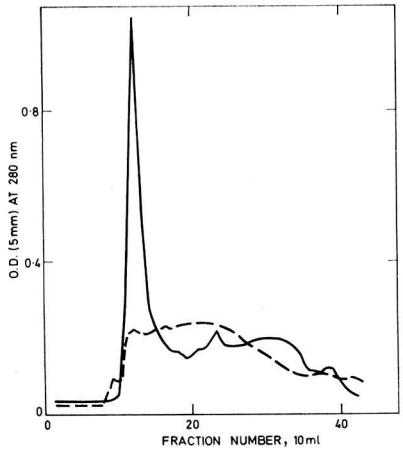

FIG. 12. *Gel filtration on Sepharose 4B of 'inert' fractions of insoluble proteins of raw and pasteurised frozen stale egg. 5 ml portions of diluted fractions used.*
Solid line—raw egg; dotted line—pasteurised egg

Discussion

Comparison of the soluble protein fractionation patterns in this study has confirmed the tentative conclusion, drawn in the previous paper,[1] that the only consistent quantitative change on pasteurisation was a reduction in those fractions eluted by 0·05 M and 0·08 M salt buffers. In the unfrozen stale egg the 0·05 M salt buffer fraction appeared unaffected but the 0·08 M salt buffer fraction was definitely reduced by pasteurisation, and in the other three cases both of these fractions were reduced.

The effect on the soluble proteins of storing new-laid eggs for 15 days at 70°F appeared to be mainly a simplification of certain fractions by reductions in the numbers of peaks in each and also a general quantitative reduction in the later fractions, i.e. those eluted by 0·30 M and 2·0 M salt buffers and hydrochloric acid. After pasteurisation the soluble protein fractions eluted from the stale egg by 0·05 M, 0·08 M and 0·10 M salt buffers were much greater, and that eluted by 0·30 M salt buffer much less, than the corresponding fractions from the new-laid egg, indicating that staling results in some proteins acquiring a greater stability towards heat treatment. This may be partly (or even wholly) due to the conversion of part of the ovalbumin to S-ovalbumin (cf. Smith & Back[2]).

It is noteworthy that the soluble protein fractions eluted by 0·05 M, 0·08 M and 0·10 M salt buffers from unpasteurised frozen new-laid and stale eggs were quantitatively significantly greater than the corresponding fractions from the unpasteurised unfrozen eggs. This also applied to the pasteurised frozen and unfrozen new-laid eggs, but differences between pasteurised frozen and unfrozen stale eggs were only small. The differences resulting from pasteurisation of both new-laid and stale eggs after freezing and thawing were generally

similar to those found with the unfrozen samples. In three cases out of four (the frozen unpasteurised new-laid egg being the exception) the large first fraction, emerging immediately after the void volume of eluant, normally found in the soluble proteins was absent, suggesting that the carotenoid pigments had been adsorbed on the insoluble proteins.

The comparisons drawn between raw pasteurised samples will be valid because in every case both samples were subjected to identical treatment before chromatography and were then chromatographed on two identical columns prepared from a single batch of re-cycled DE 52, as described in Part II of this series.[1] The other comparisons will necessarily have a slightly lesser degree of certainty, as it is not possible to check the reproducibility of columns made at two or three week intervals, since some changes in the proteins occur when egg samples are stored for such periods. However, the same conditions and materials were used for each experiment and the soluble protein fractionation patterns all have a qualitative similarity to each other, so it is considered that the above conclusions on quantitative differences are justified.

With the unfrozen eggs the insoluble portion (after dialysis) was greater for the pasteurised than the unpasteurised sample of new-laid egg, but for the stale egg the pasteurised sample had only a slightly higher weight of insoluble material than the raw sample. The insoluble portions from both raw samples were identical in weight. Fractionation patterns of these insoluble portions revealed a difference between raw and pasteurised samples of new-laid eggs, the latter showing more peaks, but not between the corresponding samples of stale egg. The main difference observed between new-laid and stale egg was that the first fraction, eluted immediately after the void volume of the column and referred to in this work as the 'inert' fraction, was much smaller for the stale than the new-laid egg, for both raw and pasteurised samples. Further examination of these inert fractions by molecular sieve chromatography revealed that, for both new-laid and stale eggs, the pasteurised samples contained a much higher proportion of compounds of high molecular weight than the raw samples. This is a more specific version of the finding, reported in Part II of this series,[1] that the insoluble portion of pasteurised egg contained more high molecular compounds than that of the raw egg, with the inference that pasteurisation results in some of the proteins, and possibly certain lipids as well, associating into some form of macromolecular aggregate.

The frozen samples both contained significantly higher amounts of insoluble material than the corresponding unfrozen samples. The fractionation patterns of the insoluble proteins from the frozen new-laid eggs showed differences between the raw and pasteurised samples that were similar to those between the corresponding unfrozen samples, but for both raw and pasteurised samples the 'inert' fractions from the frozen egg were much larger than those from the unfrozen. Gel filtration of the 'inert' fraction of the unpasteurised frozen new-laid egg showed a very high proportion of material of high molecular weight, much higher than was present in even the pasteurised unfrozen sample, and this was increased further when the thawed egg was pasteurised.

The insoluble proteins from the frozen stale egg samples were markedly different from the others. The raw sample yielded nearly twice as much insoluble material represented by the high-density fraction found at the bottom of the centrifuge tube, as the raw frozen fresh egg. The pasteurised sample had less high-density insoluble material than the corresponding raw sample but more than the pasteurised frozen new-laid egg and also yielded about 4 g of a low-density floating fraction. The insoluble fraction of the unpasteurised frozen stale egg contained a waxy substance and its chromatographic separation on DE 52 was much more difficult than usual, whereas the insoluble fraction from the pasteurised sample separated in a normal manner.

On this occasion the fractionation pattern of the insoluble proteins from the raw egg showed more and bigger fractions than did that of the pasteurised egg's insoluble proteins and the 'inert' fraction from the raw egg had a higher proportion of substance of high molecular weight than that from the pasteurised egg. This unexpected result may be a reflection of the different physical appearance and chromatographic behaviour of the two insoluble portions.

The 'inert' fractions from the raw and pasteurised frozen stale eggs had fat:nitrogen ratios of 7·7 and 10·2, respectively. These are greater than the 1:1 ratio of β-lipovitellenin, which is a probable component of the 'inert' fraction, but very much less than the 50:1 ratio of lipovitellenin, which would normally be found in the low-density floating layer after centrifugation and not in the high-density precipitate. This suggests the possibility that some (or all) of the lipovitellenin in the egg had become degraded by freezing and defrosting or by the preliminary mixing of the whites and yolks into lipoproteins of large molecular size but lower lipid content. Previous work connecting yolk gelation with lipoprotein changes has been summarised by Powrie *et al.*,[8] who themselves showed that lipoproteins extracted from frozen and thawed egg yolk had different electrophoretic properties from the corresponding lipoproteins from unfrozen yolk.

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Herts.

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RAPID METHOD FOR THE SIMULTANEOUS DETERMINATION OF GLUCOSE AND FRUCTOSE USING ANTHRONE REAGENT

By J. W. de BRUYN, H. A. VAN KEULEN and J. H. A. FERGUSON

Rapid quantitative determination of carbohydrates is possible with the aid of Dreywood's anthrone reagent, but the reproducibility of the method was found to be unsatisfactory. Better results were obtained by modifying the method and standardising it accurately. The modifications mainly bore on the composition and preparation of the reagent. The exclusion of dust during the determination was found to be very important.

Rapid simultaneous determination of glucose and fructose was possible by carrying out the reaction at 60° and 100°. An essential condition was that other carbohydrates and substances causing interference should be almost absent.

Application to the determination of sugars in tomato and apple is described.

Introduction

Dreywood's[1] anthrone reagent has been applied with various modifications for the determination of carbohydrates.[2–7] The reaction is based on the formation of a blue-green reaction product resulting from the action of anthrone on the furfural, methylfurfural or oxymethylfurfural formed from carbohydrates by sulphuric acid. Because of its sensitivity, it can be carried out with very small quantities. Undecomposed sugars respond in the same way as when they are hydrolysed.

The aim of this investigation was to ascertain whether the anthrone method could be used for a rapid determination of sugar in some horticultural crops. When current methods, using heating to 100° for some time, were applied, no consistent results were obtained.

Experimental

Standardisation of the method

Composition of the reagent

In the method of Morris[2] 4 or 5 ml of the sugar solution is mixed in a test-tube with twice its volume of reagent, which consists of a solution of 2 g anthrone in 1 l 95% sulphuric acid. For various anthrone concentrations and total volumes, twice the volume of reagent has been used by other authors; sometimes 1 ml solution is mixed with 10 ml reagent.

The temperature, after 2 ml solution had been used (without cooling) with 4 ml reagent, was about 90°. To avoid uncontrolled rises in temperature during mixing with the sugar solution the reagent was diluted with water (2 vol. reagent +1 vol. water). 5 ml of this diluted reagent was mixed with 50 μl sugar solution. The final anthrone and sulphuric acid concentrations were then almost identical with those prescribed by Dreywood and Morris.

Preparation of the reagent

In his research Bailey[6] took precautions to keep the reacting solutions dust-free.

In the present work two reaction temperatures were used, and it was found that at 100° too high values were sometimes obtained as a result of insufficiently dust-free glassware and the entry of dust during the determination.

Forty clean test-tubes were stored on a laboratory table for 48 hours, with the opening turned upwards, after which series of 20 tubes were tested for entry of dust at 100° and 60°. The E_{100}, measured against dust-free, similarly treated blanks, averaged 0·01 (0 to 0·038) and the E_{60} 0·001 (−0·003 to 0·005). The scatter of values of E_{100} was clearly larger than with dust-free treated blanks but at 60° the small amount of dust had no significant effect.

However, despite the use of dust-free glassware and test-tubes closed during the determination, the results continued to show too wide a range.

Bailey[6] found, with pentoses, a clear effect of acid concentration on the reaction. In the present experiments differences were found even with a small alteration in acid concentration. Reagents containing 73·5 and 76·0% by weight of sulphuric acid gave, with 60 μg glucose, the following extinctions (determination replicated six times) after being heated in a water-bath of boiling water for 8 minutes:

73·5% H_2SO_4: ave. 0·544 (0·539—0·546)
76·0% H_2SO_4: ave. 0·565 (0·559—0·571)

It was thought that floating dust particles from the anthrone might enter the tubes in different quantities and, after heating, increase the extinction. By heating the solution for some time a reagent of homogeneous composition was obtained. To standardise also the amount of heat to which the anthrone was exposed during preparation, the anthrone was first dissolved, without being heated, in a small portion of sulphuric acid, and the rest of the sulphuric acid was diluted with water, cooled, and then mixed with the anthrone solution. This reagent was divided into 100 ml portions which were heated in boiling water for 15 minutes.

Standard solutions containing 60 μg glucose were determined in three ways: with glassware not dust-free, heating of reagent not standardised; with glassware dust-free, heating of reagent not standardised; and with glassware dust-free, and 100 ml portions of reagent heated in boiling water in a water-bath for 15 minutes.

The frequency distribution obtained in these 3 experiments as well as in the corresponding blank determinations are summarised in Fig. 1. The determinations were made by heating the mixture in a boiling water-bath for 8 minutes, and by comparison against similarly treated blanks. The blanks

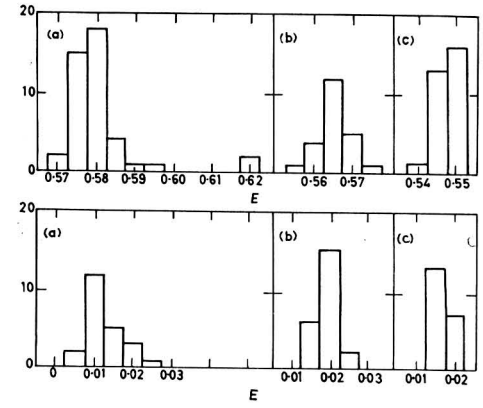

FIG. 1. *Frequency distributions of extinctions obtained in the determination of 60 μg glucose according to 3 methods (see text) and, in the lower diagrams, the corresponding blanks*

(a) Glassware not dust-free—heating of reagent not standardised
(b) Glassware dust-free—heating of reagent not standardised
(c) Glassware dust-free—100 ml portions of reagent heated in boiling water in a water-bath for 15 minutes

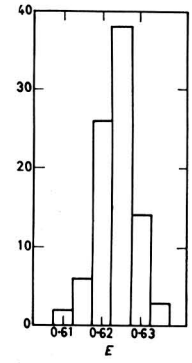

FIG. 2. *Frequency distribution of the extinction obtained in the determination of 60 μg. fructose*

Glassware dust-free—100 ml portions of reagent heated in boiling water in a water bath for 15 minutes

were measured against a reagent that had not been subjected to the 8 minutes' heating. As the scatter of the values for the blanks was not negligible, subsequent measurements were made against a mixture of 3 blanks. From this it may be concluded that the use of dust-free glassware improved the reproducibility of the results, and that after standardisation of heating of the reagent the scatter becomes somewhat smaller. An experiment in which 500 ml was heated for 15 minutes gave on average a slightly higher value than for portions of 100 ml, and the procedure was standardised at 100 ml.

When the experiments were repeated with fructose the results from standardisation were clear for dust-free glassware, but not for the heating treatment, for which the scatter was somewhat larger (Fig. 2) for some unaccountable reason. At 60° the scatter was as great for fructose as at 100°.

Other factors

It was found that despite the above precautions, the reproducibility from day to day was not satisfactory, as small shifts of level occurred. A possible cause was the difference in the temperature of boiling water at different barometer readings, because a change in air pressure of 2 cm Hg results in a change in the boiling point of about 0·8°. From Fig. 3 it can be concluded that such a difference of temperature influences the extinction values. After a decrease in air pressure of 2 cm Hg a decrease in extinction of 0·006 may be expected for 60 μg of glucose, and an increase of 0·016 for fructose. To eliminate this influence a glycerine–water mixture heated to 100° and stirred by an agitator was used instead of boiling water. Better results were, however, not obtained in this way, possibly because the temperature distribution in this viscous liquid was too irregular.

Another factor which could have caused differences in extinction level was the difference in level between the boiling water in the water-bath and the liquid in the test-tubes. Temperature measurements have shown that the contents of a tube at the same liquid level in and around the tube may be 0·8° lower than when the water level of the water-bath was 5 cm higher. If the level was decreased from 5 cm by ½ cm at a time the resulting decreases of temperature in the tube were successively 0·02, 0·02, 0·03, 0·02, 0·03, 0·04, 0·07, 0·10, 0·20 and 0·29°. Temperature measurements in simultaneously heated tubes differed only slightly (about 0·2°).

Although in these experiments the water level in the water-bath was not always exactly the same, this will presumably have caused only slight differences. The effect of differences in air pressure is not known. As the shifts of extinction-level could not be entirely eliminated it was considered advisable to add some standards to each series.

Reaction temperature and reaction time

To find out whether reaction temperatures below 100° would have advantages, 60 μg portions of sugar were determined at different temperatures and times. The results are summarised in Fig. 3. The behaviour of glucose was quite different from that of fructose, the most noteworthy difference being the extremely low extinctions of glucose at 60° compared with fructose. This difference allows the simultaneous determination of both sugars by carrying out the reaction at 60° and at 100°. For a determination of the sum of both sugars 100° seems the most suitable temperature, because at 90° and 80° the extinctions come close enough to one another only after a longer period of time. Any changes in temperature of boiling water resulting from differences in air pressure can be allowed for by the simultaneous determination of a few standards.

For the reaction time 8 minutes was chosen because rapid determinations were desired and the extinctions were favourable after this period.

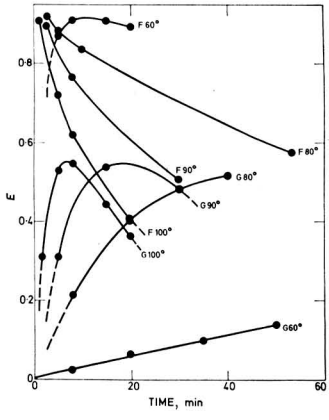

FIG. 3. *Extinctions obtained with 60 μg glucose (G) and 60 μg fructose (F) with 5 ml standardised anthrone reagent at different temperatures and reaction times*

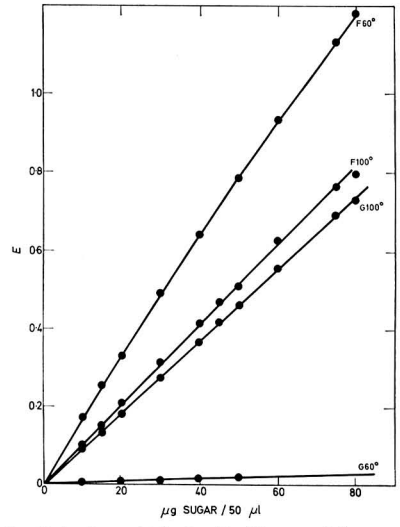

FIG. 4. *Extinctions obtained with 50 μg solution containing different quantities of either glucose (G) or fructose (F) and 5 ml of standardised anthrone reagent, after heating in a water-bath for 8 minutes at 60° or at 100°C*

Standard curves and calculations

The method has been standardised for glucose and fructose by heating 50 μl of each solution with 5 ml standardised anthrone reagent in a water-bath for 8 minutes at 60° and at 100°. The resulting extinctions are shown in Fig. 4. It appears that the points can be approximated by straight lines, at least up to concentrations of 50 μg/50 μl.

To find the equations for a simultaneous determination of the concentrations of glucose and fructose in a mixture of both, two series each of 35 mixtures were analysed in which both components occurred with 0, 10, 20, 30, 40 or 50 μg/50 μl. Extinction values at 60° (E_{60}) and 100° (E_{100}) were determined. This led to the equations:

$$G = 113{\cdot}3\ E_{100} - 73{\cdot}1\ E_{60} \quad \ldots\ldots\ldots\ldots (1a)$$

$$F = 65{\cdot}2\ E_{60} - 3{\cdot}3\ E_{100} \quad \ldots\ldots\ldots\ldots (1b)$$

in which G and F stand for the concentration in μg/50 μl of glucose and fructose, respectively.

For solutions containing either fructose or glucose the equations may be applied as well. In this instance only one reaction temperature (100°) is required. By elimination:

$$\text{if } F = 0,\ G = 109{\cdot}6\ E_{100} \quad \ldots\ldots\ldots\ldots (2a)$$

$$\text{if } G = 0,\ F = 97{\cdot}8\ E_{100} \quad \ldots\ldots\ldots\ldots (2b)$$

which agrees closely with the results shown in Fig. 4.

For an analysis of total sugar in a solution of equal concentrations of glucose and fructose a reaction at 100° will suffice, using the equation:

$$\text{if } F = G,\ F+G = 103{\cdot}3\ E_{100} \quad \ldots\ldots\ldots\ldots (3)$$

This might be applied as an approximation when the concentrations of both components do not differ too much.

There remain three kinds of errors to deal with. The first is caused by the deviation of the relationship between F and E_{60} from linearity (see Fig. 4). This led to the suggestion that solutions to be examined should contain 20–60 μg/50 μl.

The second source of error is the daily variation in level. For this reason each day a number of analyses of one or more standard mixtures of glucose and fructose should be made, from which the correction of the day should be derived, to be added to the results of (1a) and (1b), or to the other equations. For example when 3 standard mixtures were analysed: $G=20$, $F=40$; $G=30$, $F=30$; and $G=40$, $F=20$, in five replications, the corrections required for three successive days were: for glucose, −0·24, −1·02 and −0·94; and for fructose, −1·71, −1·60 and −1·29. By keeping a record of the day corrections it is hoped that a better insight into this pheomenon may be obtained.

The third is the conjectural experimental error, which was $s_1=0{\cdot}6$ for glucose and $s_2=0{\cdot}25$ for fructose. On these data the number of analyses can be fixed on which a day correction should be based. If this number is N, the standard error of the correction term is $s(c)=s\sqrt{N}$. For example, for $N=6$ $s(c_1)=0{\cdot}25$ for glucose and $s(c_2)=0{\cdot}10$ for fructose, and the error of the day corrections would be unlikely to exceed 0·5 and 0·2 respectively.

Other sugars and substances causing interference

To determine the interference effect of other soluble sugars which may occur in addition to glucose, fructose (and sucrose), the extinctions were measured of 100 μg rhamnose, galactose, xylose and arabinose after they had been heated at 60° and 100° for 8 minutes. The results were as follows:

	E_{60}	E_{100}
L-rhamnose (1 H_2O)	0·354	0·678
D-galactose	0·045	0·510
D-xylose	0·320	0·054
D-arabinose	0·080	0·018

Thus at both temperatures some of these sugars can have a large effect.

Because it was hoped that this method could be used for the determination of sugars in pectin-containing products, special attention was given to any interference by pectin. A commercial sample of pectin (from apples) was purified 4 times by precipitation, after addition of 1 ml 4N-HCl to 40 ml solution, with an equal volume of acetone. The precipitated pectin was passed through a glass filter and washed with 50% acetone. In the last filtrate the anthrone reaction, after removal of the acetone, was negative. The extinctions obtained with 100 μg purified pectin were approximately 0·08 at 100° and 0·01 at 60°. As the sugar content of fruits is usually much higher than the pectin content, the expected error will be slight, The tomato contains about 15 times as much sugar as pectin, with the result that the sugar content will appear to be about 1% too high. The pectin content of apples may be higher, but the sugar content is also higher so that here, too, the determination is hardly disturbed by the pectin.

Starch will usually have only a slight effect if clear juice of the unheated product is used. Pollution during analysis by particles of filter paper and cotton-wool should be carefully avoided.

Standardised procedure

Preparation of reagent

From 135·6 g 95·0% by weight of sulphuric acid, 5–10 ml were used to dissolve 148 mg anthrone. The rest of the sulphuric acid was mixed with 36·9 g of water and, after cooling to room temperature, mixed with the anthrone solution. Thus rather more than 100 ml of reagent was obtained. This quantity was heated in a boiling water-bath for 15 minutes and then quickly cooled. When a larger quantity of reagent was prepared, this was divided into portions of 100 ml before being heated. The reagent was used at least 3 hours after, and at most 24 hours after, preparation.

Determination

Before being dried, carefully cleaned test-tubes were inverted in order to exclude dust. All other glassware was also thoroughly cleaned and kept dust-free. With a micropipette 50 μl of the solution to be analysed, containing about 20–60 μg sugar, was placed in each tube, after which 5 ml reagent was added. Each tube was closed with a sheet of plastic (also properly cleaned), after which the contents were mixed.

The blank reference solutions contained 50 μl water and 5 ml reagent. Before measurement 3 blanks were mixed.

The tubes were placed in a rack and heated for 8 minutes in a water-bath at 60°, or in vigorously boiling water in a water-bath. The level in both water-baths should preferably be 3–4 cm above the level in the tubes. After having been heated, the tubes were rapidly cooled in cold water. Within two hours the colour was measured at 630 nm.

In each series of determinations some standard mixtures of glucose and fructose (e.g. 20+20 and 30+30 μg) were included to determine the day corrections. If only the total-sugar content was to be determined the reaction at 60° could be omitted.

Examples of application

Tomato

For tomatoes the anthrone method appears to be time-saving, the more so as purification of the solution is not necessary, in contrast to the determination according to Luff-Schoorl.

The procedure was as follows: a number of tomatoes were ground in a homogeniser for 5 minutes. A portion of the substance was centrifuged at 3,500 rev/min in closed tubes for 10 minutes, filtered through cotton-wool and subsequently twice through one filter-paper. In this way no trouble arose from particles of the filter-paper. After dilution by 40 times, the analysis was carried out on 50 μl solution.

Agreement with determinations in an extract purified with lead subacetate and sodium phosphate according to the method of Luff-Schoorl as modified by Van de Kamer[8] is good, as appears from the results of 22 determinations with both methods (see Table I). The anthrone method generally gave a somewhat lower content than the Luff-Schoorl method. The relative difference averaged 1·6%. The glucose/fructose ratio was about 1:1 (on an average glucose 46%, fructose 54% of the total).

TABLE I

Comparison of the anthrone method with the Luff-Schoorl method in tomato

The 22 items are fruits of *Lycopersicon pimpinellifolium* and crosses with it, and different varieties of *L. esculentum*.

No.	% red. sugar (Luff-Schoorl)	% sugar (anthrone method)			% deviation rel. to Luff-Schoorl
		glucose	fructose	gluc.+fruct.	
1	3·77	1·53	2·19	3·72	−1·3
2	4·54	2·08	2·32	4·40	−3·1
3	3·27	1·62	1·70	3·32	+1·5
4	2·40	1·05	1·34	2·39	−0·4
5	2·96	1·39	1·38	2·77	−6·4
6	3·32	1·52	1·82	3·34	+0·6
7	2·15	0·92	1·23	2·15	0
8	3·23	1·42	1·80	3·22	−0·3
9	3·39	1·53	1·86	3·39	0
10	3·54	1·59	1·95	3·54	0
11	3·04	1·30	1·64	2·94	−3·3
12	3·03	1·38	1·70	3·08	+1·7
13	3·59	1·53	1·93	3·46	−3·6
14	3·49	1·51	1·85	3·36	−3·7
15	4·65	2·08	2·40	4·48	−3·7
16	3·16	1·31	1·70	3·01	−4·7
17	3·00	1·33	1·63	2·96	−1·3
18	3·11	1·41	1·60	3·01	−3·2
19	3·08	1·48	1·62	3·10	+0·6
20	2·82	1·22	1·54	2·76	−2·1
21	3·62	1·83	1·79	3·62	0
22	3·16	1·52	1·55	3·07	−2·8

In the presence of sucrose, which is the principal sugar in *Lycopersicon hirsutum* and *L. peruvianum*,[9] the anthrone method was only suitable for the determination of total sugar, and of the glucose/fructose ratio (free+bound). For more detailed information chromatographic separation was necessary. In the current tomato varieties tested in the present experiments, however, as in *L. pimpinellifolium*, sucrose only occurs in minute quantities.

Apple

The apple always contains sucrose, in addition to glucose and fructose. Unripe apples contain more sucrose than do ripe ones. The amount of fructose is considerably higher than the amount of glucose at all stages of maturity. A determination of total sugar, if necessary combined with a determination of the glucose/fructose ratio, can also be carried

out rapidly on apple. From a number of apples, sectors of the exposed and the shaded sides were made into juice in a juice centrifuge (within 1 minute in order to limit evaporation). The juice was then passed twice through the same filter. After dilution by 80–200 times the determination was done with 50 μl solution.

Agreement with determinations according to the method of Luff-Schoorl, after inversion, in an extract purified with lead subacetate and sodium phosphate, was good. In 12 samples, which were analysed both in the picking-ripe and the eating-ripe stages, the relative deviation compared with the Luff-Schoorl method averaged -2%. All values were somewhat lower (1–4%).

Discussion

The determination of carbohydrates using anthrone reagent is an easy and rapid method, but seems to be employed little, probably because the results used to be inconsistent. With the modified method, however, it is possible to determine carbohydrates more rapidly than with a reduction method, and with an equal degree of accuracy.

However, the method has its limitations. Thus, in a mixture of glucose, fructose, and sucrose, only the total amount of sugar can be determined, if necessary combined with a determination of the glucose/fructose ratio (free + bound).

Furthermore the qualitative composition of the carbohydrates in the sample must be known before it can be judged whether the method is applicable. Moreover, with products to which this method is applied for the first time, it is necessary to ascertain whether disturbing substances are present.

There are several colour reactions to distinguish fructose from glucose, which are all based on the fact that fructose gives oxymethylfurfural rapidly, whereas glucose does so slowly.[10]

Guimberteau[11] used this property by determining, besides total sugar (at 100° for 12 minutes), the fructose separately by heating it at 25° for 4 hours. In the latter case the glucose does not react perceivably. The method chosen here, in which glucose reacts slightly at 60°, has the advantage of shortening the duration of heating, but this shorter time of heating necessitates an accurate control of the length of time and the temperature. Hence the number of tubes heated simultaneously must not be too large.

The high degree of accuracy required in applying the anthrone method is because a micromethod which is greatly disturbed by pollutions, notably by dust from the air. That deviations due to dust occur at 100° but not at 60° might be due to the fact that, in laboratory dust, polysaccharides derived from glucose (cellulose from clothing, towels, filter-paper, etc.) will be present, but probably no polysaccharides derived from fructose.

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LOW MOLECULAR WEIGHT THIOLS AND DISULPHIDES IN FLOUR

By F. J. R. HIRD, I. W. D. CROKER and W. L. JONES*

The reduced glutathione and oxidised glutathione contents of two grades of flours have been determined by an enzymic method. The low molecular weight thiol and disulphide content of these flours have been estimated by polarography.

It is concluded that the glutathione levels in flour are large enough to modify the rheological properties of dough. The low molecular weight compounds of flour are present in larger amounts and these may also be implicated in determining the normal rheological behaviour of dough.

Introduction

Low molecular weight thiols such as cysteine and glutathione have been shown to have marked effects on the mixing properties of dough and the relaxation of strains introduced into dough.[1, 2] A comparison of the effects of reduced and oxidised glutathione,[3, 4] indicated that oxidised glutathione also exhibited marked rheological effects at levels as low as 5 μmole/50 g flour.

Glutathione is known to be present in large amounts in wheat germ and its presence in flour has been detected.[5] The presence of low molecular weight thiols and disulphides in flour was shown by polarographic techniques[3] and as low molecular weight disulphide compounds,[6, 7] participate readily in thiol-disulphide interchange reactions (Frater, R., & Hird, F. J. R., unpublished). It was thought that their presence in flour may have special significance. The following investigation on the levels of glutathione, oxidised glutathione and low molecular weight disulphides on various samples of flour has been made to estimate their quantitative significance.

Experimental

Enzymic estimation of reduced and oxidised glutathione

Reduced glutathione was estimated in a pH-stat (Radiometer Copenhagen, Denmark) by the glyoxalase method as previously described.[8]

Oxidised glutathione was estimated by the same method following enzyme reduction by reduced nicotinamide-adenine dinucleotide-phosphate ($NADPH_2$) in the presence of glutathione reductase.[8]

Preparation of flour extracts for enzymic estimation

Flour (20 g) was mechanically shaken with 30 ml EDTA solution (10 mM) at pH 7·0 for 15 min in a capped polythene centrifuge tube containing a large glass marble. The suspension was then centrifuged at 9000 *g* for 10 min and the supernatant fluid containing the compounds to be investigated was decanted to yield 14 ml of extract. The extract was stored in ice for the duration of the experiment and 1 ml samples were taken for analysis. The results were calculated on the basis of 30 ml water being available as a solvent. Recovery of oxidised glutathione (0·1 μmole) added to the extract ranged from 82–98%.

Preparation of flour extracts for polarographic estimation

To avoid the problem of incomplete extraction and of estimation of the volume of available solvent following extraction, the flour samples were used as suspensions. Flour (5 g) was extracted as above by suspension in 30 ml of EDTA solution (total vol. 33 ml) at pH 7·0 and a 10 ml sample taken for analysis in the polarographic system.

Estimation of low molecular weight thiols and disulphides by polarography

At pH 9·0 reduced and oxidised glutathione gave well defined current voltage waves with a half-wave potential at −0·5 V – these being cathodic and anodic respectively. The height of these waves is linearly related to concentration. In flour extracts, however, an unknown compound combines with disulphide compounds so that the current voltage wave at −0·5 V disappears.[8] The addition of sulphite forms an equivalent of thiol from each disulphide bond and this now allows the wave height of the thiol to be measured.

The polarograph (Metrohm Polarecord Type E 261) was used in conjunction with an Ag/AgCl reference electrode. The reaction vessel contained KCl (0·7 g) as the carrier electrolyte, the sample being analysed and tris buffer (pH 9·0, 0·1 M) to a final volume of 11 ml. When sulphite was used 0·5 g anhydrous Na_2SO_3 was added as a solid. The temperature was maintained at 38°. Current voltage waves were run in the absence of sulphite, in the presence of sulphite and after the addition of a standard amount of either glutathione or oxidised glutathione. The quantity of diffusible thiol (in the absence of sulphite), the quantity of diffusible disulphide (in the presence of sulphite) were calculated from the wave height in relation to the increase in wave height on the addition of 1 μmole of glutathione or oxidised glutathione to the system. The recovery of oxidised glutathione added to the flour varied from 77–88%. No correction has been made to allow for the incomplete recovery.

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Estimation of total thiol groups and disulphide bonds in flour

These estimations were made amperometrically with methylmercuric iodide in the presence of 8 M urea by the back titration method already described.[9]

Materials

The flours were unbleached commercial samples of Victorian soft wheats taken at different points of the three mills in order to give high grade and low grade flours. It was thought that these two grades may exhibit differences in low molecular weight thiols and disulphides as the extensograms of such flours are consistent with variations in the amounts of these compounds—assuming that their presence would affect the rheological properties of dough. Glutathione reductase and reduced NAD were purchased from Sigma Chemical Co. Glutathione was purchased from S.A.F. Hoffmann-La Roche and Co. Ltd.

Results

Analytical and rheological characteristics of flour samples

Table I lists the protein, thiol and disulphide content of the six flour samples and shows that the disulphide and thiol content increase with the protein content and are higher in the low grade flours.

The extensograph (Fig. 1) given only for sample No. 2 (the other samples giving similar results) show the greater extensibility and lower resistance in the low grade flour. No attempt is made to relate the negative correlation between rheological characteristics and the analytical figures as it is known that features other than those listed in Table I are important in determining rheological properties.

As measured polarographically, the diffusible (low molecular weight) thiols and disulphides are presented in Table II. The figures presented are based on the unknown compounds having identical rates of diffusion to the electrode as glutathione. While they must therefore be approximations to absolute values, the results enable comparisons of the various flours to be made. A constant feature of the results is that the disulphide form is always greater than the thiol form. If they were as active as glutathione such concentrations of thiol and disulphide would have marked effects on the rheological properties of flour-water systems. There is however, no uniform relation between concentrations and grade of flour.

Table III gives the estimates of both forms of glutathione in flour as measured enzymically by use of the yeast enzyme, glyoxalase. Consistently there is an association with the total glutathione level and the low grade flour. The amounts present are probably of significance in modifying the rheological characteristics of dough.

Discussion

The peptide nature of the low molecular weight thiols and disulphides present in flour is a subject of a separate paper (Jones, I. K., & Carnegie, P. R., personal communication). From the specificity of the glyoxalase reaction however, it can be assumed that a small proportion of the total thiol and disulphide consists of glutathione and its oxidised form.

TABLE I

Protein, ash, disulphide and thiol content of flours

Flour Sample	Protein Content, %	Ash Content, %	Disulphide content, μmole/g flour	Thiol content, μmole/g flour
1. High grade	7·3	0·39	7·65	1·09
Low grade	8·4	0·48	10·12	1·16
2. High grade	8·4	0·40	9·09	1·14
Low grade	9·9	0·53	10·79	1·32
3. High grade	8·7	0·40	9·43	1·05
Low grade	9·7	0·56	9·78	1·23

Flours were conditioned to 12% moisture and results calculated on wet basis.

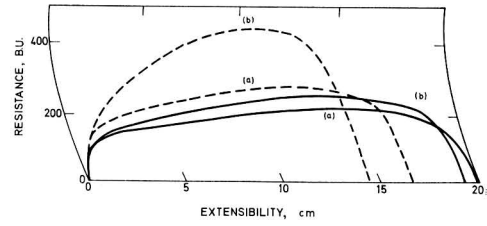

FIG. 1. *Tracings of extensograms of flour sample No. 2.*

- - - High grade (a) Dough pulled at 45 min (b) Dough pulled at 135 min
—— Low grade (a) Dough pulled at 45 min (b) Dough pulled at 135 min

TABLE II

Diffusible thiols and disulphides of flour as measured polarographically

	Thiol* (μmoles/gm)	Disulphide* (μmoles/gm)	Total – SH +–SS– with GSH as reference standard*	Total with GSSG +sulphite as reference standard**
High Grade No. 1 Mill	0·12	0·42	0·54	0·47
Low Grade No. 1 Mill	0·16	0·40	0·56	0·52
High Grade No. 2 Mill	0·11	0·36	0·47	0·53
Low Grade No. 2 Mill	0·16	0·50	0·66	0·53
High Grade No. 3 Mill	0·14	0·41	0·55	0·50
Low Grade No. 3 Mill	0·11	0·35	0·46	0·52

Calculations based on increase in wave height following addition of 1 μmole GSH* or GSSG** to analytical systems

TABLE III

Estimation of reduced and oxidised glutathione in flour as measured enzymically

Sample	GSH, μmole/g flour	GSSG, μmole/g flour	Total glutathione equivalents	Recovery of 0·1 μmole GSSG added to extract
High Grade	0·042	0·033	0·108	82%
Low Grade	0·045	0·040	0·125	84%
High Grade	0·065	0·022	0·109	88%
Low Grade	0·057	0·053	0·163	90%
High Grade	0·057	0·029	0·115	98%
Low Grade	0·054	0·038	0·130	90%

Previous work on the effects of the two forms of glutathione on the rheological properties of dough[4] indicates that the levels found in flour would be expected to be effective in altering the shape of farinograph curves. Thus, the presence of glutathione in normal flour would in part determine the rheological properties as measured by the extensograph and farinograph. The glutathione present in flour possibly arises from all cellular tissue of the grain but there is evidence that more is present in the lower grade flour. As the origin of this fraction is closer to the bran this is perhaps explicable.

The levels of the two forms of glutathione found in the present investigation 0·11–0·16 μmole/g are somewhat lower than those found by Kuninari & Matsumoto,[5] namely, 0·26–0·30 μmole/g. There is however the probability that only part of these latter figures is glutathione, as the alloxan method is not specific. Proskuryakov & Zueva,[10] have estimated low molecular weight sulphur compounds in extracts of flour by a different technique and arrived at figures of 0·2–0·44 μmole/g for thiol and 0·35–0·5 μmole/g for disulphide. These figures are comparable with those obtained in the present investigation.

The presence in flour of low molecular weight thiol and disulphide compounds is of considerable significance for if active, they are present in amounts which would exert rheological effects. The diffusible nature of small molecular weight compounds gives them additional significance. Furthermore, if intermolecular disulphide bonds are important in maintaining a crosslinked structure in dough, univalent thiols will react with such disulphide bonds and interrupt the system of linkages. The higher concentrations of these low molecular weight compounds associated with the bran (unpublished results) must then place additional importance on milling characteristics of the grain. In the present work there appears to be a correlation between the grade of flour and the amount of glutathione present but other possible factors have not been evaluated and firm conclusions are therefore unwarranted.

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ANTIOXIDANT PROPERTIES OF LUCERNE EXTRACTS

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Aqueous extracts of fresh and dehydrated lucerne were found to exhibit heat-stable antioxidant activity toward the autoxidation of linoleic acid, the lipoxidase-catalysed oxidation of linoleic acid and the lipoxidase-induced carotene oxidation. EDTA increased the antioxidant activity of lucerne extract in all three systems. Alone, EDTA inhibited linoleate autoxidation, but not lipoxidase-catalysed linoleate oxidation, and was only partly inhibitory toward lipoxidase-induced carotene oxidation.

The presence of ferulic acid in the acid hydrolysate of lucerne extract could be demonstrated by both paper and gas chromatography. This acid, and the related coumaric and sinapic acids, were shown to inhibit lipoxidase activity. It is suggested that a ferulic acid derivative may play a rôle in the antioxidant effects observed with aqueous lucerne extracts.

Introduction

Lucerne (alfalfa) is an economically important fodder plant and constitutes a rich source of carotenoids. Because of oxidative processes, these carotenoids are rapidly destroyed in the fresh plant after cutting and—more slowly—in the dehydrated meal. In the course of a bio-chemical study of carotenoid destruction in fresh and dehydrated lucerne, it was found that aqueous extracts of lucerne exhibited pronounced antioxidant properties. It is the purpose of the present paper to describe some of the antioxidant effects displayed by such extracts.

The following reactions were used as criteria: autoxidation of linoleate in buffered aqueous solution, as measured by oxygen absorption; lipoxidase-catalysed linoleate oxidation, also measured by oxygen absorption; and lipoxidase-catalysed oxidation of carotene in the presence of linoleate, as indicated by the colorimetric measurement of carotene disappearance. In addition to the lucerne extract, the metal-chelating compound ethylenediaminetetraacetic acid (EDTA) was also tested in the above three systems.

Experimental

Preparation of lucerne extract

Two hundred g of lucerne meal were first defatted by benzene extraction. The lipid-free meal was refluxed with 1200 ml 95% ethanol for 6 h. The mixture was filtered and the residue was similarly extracted three more times. The lucerne meal residue was again refluxed for 6 h with 80% ethanol, and finally with 50% ethanol. The last of these extracts, after filtration, was evaporated to dryness and taken up in 200 ml water, so that 1 ml extract corresponds to 1 g lucerne meal.

Hydrolysis of lucerne extract

Ten ml of lucerne extract, obtained as described above, were hydrolysed by refluxing for 10 min after addition of conc. HCl to a final concentration of 2 N. The hydrolysate was repeatedly shaken with ether and the ether extract was washed free of acid with water, dried over Na_2SO_4, evaporated to dryness and taken up in either 1 ml ethanol for paper chromatography, or 1 ml ethyl ether for gas chromatography.

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Paper chromatography of lucerne extract

Circular paper chromatography of the ethanol solution obtained as described above was carried out on Whatman No. 1 paper impregnated with 0·1 M phosphate buffer pH 7·4, using a mixture of secondary butanol and phosphate buffer (pH 7·4, 4:1 v/v) as solvent, according to van Sumere.[8] Spots were identified both by illumination with u.v. light and by spraying with diazo-Na_2CO_3 reagent.[8] Under these conditions, ferulic acid yielded an R_f value of 0·32.

Gas chromatographic detection of ferulic acid in lucerne extract

The ether solution obtained as described above was chromatographed in a Perkin Elmer 801 instrument fitted with 6 ft. × $\frac{1}{8}$ in. glass column packed with 10% DC-710 on Chromosorb W HMDS 60/80. The operating temperatures for the injector, column and detector were 210, 175 and 200° C, respectively. Nitrogen was used as a carrier gas at a rate of 60 ml/min. The retention time for ferulic acid under these conditions was 4·6 min.

Oxygen absorption by linoleic acid

Oxygen absorption was measured in a conventional Warburg apparatus. The following reagents were introduced into a 12 ml Warburg flask: 1·9 ml 0·2 M phosphate buffer pH 6·9 containing linoleic acid (see below) to yield a final concentration of $4{\cdot}75 \times 10^{-3}$ M linoleic acid in the system; 0·1 ml EDTA solution to give a final concentration of 10^{-3} M (unless otherwise stated); 0·5 ml lucerne extract. In the lipoxidase assay the side arm contained 0·5 ml enzyme (lipoxidase) solution prepared by stirring 0·25 g defatted soybean meal with 100 ml ice-cold water during 30 min and filtering the extract. After 20 min equilibration at 37°C, the solutions were mixed and the readings were started. The linoleic acid solution used in this system was prepared by a modification of the method of Surrey:[9] 0·5 ml linoleic acid was dispersed in water with the help of an equal amount of 'Tween 20'. The solution was neutralised with 1 N-NaOH and diluted with 0·2 M phosphate buffer pH 6·9 to 200 ml.

Coupled oxidation of carotene

The system for the measurement of the lipoxidase-induced coupled oxidation of carotene was made up as follows: 12·5 ml 0·1 M phosphate buffer pH 6·9, 12·5 ml lucerne extract (or H_2O in the control), 12·5 ml β-carotene in aqueous 'Tween 20' solution (final concentration of 'Tween' in the system 0·66%),

2·5 ml ethanolic solution of linoleic acid giving a final concentration of 2×10^{-3} M linoleic acid in the system, 12·5 ml enzyme solution, prepared as described in the preceding paragraph. EDTA, when added, was dissolved in appropriate amounts in the carotene solution. All solutions, except the enzyme solution, were mixed and brought to temperature equilibrium in a water bath at 37°C, after which the enzyme solution was added. At different time intervals, portions of 1·6 ml were withdrawn into test tubes containing 10 ml chloroform–ethanol (10 : 3, v/v). This solvent was adopted, since it was found that it stopped the reaction completely and that it was also very effective in the one-step extraction of carotene. Each tube was vigorously shaken and allowed to stand a few minutes until separation of two phases. The lower chloroform layer had a volume of 9·3 ml, of which about 7 ml were transferred by pipette to another test tube and clarified by adding a little anhydrous sodium sulphate powder. The absorbence of the clear solution thus obtained, was measured at 460 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. The carotene value was then computed from a standard curve of carotene in chloroform solution.

Results and Discussion

Preliminary observations showed that the lipoxidase-induced coupled oxidation of linoleic acid and carotene is strongly inhibited in the presence of an aqueous extract of fresh lucerne. The antioxidant effect of the lucerne extract was not diminished by boiling. Because of this finding, and in order to provide a more uniform source of antioxidant factor(s), commercial dehydrated lucerne meals were used in subsequent experiments. When lucerne meal was extracted according to the procedure described under Experimental, it was found that 90% of the antioxidant activity was present in a 50% ethanol extract obtained after the meal had been extracted successively with benzene, 95% ethanol and 80% ethanol, the activity of a direct water extract of lucerne meal being taken as 100%. Since the aqueous-ethanolic extract contained considerably less pigments and protein than did the water extract, it was used as the antioxidant source throughout this work.

The effect of lucerne extract and EDTA on the autoxidation of linoleic acid is shown in Fig. 1. It appears that linoleate autoxidation is strongly suppressed by the lucerne extract and, to a somewhat smaller extent, by EDTA. Practically complete inhibition is obtained by the concomitant action of both lucerne extract and EDTA.

Fig. 2 illustrates the inhibitory action of lucerne extract on the lipoxidase-catalysed oxidation of linoleate. EDTA enhances this antioxidant effect to a considerable extent, when added at a concentration of 10^{-2} M, but not when its concentration is 10^{-3} M. In the absence of lucerne extract, EDTA has almost no influence on the action of lipoxidase.

As can be seen from Fig. 3, the lipoxidase-induced oxidation of carotene is likewise suppressed by lucerne extract, and EDTA again enhances this effect. EDTA alone exerts an inhibitory effect, intermediate between those found in the two other assay systems: EDTA inhibits the lipoxidase-induced carotene oxidation, but only if added at high concentration, whereas linoleate autoxidation is sensitive to lower concentrations of EDTA, and the lipoxidase-catalysed linoleate oxidation is not affected by this chelating agent.

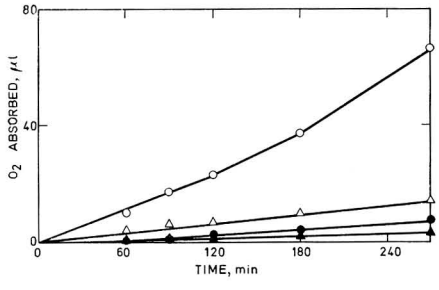

FIG. 1. *Effect of lucerne extract and EDTA on the autoxidation of linoleate at pH 6·9 and 37°C*

Reaction volume 3·0 ml. Concentration of linoleic acid 4.75×10^{-3} M
○ No addition; ● 0·5 ml lucerne extract; △ EDTA 10^{-3} M; ▲ 0·5 ml lucerne extract plus EDTA 10^{-3} M

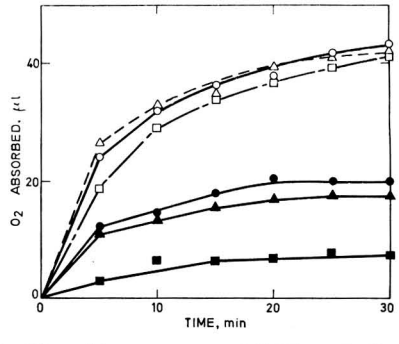

FIG. 2. *Effect of lucerne extract and EDTA on the lipoxidase-induced oxidation of linoleic acid*

Lipoxidase added as 0·5 ml of a 0·25% w/v aqueous soybean meal extract. All other conditions as in Fig. 1
○ No addition; ● 0·5 ml lucerne extract; △ EDTA 10^{-3} M; ▲ 0·5 ml lucerne extract plus EDTA 10^{-3} M; □ EDTA 10^{-2} M; ■ 0·5 ml lucerne extract plus EDTA 10^{-2} M

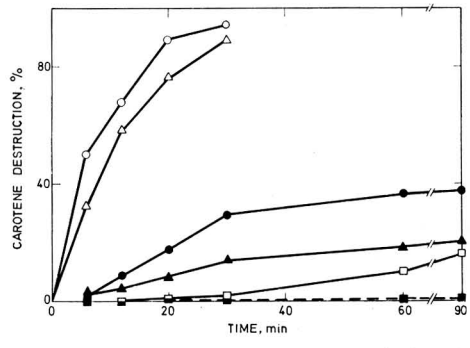

FIG. 3. *Effect of lucerne extract and EDTA on the lipoxidase-induced oxidation of carotene at pH 6·9 and 37°C*

Reaction volume 52·5 ml. Concentration of linoleic acid 2×10^{-3} M. Initial carotene concentration 18·0 µg/ml reaction mixture
○ No addition; ● 12·5 ml lucerne extract; △ EDTA 10^{-3} M; ▲ 12·5 ml lucerne extract plus EDTA 10^{-3} M; □ EDTA 10^{-2} M; ■ 12·5 ml lucerne extract plus EDTA 10^{-2} M

The diverging effects produced by EDTA in the three assay systems, and the increased antioxidant activity of lucerne extract in the presence of EDTA, point to the presence of metallic contamination in the lucerne extract and, to some extent, also in the assay systems. In autoxidation processes, in which the peroxidic end product fulfills an important autocatalytic function, metals would act as pro-oxidants by virtue of their positive catalytic effect on the homolytic scission of peroxides. On the other hand, in lipoxidase-catalysed linoleate oxidation, radical generation occurs within the lipid-enzyme complex,[1] without significant contribution from the peroxidic end product, and metal ions would therefore not be expected to affect this system. Finally, it appears likely that antioxidants and carotene are affected by peroxide-generated free radicals[2] and such a reaction would be sensitive to metal catalysis, hence the various effects of EDTA in the different systems.

Attempts were made to obtain some information on the chemical nature of the water-soluble antioxidant(s) present in lucerne. The possible presence of flavonoids was examined, since such compounds are present in many plants and have also been shown to be responsible for the antioxidant activity of certain aqueous plant extracts. Lucerne extract obtained as described under Experimental was therefore passed through a column of Amberlite IRC-50, under conditions under which flavonoids would be retained by the column.[4] The eluate was as active toward lipoxidase as the original extract, therefore it seemed unlikely that flavonoids could be responsible for the antioxidant activity.

A second class of compounds of possible significance in this respect is the group of hydroxycinnamic acid derivatives. Dicks & Friend[5] have shown that such compounds are active in the inhibition of crocin oxidation. Hydroxycinnamic acid derivatives have been discovered in extracts of oats and the antioxidant potency of such extracts has been ascribed to the presence of compounds of this type.[6] The antioxidant effect exerted by pea slurries is due to similar compounds, according to recent observations by Rhee & Watts.[7] Lucerne extract was therefore submitted to circular paper chromatography, using coumaric (*p*-hydroxycinnamic acid), ferulic (4-hydroxy-3-methoxycinnamic acid) and sinapic (4-hydroxy-3, 5-dimethoxycinnamic acid) acids as reference standards, but no such compounds could be detected. However, when the lucerne extract was first hydrolysed in the presence of hydrochloric acid, a spot corresponding to ferulic acid appeared. The identity of this compound was confirmed by gas chromatography of the ether extract obtained from the hydrolysate.

The inhibitory action of coumaric, ferulic and sinapic acids on lipoxidase was examined and compared to the effects obtained with lucerne extract and with the commercial antioxidant 'BHT' (2, 6-di-tert-butyl-4-methylphenol). When the hydroxycinnamic acids were added to the lipoxidase-linoleate system at a concentration of 2×10^{-4} M, and to the lipoxidase-linoleate-carotene system at a concentration of 10^{-4} M, considerable inhibition was obtained in both systems, the effect increasing progressively in the order coumaric, ferulic and sinapic acids, without however, reaching the antioxidant effects achieved with 'BHT' or lucerne extracts (Figs 4 and 5).

Further information on the antioxidant activity of lucerne extract was obtained by dilution studies, in which the effect of decreasing amounts of lucerne extracts was investigated. Even after tenfold dilution, lucerne extract lost none of its inhibitory activity in the lipoxidase-linoleate system. However, in the lipoxidase-induced carotene oxidation, the lucerne extract proved to be less effective; although a twofold dilution did not result in any significant loss of antioxidant activity, a tenfold dilution led to half the original inhibitory activity (Fig. 6). According to van Sumere,[8] lucerne contains 0·012% ferulic acid. Calculation shows that the lucerne extract would contribute 28 μg ferulic acid per ml reaction mixture, or 14 μg, if half the amount of extract is used. These values are of the same magnitude as the amount of pure ferulic acid

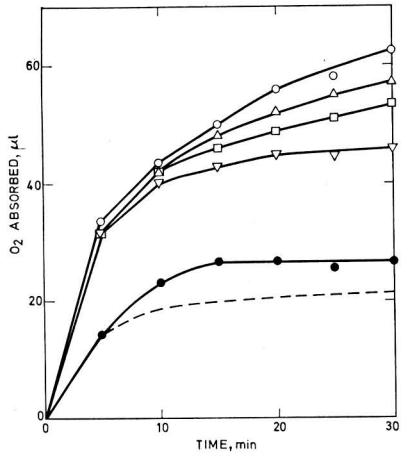

FIG. 4. *Effect of hydroxycinnamic acids (coumaric, ferulic and sinapic), 'BHT' and lucerne extract on the lipoxidase-catalysed oxidation of linoleic acid*

Incubation conditions as in Fig. 2. Concentrations of the antioxidants 2×10^{-4} M; lucerne extract added in amount equivalent to 0·5 g lucerne meal
○ No additions; △ coumaric acid; □ ferulic acid; ▽ sinapic acid; – – – 'BHT'; ● lucerne extract

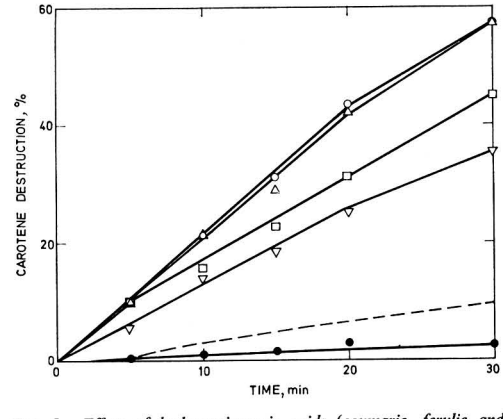

FIG. 5. *Effect of hydroxycinnamic acids (coumaric, ferulic and sinapic), 'BHT' and lucerne extract on the lipoxidase-catalysed oxidation of carotene*

Incubation conditions as in Fig. 3. EDTA present at a concentration of 10^{-3}M. Concentrations of antioxidants 10^{-4} M. Lucerne extract 12·5 ml in total reaction volume of 52·5. Initial carotene concentration 20·0 μg/ml reaction mixture. Symbols as in Fig. 4

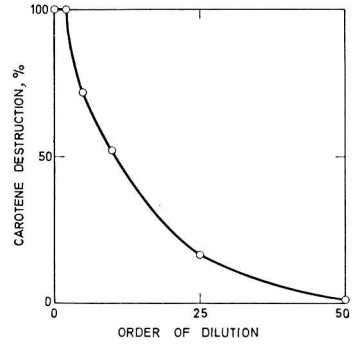

FIG. 6. *Effect of varying amounts of lucerne extract on the lipoxidase-catalysed oxidation of carotene*

Incubation conditions as in Fig. 3. Incubation time 20 min. EDTA present at a concentration of 10^{-3} M. Initial carotene concentration 19·2 μg/ml reaction mixture

TABLE I

Comparison of the inhibitory effects of hydroxycinnamic acids, 'BHT' and lucerne extract on the lipoxidase-catalysed oxidation of linoleate (oxygen absorption) and carotene (colour disappearance)

The values were calculated from the data presented in Figs 4 and 5

Antioxidant	% inhibition* of O_2 absorption	% inhibition* of carotene destruction
Coumaric acid	9·5	0
Ferulic acid	15·9	22·7
Sinapic acid	27·0	38·6
Lucerne extract	57·9	91·3
'BHT'	65·8	84·2

*decrease in O_2 absorbed or carotene destroyed as % of amounts in controls after 30 min.

added in our comparative test (19·4 μg/ml). As can be seen from Table I, ferulic acid caused a reduction of only 23% in the destruction of carotene, compared to the much greater reduction of 91% obtained with the lucerne extract. It obviously cannot be claimed that ferulic acid itself is the only or even the main antioxidant present in our extract but the order of antioxidant activity displayed by the hydroxycinnamic acid derivatives as a class strengthens the assumption that they may play a major rôle in this respect.

Further characterisation of the antioxidant compound(s) present in lucerne is being attempted.

Acknowledgment

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PESTICIDE RESIDUES IN FOODSTUFFS IN GREAT BRITAIN

VIII.*—Organochlorine pesticide residues in eggs and poultry

By ELIZABETH FINDLAY and G. A. HAMILTON

In 163 samples of eggs taken from nine sources only one sample exceeded 0·1 ppm organochlorine pesticide. In analyses of 114 samples of poultry meat, liver or fat, the content of γ-BHC in 7 samples exceeded 0·1 ppm that of dieldrin in 2 equalled or exceeded 0·1 ppm, and that of pp′-DDE in 7 exceeded 0·1 ppm, but all the samples contained less than 0·1 ppm pp′-DDT. Samples of poultry feed were also analysed to evaluate the importance of the food as a source of pesticides.

Introduction

It has been shown that chlorinated hydrocarbon pesticides are present in a wide range of foodstuffs, and the work reported here was done to find out to what extent they are present in eggs and poultry, because these foods together make up more than 20% of the protein consumed in the United Kingdom. High levels of pesticide would be undesirable, and since residue levels might be affected by husbandry methods, a year's survey was carried out on eggs and poultry from differing methods of production. This work was done on behalf of the Panel of Residues of Pesticides in Foodstuffs.[1]

Eggs and table poultry are produced by several methods varying widely in type and size of unit. However for the purposes of the survey, egg production was divided into three categories, battery, deep-litter and free-range, and the table poultry into 'chicken' from broiler production and 'fowls' from egg-laying flocks. In the U.K. battery hens produce 67% of the eggs, with deep-litter and free-range hens producing 25% and 8%, respectively.[2] Chicken produced in U.K. in 1965–66 by broiler methods accounted for 78% of the poultry meat production.[3]

The sources of pesticide available to the birds were from the environment, food, and water. Because of the location of the laboratory, the sampling was confined to the north of England and the south of Scotland; this biased the sampling, as none of the establishments used insecticides in connexion with poultry husbandry, in contrast with many poultry producers in the south of England who find it necessary to use pesticides to control flies, mites, etc. This meant that, with the exception of the free-range poultry, the birds could only obtain insecticides from their food, water and from the atmosphere. Since it has been shown that there is little pesticide present in water[4,5] and in the atmosphere,[6,7] the only important source of contamination was food, so that where possible, food samples were taken for analysis at the same time as the eggs.

The free-range birds were in direct contact with the field environment, so they could be exposed to insecticides from many sources, including treated cereal seed or any other flora or fauna contaminated as a result of the use of insecticides for general agricultural purposes.

Sampling and analysis

Normally two dozen eggs were collected from the place of production. These were divided into two separate dozens, weighed and broken, and the contents were homogenised in a blender. One sample was taken from each dozen for analysis. On some occasions, at the free-range farms, less than two dozen eggs were available. In such cases, the contents of all the eggs were homogenised together, and duplicate samples were analysed. No attempt was made to examine whites and yolks separately.

It was more difficult to arrive at a representative sample for poultry analysis. Three birds were taken from each broiler flock (chicken) and one bird (fowl) from the egg producers. As many workers have found pesticides concentrated in the fat, it was decided to include a proportion of skin with its subcutaneous layer of fat, with a thick slice of meat from the breast or leg where applicable. This was minced finely twice before being sub-sampled. On two separate occasions the meat samples from two birds and three birds were combined at the mincing stage before being sub-sampled. The liver and abdominal fat from some of the broiler birds were also analysed.

The samples of feed were extracted by being tumbled for two hours with acetone–hexane mixture before clean-up and analysis. The method of clean-up procedure was basically that of de Faubert Maunder *et al.*[8] and the pesticide residues were estimated by gas chromatography using an electron capture detector.[9]

Results

The results are recorded in Tables I–III. The recoveries of known amounts of pesticide were usually within 80–90%, depending on the insecticides. No adjustment has been made for recovery. One sample of chicken fat contained a measurable amount (0·03 ppm) of α-BHC.

Conclusions

The levels of pesticide residues found in eggs were very low and showed very little variation. The only exception was in Free Range Farm 2 where there was an increase in the HEOD (dieldrin), pp′-DDE and pp′-DDT levels in October and November, but these had returned to lower levels by December.

* Part VII: *J. Sci. Fd Agric.*, 1968, **19**, 451

TABLE I

Organochlorine insecticide residues in eggs—summary of results
(parts per million)

Source	No. of samples		γ-BHC	Dieldrin (HEOD)	pp′-DDE	pp′-DDT
Large battery	24	range	<0·01–0·03	<0·01–0·01	<0·01–0·01	<0·01–0·01
		mean	0·01	<0·01	<0·01	<0·01
Small battery	24	range	<0·01–0·03	<0·01–0·01	<0·01–0·04	<0·01–0·02
		mean	0·02	<0·01	0·02	0·02
Large deep-litter	24	range	0·01–0·03	<0·01–0·01	0·01–0·03	0·01–0·04
		mean	0·02	<0·01	0·02	0·02
Small deep-litter	24	range	0·01–0·02	<0·01–0·01	0·01–0·02	<0·01–0·04
		mean	0·02	<0·01	0·02	0·02
Free-range 1	24	range	0·01–0·03	0·01	0·01	<0·01–0·01
		mean	0·02	0·01	0·01	<0·01
Free-range 2	12	range	0·01–0·03	0·01–0·06	0·01–0·06	0·01–0·15
		mean	0·02	0·02	0·03	0·05
Free-range 3	13	range	0·01–0·02	<0·01–0·02	0·01–0·02	0·01–0·02
		mean	0·02	0·01	0·02	0·02
Free-range 4	14	range	0·01–0·04	<0·01–0·01	<0·01–0·01	<0·01–0·01
		mean	0·02	<0·01	0·01	<0·01
Free-range 5	4	range	0·01	0·01–0·04	0·01	<0·01–0·01
		mean	0·01	0·03	0·01	<0·01

TABLE II

Organochlorine insecticide residues in poultry—summary of results
(parts per million)

Source	Sample	No. of Samples		γ-BHC	Dieldrin (HEOD)	pp′-DDE	pp′-DDT
Large broiler	Breast	24	range	0·03–0·20	<0·01–0·08	<0·01–0·02	0–0·06
			mean	0·06	0·01	0·01	<0·01
	Leg	24	range	<0·01–0·35	<0·01–0·13	<0·01–0·03	0–0·04
			mean	0·07	0·01	0·01	<0·01
	Liver	7	range	0·03–0·06	0·01–0·04	<0·01–0·20	0–<0·01
			mean	0·04	0·02	0·05	<0·01
	Fat	5	range	0·04–0·66	0·03–0·10	0·04–1·0	0–0·04
			mean	0·18	0·06	0·28	0·01
Small broiler	Breast	7	range	0·04–0·09	<0·01–0·05	0·01–0·04	0–0·04
			mean	0·06	0·01	0·02	0·01
	Leg	7	range	0·04–0·09	<0·01–0·01	0·02–0·08	0–0·07
			mean	0·06	<0·01	0·03	0·02
Experimental broiler	Breast	6	range	0·02–0·04	<0·01	<0·01–0·09	<0·01–0·04
			mean	0·03	<0·01	0·03	0·01
	Leg	6	range	0·02–0·06	<0·01	<0·01–0·05	<0·01–0·09
			mean	0·04	<0·01	0·02	0·03
Fowls from large deep litter	Breast	9	range	0·03–0·09	<0·01–0·01	<0·01–0·03	0–0·04
			mean	0–0·05	<0·01	0·01	<0·01
	Leg	9	range	0·02–0·78	<0·01–0·01	<0·01–0·12	0–0·03
			mean	0·14	<0·01	0·03	0·01
Fowls from free-range farm 2	Breast	5	range	0·03–0·06	<0·01–0·02	<0·01–0·15	0–0·01
			mean	0·04	<0·01	0·04	<0·01
	Leg	5	range	0·03–0·08	<0·01–0·03	<0·01–0·15	0–0·02
			mean	0·05	0·01	0·06	0·01

TABLE III

Organochlorine insecticide residues in poultry feeds—summary of results
(parts per million)

Feed supplied to hens in	No. of samples		γ-BHC	Dieldrin (HEOD)	pp′-DDE	pp′-DDT
Large battery	10	range	0·01–0·10	0–<0·01	<0·01	<0·01–0·02
		mean	0·04	<0·01	<0·01	<0·01
Large deep litter	14	range	0·01–0·12	<0·01–0·01	<0·01–0·01	<0·01–0·03
		mean	0·05	<0·01	<0·01	0·01
Large broiler	4	range	0·02–0·03	<0·01	<0·01–0·01	0·02–0·03
		mean	0·03	<0·01	<0·01	0·02
Experimental broiler	12	range	0·01–0·05	0–<0·01	0–<0·01	<0·01–0·08
		mean	0·04	<0·01	<0·01	0·02

In the poultry samples the variation that occurred was in two chickens taken from the large broiler producer. One contained 0·13 ppm dieldrin in the leg meat and 0·09 ppm in the fat, and the second bird contained 0·1 ppm in the fat. In both these cases the less toxic pesticides, γ-BHC, pp′-DDE, an pp′-DDT, were also present at the higher levels.

The fact that eggs and chickens contained relatively little pesticide was not unexpected. As mentioned earlier, none of the establishments used pesticides for control of insects or mites, and other workers have shown it would have been unlikely for the birds to have obtained pesticides from their water or from the atmosphere. Therefore, as far as the confined birds were concerned, the only available source of pesticide was in the food. The analyses of the feed samples showed that the residues were low and of a similar order to the levels found in the corresponding eggs and poultry.

From these results it is evident that where pesticides are not used for the specific control of poultry pests, the usual methods of egg and poultry production do not give rise to levels of pesticide which may be considered a health hazard to those eating poultry products.

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NON-DESTRUCTIVE METHOD OF ASSESSING THE TOUGHNESS OF INDIVIDUAL TURKEYS

By M. D. RANKEN and D. H. SHRIMPTON*

Occasionally turkeys are exceptionally tough despite apparently normal processing, ageing, cooking etc. In an attempt to identify them under manufacturing conditions an electrical method was investigated. A potential of 14 volts a.c. (50 cycles) was applied across two points inserted in the turkey breast and the 'Probe Reading', or current passing, was recorded in mA.

Probe Readings $\geqslant$80 occurred in only about 1% of the turkeys examined. This group contained almost all the tough birds encountered, but also some 2–4 times as many tender ones. Attempts to tenderise the tough birds by increased ageing or to distinguish them by pH measurements or observations of muscular contractibility were not successful.

Introduction

It is commonly accepted that table poultry may exhibit wide differences in toughness or tenderness and de Fremery and others have shown that the ultimate degree of toughness can sometimes be related to acceleration of *post mortem* glycolysis by the processing treatments applied.[1,2] Fairly large differences can be caused, particularly by varying the ageing time, and in recent American studies on toughness measurement this method was used to obtain specimens with a wide range of toughness scores.[3,4]

In commercial practice it is usual to keep close control over all those factors which are so far known to influence toughness and yet domestic consumers occasionally complain about toughness, especially of turkeys, in circumstances which appear to be no different from those which normally produce a satisfactory product.[5] Apparently wide 'within batch' variation has been reported in the U.K.[6] and the U.S.A.[7] and in many instances of consumer complaint this is a more likely explanation than the coincidence of minor deleterious variations in processing techniques.[8] To identify a few unusually tough birds, it is essential to devise a test which is non-destructive, rapid and sufficiently simple to be carried out in a packing station. Because the birds sought are exceptional, and so few relative to the large numbers of average birds processed every hour, these criteria are more important than precision in a test.

A comparable problem arose in the development of bacon factories in the 1930s and Callow chose an electrical resistance method to identify carcasses which were troublesome.[9] His apparatus appeared to satisfy the conditions outlined above and, after a preliminary trial with Callow's original equipment had suggested that the method might be effective, a simplified version was made for the tests reported here.[10]

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Experimental

The probe instrument

An instrument, following Callow's principle, was made which permitted an alternating potential difference to be applied between two electrodes inserted into a chosen muscle site. Alternating voltage was used to reduce polarisation effects. The resulting current was taken as a measure of the condition of the meat.

The apparatus consisted of: (a) a transformer to reduce the mains voltage to 14 v. 50 cycles a.c. (the voltage was not stabilised and might fluctuate slightly), (b) a well damped ammeter with a range of 0–500 or 0–200 milliamps a.c. and (c) a probe consisting of two steel points $\frac{3}{4}''$ apart, held in an insulated handle; each point had an individual insulating collar which set the depth of insertion at $\frac{5}{8}''$ and also prevented current from short-circuiting across the skin of the bird and each point was wired to one transformer outlet, one directly and the other through the ammeter in series.

This is a fairly crude instrument compared with later developments of Callow's device[11] but its simplicity and robustness commended it for factory use.

To use it, the current was switched on and the points inserted in the turkey breast across the middle of an imaginary line joining the anterior point of the sternum with the point of the shoulder (joint of the coracoid and humerus). The ammeter reading was noted. Some error in position could be tolerated and repeatable readings within about 5 mA were obtained. A small deposit formed on the points during use and it was necessary to clean them with emery cloth after every 50 readings or so.

For simplicity, the reading of the milliammeter is referred to as the 'Probe Reading'.

Sampling

Tests were carried out in a commercial plant handling turkeys of a uniform breed, a Beltsville type developed by Yorkshire Turkey Breeders Ltd., Dalton, England. The process consisted of the conventional sequence of electrical stunning, bleeding, hard scalding at 140°F, mechanical plucking, evisceration, washing and mechanical ice-water chilling. Up to this point, turkeys passed along the line at about 1,200 per hour or one bird every three seconds. They were then transferred to ice-water tanks for ageing overnight (16–24 hours) or over the week-end (64–72 hours), then trussed, packed and frozen.

Birds required for subsequent measurement of toughness were taken through the whole process and held in cold store at −10°F until required.

Probe readings were normally made just before transfer to the ageing tanks, at about 45 minutes after slaughter, and this value is referred to as the '45 minute Probe Reading' or 'P_{45}'. Some measurements were made at other points in the sequence: before chilling, at about 20 minutes after slaughter (P_{20}'), or after ageing (P_{16h} or P_{64h}).

Measurement of pH

An attempt was made to measure the pH of breast muscles rapidly by using BDH Narrow Range Indicator Paper (pH 5·5–7·0) pressed on to the skin or into a small incision in the meat: neither method was successful.

For this work a portable pH meter was used with a combination spear type glass electrode which was inserted directly into the meat.

Measurement of toughness

The frozen carcasses to be tested were thawed at room temperature for 18 hours. One or both of the *pectoralis major* muscles were removed, individually wrapped in aluminium foil, cooked in free steam for 1 hour, and allowed to cool. The surface layer (1 mm) was discarded and the remainder divided into an 'outer' portion 1 cm thick, and the remaining 'inner' portion. The two portions of each muscle were then treated as separate samples.

For most of the work a small trained panel of 4–6 people was used, tasting 6–10 samples at each session.

The samples were cut into 1 cm cubes and each taster was given 4 random cubes from each. The samples were coded and presented in random order: tasters were required to taste one cube from each sample in turn and give it a score on the scale

1 = very tender
2 = tender
3 = slightly tender to slightly tough
4 = tough
5 = very tough

repeating the process until all cubes were scored. The average scores were calculated for each taster and for the panel as a whole.

At a later stage a Grünewald tenderometer[12] was used with a 5 kg spring and the 'pressure ridge' attachment to shear across pieces of meat of cross-section 1 cm × 1 cm. The maximum depth, in mm, of the curve drawn by the instrument was noted and the mean of six such values taken as the Shear Value.

By including pieces sheared by the tenderometer among the cubes submitted to the panel for tasting, it was possible to make a comparison between the two methods.

Results and Discussion

Probe readings

Probe readings were obtained from the breasts of 2,649 turkeys examined over a period of six weeks. The values for 98·9% of all the birds were less than 80 and only 1·1% of the birds gave higher probe readings, between 80 and 129.

Toughness

Two breasts from the same bird normally gave similar scores and the outer part of a muscle was usually found to be tougher than the inner part, as reported by Wise & Stadelman.[13] Table I shows a few selected examples but also indicates some exceptions: bird No. 3 is exceptional in both respects.

There is some difficulty in describing the overall toughness of a turkey breast as a single score: the outer part is small compared with the remainder but the effect on a consumer who discovers the first slice from the breast of a turkey to be tough is likely to be considerable. It was decided to take the simplest course and to report the 'average' toughness or Mean Panel Score as the arithmetic mean of the scores for the inner and outer breast.

The relationship between Mean Panel Score and Shear Value was found to be:

$$\text{Mean Panel Score} = 0{\cdot}124 \times \text{Shear Value} + 0{\cdot}674$$

with correlation coefficient $r = +0{\cdot}859$ (30 comparisons). The value of the correlation coefficient is similar to that found by Shrimpton[14] for chicken breast muscle using the

TABLE I

Probe Readings and toughness of the *pectoralis major* muscles of 7 selected turkeys

Comparisons between inner and outer portions of the muscle and between left and right breasts of the same bird

Bird No.	Probe-reading P_{45}'		Toughness (Panel Score)			
			Inner portion		Outer portion	
	Left breast	Right breast	Left breast	Right breast	Left breast	Right breast
1	20	25	1·10	1·00	1·45	1·20
2	20	25	1·56	1·37	1·19	1·50
3	26	26	3·12	2·12	2·69	1·50
4	30	20	1·62	1·12	2·00	2·00
5	34	34	1·05	1·25	1·75	1·70
6	48	48	1·60	1·75	2·10	2·00
7	110	110	2·65	2·95	3·55	4·05

same instrument. In some of the later work Shear Value only was determined: in such cases it was converted, for ease of comparison, into a Mean Panel Score using the above equation.

For many purposes the scoring was further simplified and turkeys were classified as 'tender', 'intermediate', or 'tough', on the following (arbitrary)basis:

Mean Panel Score 1·0–2·79 Tender
,, ,, ,, 2·8–3·19 Intermediate
,, ,, ,, 3·2–5·0 Tough

Relationship of Probe Reading to toughness

Table II shows the Probe readings at 45 min after slaughter (P_{45}') and the Average Toughness Scores of a selected group of birds, examined early in this investigation. The P_{45}' values of particular turkeys are only roughly related to their Panel Scores ($r = +0{\cdot}412$, 92 comparisons) but the probability of finding a 'tough' turkey is much greater at high values of the Probe Reading.

As is indicated in Table I, the outer breast meat makes a large contribution to the average toughness as defined here; in fact in only one of the seven birds classed in Table II as 'tough' overall was the inner breast meat any worse than 'intermediate'.

Toughness can be seen to be completely confined to birds with P_{45}' values of 80 or over, though many of the birds in that group were satisfactorily tender. Table I shows that approximately 1% of the carcasses had Probe Readings $\geqslant 80$: by comparison with Table II, it would be expected that about one third of these would be classified as 'tough', which gives an estimate of 0·3% 'tough' birds overall. The definition of 'toughness' used here is strict, so the proportion of birds likely to give rise to strong reaction from consumers may be a good deal lower. Since customers may be expected to vary in both their assessment of toughness and their readiness to complain, it is not possible to estimate how much lower.

Subsequent batches of birds exhibited a similar pattern except that the proportion of tender birds in the 'high Probe Reading' group ($P_{45}' \geqslant 80$) sometimes rose to 80%, and a single tough sample was encountered with $P_{45}' = 60$.

The Probe Reading changed with time after slaughter but values at 20 minutes or 16 hours after slaughter were no more closely related to individual toughness scores than the P_{45}' value, and were less efficient as predictors of likely toughness.

TABLE II

Probe Readings and Average Toughness of the *pectoralis major* muscle of 47 selected turkeys

Probe Reading P_{45}'	Average Toughness (Mean Panel Score)		
	Tender 1·0–2·79	Intermediate 2·8–3·19	Tough 3·2–5·0
20	1·20		
20	2·30		
20	2·35		
20	1·56		
22	1·75		
25	1·43		
25	1·10		
26	1·81		
28	2·15		
30		2·93	
30	2·60		
34	1·48		
40	2·25		
40		2·85	
48	1·88		
50	1·95		
50	1·92		
50	2·50		
60	2·18		
60	1·90		
60	2·53		
65	2·63		
70	1·83		
77	2·73		
77	1·98		
80	2·13		
80			3·65
85			3·35
90	2·13		
90	1·78		
90		2·95	
90		3·06	
90	2·78		
90	1·62		
100	2·08		
100	1·50		
110			3·60
110			3·50
110	2·38		
115	2·63		
120			4·03
120	1·48		
120			3·53
120	2·10		
120			3·44
120	2·75		
140		3·15	

Ageing time

Since ageing time is known to have an effect on toughness, it seemed possible that an increase over the usual period might tenderise the tough birds in the 'high Probe Reading' group. Birds were selected whose P_{45}' values were either very high or very low and they were aged for various times from 0 hours to 64 hours before being frozen, thawed and examined by both the tenderometer and the tasting panel. The results were unexpected. Birds with low Probe Readings were tender, even if not aged at all; birds with high Probe Readings were not tender unless they were aged. Some birds with high Probe Readings remained tough after ageing, but the proportion was not significantly altered over the range of ageing times studied.

Ways were then sought to resolve the 'high Probe Reading'

group and to try to distinguish in advance those birds which would tenderise in 16 hours ageing from those which would not.

Ultimate pH and rate of attainment

The pH was measured at various times after slaughter. Whatever the Probe Reading, the ultimate pH was in the range 5·6–6·0 and there were no significant differences within the 'high Probe Reading' group.

Although the very tough birds occurred in the group whose pH fall was fastest, there were also tender birds in the same group. It is possible however, that this exception to de Fremery's theory[1] is apparent rather than real and results from an inability to determine muscle pH sufficiently rapidly after death under packing station conditions.

The Probe Reading is a measure of 'current passing' or, roughly speaking, a reciprocal resistance, and these results show that as the pH of a turkey falls with time, the resistance rises. Callow's findings[9] were different—in pigs the resistance fell with pH and the final resistance was related directly to the final pH and the texture of the meat. The explanation may lie in the fact that he used a higher voltage possibly leading to some breakdown of cell walls as the contents acidified; in the present tests using low voltage, the extracellular fluid alone may have been the main conductor. It is possible, too, that the effect observed in turkeys may be of a smaller order of magnitude than that observed in pigs and so have been masked by Callow's technique.

Rigor mortis

The changes in pH and in muscular contractability ran parallel with changes in Probe Reading, suggesting that the Probe Reading might be used as a convenient measure of the progress of *rigor mortis*. Exceptionally tough turkeys were encountered among the low proportion whose Probe Readings were high and which had gone quickly into *rigor*, but it was not possible to use any readily measurable feature of *rigor mortis* to distinguish them from other turkeys with similar Probe Readings which became tender after 16 hours ageing. It seems, therefore, that the toughness of the exceptional birds is either related to an extremely fast *rigor mortis* or pH fall which so far has not been observed, or it is governed by some other factor as yet unknown.

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LESSER KNOWN NIGERIAN EDIBLE OILS AND FATS
I.—Characteristics of melon seed oils

By PAMELA GIRGIS and F. SAID

Some of the characteristics of a melon seed oil, which is shown to be of the semi-drying type containing mainly linoleic and oleic acids, are described.

Introduction

Many members of the Cucurbitaceae or gourd family occur in Nigeria, and the seeds of several varieties are used in native cooking as a source of oil. None of these oils has yet been utilised on an industrial scale. The watermelon, *Citrullus lanatus* (Thunb.) (syn. *Colocynthis citrullus* (Linn.)[1]) provides an important source of such seeds. The plant is grown in all regions of Nigeria, the total gross production of melon seeds in the country on the basis of the 1963 census being 73,000 tons per year.[2] The seeds are all consumed within the regions in which they are produced. Both the seeds and the seed oil can be obtained in local markets under the names egusi (Yoruba), ogili (Ibo), ogi (Benin) guna agushi (Hausa) and dende (Fulani). The purpose of this paper is to report on the general characteristics of melon seed oil available in the Ibadan locality.

Experimental

An authenticated sample (specimen FHI 18101, Dept. of Forestry Research HQ, Ibadan) of whole sun-dried seeds was reduced to a fine meal, and extracted to exhaustion in a Soxhlet extraction apparatus using petroleum ether (b.p. range, 40–60°). The oil obtained after evaporation of the petroleum ether was clear and pale yellow in colour, and had a faintly nut-like odour and a bland taste. The locally produced oil is generally obtained from the seed kernels (cotyledons), which are crushed and boiled with water, until the oil floats to the surface and is separated. It varies in colour from pale yellow to dark brownish-yellow.

Measurements of infra-red spectra were made on capillary films of the oil held between sodium chloride plates and using a Perkin–Elmer Model 137 Infracord Spectrophotometer. The fatty acid composition of the oil was determined by the spectrophotometric method[4] of analysis of the alkali-isomerised oil as revised by Hilditch *et al.*[5]

Results and Discussion

Table I shows some of the characteristics of the solvent-extracted oil and of the locally produced oil, together with those reported elsewhere[3] for the seed fat of the watermelon, *Citrullus vulgaris* (variety Cuban Queen) grown in Florida.

The solvent-extracted oil and the locally produced oil showed identical infra-red spectra and resembled each other closely in all characteristics except the acid value. This was invariably high for the solvent-extracted oil, and was not lowered when the outer husks of the seeds were removed before grinding and extraction. Both the local oil and the solvent-extracted oil showed good keeping properties over a period of four months, but the latter developed a slight turbidity. The oil showed negative colour reactions in Halphen's test for cottonseed oil and Baudouin's test for sesame oil. It was insoluble in alcohol. The fatty acid composition of the oil is shown in Table II together with the composition reported[3] for melon oil obtained in Florida. Melon seeds obtained in Ibadan contain a much higher percentage of oil than the seeds grown in Florida (see Table I) but there are no striking differences in the oils obtained from either variety.

TABLE I
Characteristics of melon seed oil

	Citrullus lanatus from Ibadan, Western Nigeria		*Citrullus vulgaris*[3] from Florida
	Solvent-extracted-oil	Locally-produced oil	
Oil, % in seed	51		26·5
Fat characteristics:			
Acid value	13–17·9	1·1–2·9	0·4
Saponification value	191·7	189·5–190·5	197·4
Hydroxyl value	1·7	2·6	7·5
Iodine value	112·9	114·7–121·1	133·8
Unsaponifiable matter (%)	0·98	1·04	1·2
Refractive Index, $n_D^{40°}$	1·4722	1·4725	1·4669
Weight per ml, 20° C	0·9167	0·9170	0·9197*

* Specific gravity, 25/25°C

TABLE II
Fatty acid composition of melon seed oil
(wt.,% of total)

	Ibadan	Florida[4]
Unsaturated acids:		
Oleic	19·0	13·5
Linoleic	55·1	70·8
Saturated acids	25·9*	15·7

* by difference

Conclusion

Melon seed oil is found to be a semi-drying oil, consisting mainly of the glycerides of oleic and linoleic acids. Because of its high content of unsaturated fatty acids, it should prove a good substitute for maize oil for use in diets intended to reduce high levels of blood cholesterol.

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ERRATA

In the paper by Pianka *J. Sci. Fd Agric.*, 1968, **19,** 475.

Page 475. Line 3 from bottom of left-hand column:
for 'Y = CH_2OH or CH_2Cl' *read* 'Y = OH or Cl'
Right-hand column formulae: a hydrogen atom should have been attached at position 5 in the formulae numbered **IX** and **X**

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

OCTOBER, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Soils of the Namib desert, South-West Africa. H. Scholz (*Z. PflErnähr. Bodenk.*, 1968, **119**, 91–107).—The area under survey was of three types: sandy desert with dunes and without vegetation, plain and slightly undulating gravel desert and dry valleys. These three types consisted mainly of sand desert or 'ergs', stone capped soils or 'regs', or soils capped with calcareous crusts and alluvial sands or terrace loams respectively. M. LONG.

Mechanism of mottling and nodule formation in poorly drained soils. H.-P. Blume (*Z. PflErnähr. Bodenk.*, 1968, **119**, 124–134).—On the basis of measurements of water tension, air content, O_2 diffusion rate and oxidation rate in both poorly and well-drained soils and the measurement of free oxide content and other soil constituents in both bleached and rust-coloured concretions a theory was developed to account for their formation. The surface Fe of aggregates is reduced in poorly drained soils and tends to move to the interior of the aggregate. On drying, O_2 diffuses into the aggregate and oxidises the iron. Where the diffusion rate is high concretions form and when it is low, mottling occurs as in this case the dissolved iron has time to move back to the surface. (27 references.) M. LONG.

Quantitative relations between climate and soil formation. H. Kohnke, R. G. Stuff and P. A. Miller (*Z. PflErnähr. Bodenk.*, 1968, **119**, 24–33).—Replacement of average temp. by the 'biotemperature' suggested by Holdridge in the Lang rainfall factor leads to a biofactor which is related to pH, exchangeable H, cation exchange capacity, N content and C content in the top 25 cm of soils from many countries. However no relationship was found with properties of the lower layers and the factor failed to account for the boundary between the arid and humid soils in the United States, thus over-emphasising the effect of temp. (14 references.) M. LONG.

Utilisation of the nitrogen in the alkaloids brucine and strychnine by two different cultivated plants. R. Dörr (*Z. PflErnähr. Bodenk.*, 1968, **119**, 107–114).—Pot trials carried out with maize and barley using a brown rendzina and a brown earth pseudogley showed that the N of brucine and strychnine is utilised by these plants, amounting to 31% of the plant N. The concn. of alkaloid in shoots did not exceed 0·1%. M. LONG.

Studies on soil polysaccharides. I. S. A. Barker, M. H. B. Hayes, R. G. Simmonds and M. Stacey (*Carbohydrate Res.*, 1967, **5**, 13–24).—Polysaccharide material was extracted from an org. soil with *N,N*-dimethylformamide, *N*-methyl-2-pyrrolidone, Na-EDTA, Amberlite IR-120 (H^+) resin plus water, 8 M urea and 0·6 N H_2SO_4. Using gel-filtration technique it was possible in the case of acid extracts to separate the brown acid-sol. components of soil org. matter for high mol. wt. polysaccharides which contained arabinose, galactose, mannose, rhamnose (**I**), xylose and glucuronic acid as major constituents, and fucose, ribose and possibly methylated xylose and glucose as minor constituents. The most acidic fraction contained an unidentified acid component which was not uronic acid; 25–50% of **I** was present as α-L-linked terminal units. (57 references.) C.V.

Determination of total phosphorus in soils by different methods. G. Möller (*Z. PflErnähr. Bodenk.*, 1967, **118**, 44–52).—Differences in total P determinations in a series of soils by various published methods are recorded. Acceptable results were obtained only after complete destruction of silicates and org. matter in the samples. All other methods gave low results. For determination of the P requirement of soils complete destruction is not necessary; the silicate-bound P does not become available to plants until much later. For routine purposes, Kjeldahl digestion (micro- or macro-scale) is suitable especially if N is also to be determined. The colorimetric vanadate–molybdate method may be used in presence of max. amounts of interfering ions. (17 references.) A. G. POLLARD.

Correlations between results obtained from twenty methods for determining the phosphate requirement of soils and the effect of soil pH on the values obtained. E. Rauterberg and D. Ali Dervish (*Z. PflErnähr. Bodenk.*, 1967, **118**, 85–100).—Methods compared included 18 chemical methods together with the Neubauer method and the Ehrendorfer technique. Correlation coeff. between methods were calculated separately, for soils of pH ≤5 and for those of pH 5 or under. (23 references.) A. G. POLLARD.

[A] **Solubility of soil phosphorus, iron, calcium and magnesium in solutions of ammonium ethylenediamine tetra-acetate.** [B] **Extraction of soil phosphorus with ammonium ethylenediamine tetra-acetate under various conditions.** E. Rauterberg and D. Ali Dervish (*Z. PflErnähr. Bodenk.*, 1967, **118**, 73–80, 80–85). [A] Soil samples (15 g) are shaken (2 h) with 150 ml of 0·05 M-NH_4-EDTA. The extract is removed centrifugally, any fine particles of humus being removed by paper filtration. The extraction process is repeated. P, Ca, Fe and Mg are determined in successive extracts. The ability of an individual soil to provide plant nutrients can be calculated from the amounts present in successive extracts until differences between amounts of particular nutrients in two successive extracts become insignificant. A useful approximation can be found from values obtained in the first two extracts (*a* and *b*) by the equation $X = a^2/(a-b)$, X being the element concerned. (12 references.)

[B] Optimal operational conditions for use of this method are examined. Neither the ratio, soil: extractant solution, the concn. of the extractant nor the time of contact of extractant with soil had any appreciable effect on results obtained. A. G. POLLARD.

Extractive removal of iron, copper, manganese and aluminium ions interfering in complexometric determination of calcium and magnesium in soil extract. A. Sapek and T. Żminkowska (*Chemia analit.*, 1967, **12**, 907–909).—The method is based on the extraction of soil with 0·1 N-HCl, followed by removal of complexes of Cu and Fe with Na diethyldithiocarbamate, and those of Fe, Mn and Al with cupferron by extraction into EtOAc. The remaining aq. phase is divided in two parts; one part is used to determine Ca by titration with 0·01 N-EDTA in the presence of 'Calces', the other for titrimetric determination of the total content of Ca and Mg with 0·01 N-EDTA in the presence of Eriochrome Black T. The error is ≯±2%. B. KAMIŃSKI.

Determination of some components of soil extracts by ion-exchange chromatography–neutron activation method. W. Żmijewska and J. Minczewski (*Chemia analit.*, 1967, **12**, 675–681).—The soil samples were macerated with water, and the dry residues of the aq. extracts irradiated in a neutron flux for 15 to 24 h to determine K, Na, Mn, Cu and Zn, and for 14 days to determine Fe, Co and Zn. The samples were dissolved in aq. HNO_3, then mixed with carriers, and subjected to column chromatography on Dowex 1 × 10 anionic resin. The eluates, containing separated metals in HCl of various concn. were subjected to γ-scintillation spectrometry. ^{42}K and ^{24}Na were separated by additional column chromatography on Amberlite IR-120. If the soil contains Sb it can be eluted from the column with N-HCl after eluting ^{64}Cu with 3 N-HCl. The min. detectable amounts (in μg per ml) are: Mn: 0·0005, Na, Cu and Co: 0·001, Zn: 0·01, Fe: 0·5, and K: 0·1. The coeff. of variation varied from 2·3 to 19·0%. Results for four soil samples are tabulated. B. KAMIŃSKI.

Phosphatases in the enzyme system of cultivated soils; methods for determining their activity. G. Hoffmann (*Z. PflErnähr. Bodenk.*, 1967, **118**, 153–160).—The importance of the phosphatases in the utilisation of org. P compounds by plants is discussed, together with means of assessing the activity of cell-free phosphatases occurring in soil. The org. part of the substrate ester should be

determined, rather than the rate of liberation of phosphate, for this purpose. (40 references.) A. G. POLLARD.

Photometric method for determining phosphatase activity in soils. G. Hoffmann (*Z. PflErnähr. Bodenk.*, 1967, **118**, 161–172).—Three groups of phosphatases having pH optima at pH 5·0, 7·0 and 9·6 respectively, occur in soil. Activities may be determined by the amount of phenol liberated from Na_2 phenylphosphate at the respective pH levels in a 3-h incubation at 37°. The released phenol is converted into indophenol blue which is measured colorimetrically. (22 references.) A. G. POLLARD.

Emergence and seedling growth related to oxygen content and oxygen diffusion rate in soils. K. Kaack and K. J. Kristensen (*Agron. J.*, 1967, **59**, 541–544).—Winter wheat germinated at nearly zero O_2 concn. [O_2] in the soil atm. and showed max. emergence with about 10% O_2 in the soil atm. Mustard did not germinate until soil atm. [O_2] exceeded 2%, and emergence increased with [O_2] up to 20%. Emergence and growth correlated better with [O_2] of soil atm. than with O_2 diffusion rate in the soil. At any [O_2] in the soil atm., emergence and growth were usually better at 70 cm than at 5 cm soil moisture tension. A. H. CORNFIELD.

Activity (*C*) of calcium as a plant nutrient. E. Sadeghian and H. Linser (*Z. PflErnähr. Bodenk.*, 1967, **118**, 112–116).—The activity of Ca is determined in pot experiments on the basis of the Mitscherlich equation. The *C* values differed with the plant species, e.g., cruciferae, 6–8: gramineae and leguminoseae, 10–14; lupins 6–7. A. G. POLLARD.

Water-soluble silicic acid in loess soils. P. Schachtschabel and C. G. Heinemann (*Z. PflErnähr. Bodenk.*, 1967, **118**, 22–35).—The influence of some soil factors on the SiO_2 concn. in aq. equilibrium extracts is examined in a series of samples from the A_p horizons of grey-brown podzolic soils and some imperfectly drained chernozems. Water-sol. Si (*WS*) was determined by extraction with 0·1% aq. NaN_3, the suspension (soil : water = 1 : 5) being shaken twice daily for 21 days. Other extracts were made with buffer solutions at pH 5·0 or 7·5. Addition of NaN_3 lowered the amount of Si extracted by 10–20%. The solubility of SiO_2 gel in the pH range 4–8 was independent of the pH of the buffer solution used but the solubility of Si in soil fell somewhat with rise in soil pH. B- and C-horizons in which the C contents were much smaller than in -A showed significant negative correlations between *WS* and the soil pH; in the A horizons *WS* was significantly and negatively correlated with the org. C constant. The A_p horizons of grey-brown podzolic soils and of chernozems contained 14–15% of *WS*, the B horizons had 7–8 mg of *WS* and the C horizons of chernozems contained 4 mg of *WS*/l. Negative correlations were established of *WS* with oxalate-sol. Al and with dithionite-sol. Fe. The data suggest that much of the *WS* in loess soils is bound to reactive Al- and Fe-oxides. (11 references). A. G. POLLARD.

Effect of time and depth of sampling upon soil test results. F. D. Childs and E. M. Jencks (*Agron. J.*, 1967, **59**, 537–540).—Available P and K values of acid soils (pH 4·7–5·6) as determined by extraction with 0·05 N-HCl–0·25 N-H_2SO_4 were significantly higher when the soils were sampled in the winter than when sampled in the summer months. Soil pH was highest and CaO requirement was lowest during the winter. Available P and K were somewhat lower in the 0–15 cm soil depth. Differences in available P and K and in CaO requirement between seasons were sufficiently large to influence fertiliser and lime recommendations. A. H. CORNFIELD.

Optimal proportions of the six major nutrients (N, S, P, K, Ca and Mg) for *Aspergillus niger* as determined by the Homès systematic variation technique. M. Foroughi (*Z. PflErnähr. Bodenk.*, 1967, **118**, 1–11).—The *A. niger* test for nutrient levels in soil or extracts becomes more accurate as the ratios of the major nutrients in the culture solution approach the optima for the fungus, i.e., produce the max. yield of mycelium. Data presented show that best yields were obtained with cation ratios, K : Ca : Mg = 34 : 33 : 33, and anion ratios, N : S : P = 45 : 27 : 28. The optimal ratio of nutrient anions : cations was 1·1 : 0·9. The best total ion concn. was 150 mequiv. with 50 g glucose/l or 250 mequiv., with 75 g or more of glucose. (20 references.) A. G. POLLARD.

Reflectance of cotton leaves in relation to soil salinity. J. R. Thomas, C. L. Wiegand and V. I. Myers (*Agron. J.*, 1967, **59**, 551–554).—Reflectance from single cotton leaves increased with soil salinity and leaf moisture deficit and with Cl content. Field cotton on a saline soil showed greater reflectance than that on a non-saline soil. Cotton yields declined significantly with increasing reflectance from the field. Differences in reflectance accounted for 51–75% of the variation in yields. A. H. CORNFIELD.

Dating of soils by the radiocarbon technique. I. Methods and existing ^{14}C data. II. Some results. H. W. Sharpenseel, M. A. Tamers and F. Pietig (*Z. PflErnähr. Bodenk.*, 1968, **119**, 34–44, 44–52).—I. Radiocarbon provides the best method for the dating of soils. A procedure is described in which plant residues are removed after dispersal of the sample in water and sieving. Soil C is measured as benzene after oxidation to CO_2, subsequent absorption in NH_4OH, pptn. as $SrCO_3$, liberation of CO_2 with $HClO_4$, and reaction with molten Li to form Li_2C_2; treatment of Li_2C_2 with water yields C_2H_2, which is converted into benzene. Consideration is given to the interference of free carbonates, plant roots, cell debris and rejuvenation by percolation of C-containing water and coprogenic products of the soil fauna. All existing data are collected but are insufficient to draw any conclusions. (13 references.)

II. Data for chernozems, grey-brown podsols, rendzinas, low moor, 'plaggen' soils, sea marsh soils and humus-iron podsols are presented and discussed. (14 references.) M. LONG.

Effect of mineral fertilisers on the humus content of arable soils. K. Nehring and W. Wiesemüller (*Z. PflErnähr. Bodenk.*, 1968, **119**, 11–24).—N deficiency or lack of fertiliser reduced biological activity and humus quality in soils used for permanent fertiliser trials, although the total humus content hardly varied in soils subjected to complete fertilisation or from which P and K were withheld. Acidification of the soil also had no effect. With low levels of biological activity, easily decomposed low-mol. compounds accumulated and a highly significant correlation was found between biological activity and fulvic acid content. Liming and complete fertilisation stimulated the activity of micro-organisms, so that the readily decomposed fractions were attacked. Where liming was practiced the grey humic acid content was higher as was the case with non-extractable matter. Humic C, org. matter and exchange capacity of the limed plots were all higher than on the unlimed variants. Farmyard manure (**I**) increased the brown humic acid and hymatomelanic acid contents of the total humus whilst grey humic acids and fulvic acids were decreased. Org. matter in the **I** plots was the most stable and had the highest methoxyl content. M. LONG.

Effect of application of gypsum and fertilisers on ammonification and nitrification in salty soils. V. Rankov (*Microbiology*, [*USSR*], 1967, **36**, 120–124).—Addition of gypsum (**I**) together with org. and mineral fertilisers (**II**) increases the number of NH_3-fixing and nitrifying bacteria, intensifying both these processes. Treatment with **I**, but without **II**, at first has a depressant effect, lowering the activity and reducing the no. of organisms taking part; with prolonged action however both processes are to some degree stimulated. The use of **I** + **II** results in a change in the bacterial population; *Pseudomonas* is increased and the number of *Bacillus megathorium* and *B. cereus* rises significantly. The population of *B. mycoides* increases to a lesser degree. (14 references.) C.V.

Use of urea in compound fertilisers. T. P. Hignett (*Chem. Age India*, 1967, **18**, 800–809).—The uses and physical characteristics of granular N-P fertilisers containing urea are described. (15 references.) K. GRAUPNER.

Evaluation of asphalt coatings on phosphorus fertilisers. J. K. Hall and D. E. Baker (*Agron. J.*, 1967, **59**, 503–505).—Reducing the dissolution rate of PO_4^{3-} from superphosphate by coating it with asphalt did not result in increased recovery of added P by successive crops of millet–maize–millet. Growth response and fertiliser-P uptake were less from coated superphosphate than from uncoated $CaHPO_4$ or $Ca(PO_3)_2$. Where the coating reduced availability of fertiliser P, a higher proportion of P taken up by the plant was derived from native soil P. The asphalt coatings did not affect the uptake of P from $(NH_4)_2HPO_4$ or $Mg(NH_4)PO_4$. A. H. CORNFIELD.

Mechanism of the action of cyanamide on the respiration metabolism of parasitic fungi. A. Amberger (*Z. PflErnähr. Bodenk.*, 1968, **119**, 1–10).—Cyanamide (**I**) interferes markedly with the respiration metabolism of *Cercosporella herpotrichoides* and *Fusarium nivale*; H_2O_2 arising from glucose oxidation is no longer decomposed and peroxidase is not detectable in the mycelium. Sol. thiol and dehydroascorbic acid contents are greatly reduced by **I**, small concn. almost completely blocking the ascorbic acid oxidase activity. From these observations it is concluded that the toxic activity of **I** arises from the inhibition of essential enzyme and redox systems. (13 references.) M. LONG.

Controlled release fertilisers. R. Powell (*Chem. Process Rev.*, 1968, No. 15, 279 pp.).—Compounds of low solubility [urea (**I**)–formaldehyde (**II**) condensation products, **I**–**II** in combination with

other materials, other I-aldehyde products, oxamide, ammoniated coal, I-pyrolyzate and NH_4-polyphosphate and other phosphates] are discussed; coated granules (wax, polymer, sulphur) and various forms of incorporation in a matrix, gel, mulch or other material are reviewed and the prevention of N-losses through chemical and bacterial action in the soil is studied. A brief note is included on a rapid-release high-N fertiliser. References (100), most of them U.S. patents are given. C.V.

Effect of phosphate in multiple fertilisers. A. Buchner (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 868–871).—The biological fixation and chemical reaction of phosphates in the soil are explained. The phosphate equilibrium system in soil is illustrated and experimental results on the effect of water-sol. and citrate sol. phosphate are reported. Field trials showed that Nitrophoska with grain sizes of 1–4 mm dia. and a major part of 1·5–3·5 mm dia. and with 100% relative citrate and 35% relative water solubility of the phosphoric acid was very suitable for soils poor in P_2O_5. (30 references.) (In German.) M. SULZBACHER.

Effect of wet-process phosphoric acid technology on fertiliser production and distribution costs. J. G. Kronseder (*Chem. Age India*, 1967, **18**, 826–834).—Wet-process acid, containing 54% P_2O_5 or more, is of increasing importance in reducing fertiliser transportation costs. The present position, and future prospects of the process are discussed with detailed production cost tables. K. GRAUPNER.

Recovery of potassium fertiliser by flotation. M. V. Chandorikar and D. J. Mehta (*Chem. Age India*, 1967, **18**, 812–816).—Contact angles of NaCl, KCl and kainite in marine bitterns containing octadecylamine acetate and sulphonated castor oil were measured. This study led to the definition of optimum conditions for flotation which have been applied in experiments for recovery of K as KCl from sylvinite and as kainite from mixed salt of average composition KCl 17–20, $MgSO_4$ 25–30, NaCl 25–30, $MgCl_2$ 4–5%. Large scale results were also promising. K. GRAUPNER.

Estimation of phosphate, sulphate and fluosilicate in wet process phosphoric acid by high frequency titrimetry. A. D. Pandey and A. K. Roy (*Technology, Q. Bull. Fertil. Corp. India*, 1966, **3**, 183–188).—A rapid method, based on the apparatus previously described (*Idem, ibid.*, 1965, **2**, 125), but using thin-walled polythene cells is described. The sample is titrated against standard NaOH; the first break in the titration curve corresponds to complete neutralisation of H_2SO_4 and HF, and the second break to one-third neutralisation of H_3PO_4. Sulphate is estimated separately by titration against standard $BaCl_2$. The total time required is 30 to 45 min. and the results are sufficiently accurate for plant control purposes. (11 references.) E. C. APLING.

Simultaneous spectrographic determination of some trace elements in fertiliser raw materials: I. Limestones. R. C. P. Sinha and B. K Banerjee (*Technology, Q. Bull. Fertil. Corp., India*, 1966, **3**, 200–202).—Determinations of the trace elements Pb, Cu, Ga, Ca, V, Mn, Ba, Sr, and Ti are reported for seven Indian limestones. Other elements detected qual. in each sample are also listed. (11 references.) E. C. APLING.

Emission spectroscopic estimation of fluorine in rock phosphates. A. K. Chakraborty, K. C. Singhal and B. K. Banerjee (*Technology, Q. Bull. Fertil. Corp. India*, 1966, **3**, 203–209).—A method for estimation of high amounts of F in rock phosphate using a CaF 5291 Å band head is described. Emission is smoothed by mixing the sample with $CaCO_3$, graphite and ZnO (1 : 1 : 1 : 3) and placing it in a specially shaped electrode. Co is used as internal standard, and the relative deviation is ±5%. E. C. APLING.

Composting of organic material. J. R. Snell (B.P. 1,066,158, 5.10.65. U.S., 20.10.64).—Org. material is comminuted, and its moisture content adjusted to the optimum level for digestion with aerobic thermophilic micro-organisms. It is then fed into the claimed apparatus. The method and apparatus possess the following advantages: rapid, continuous composting in minimum space; uniform O_2 supply and avoidance of undesirable localised cooling; novel feeding and discharging devices; improved agitation means; improved quality of compost (reduced moisture, max. N-content). (17 drawings.) J. M. JACOBS.

Plant Physiology, Nutrition and Biochemistry

Current literature survey of photosynthesis. X. L. P. Vernon and D. J. Earheart (*Photochem. Photobiol.*, 1967, **6**, 163–175).—(286 references.) C.V.

Comparison of kinetics of photosynthetic carbon dioxide fixation in maize, sugar-cane and tobacco and its relation to photorespiration. A. Goldsworthy (*Nature, Lond.*, 1968, **217**, 62).—It has been suggested that the increased affinity for CO_2 in maize (I) and sugar-cane (II) in comparison with tobacco (which shows normal photorespiration and photosynthesis) could be accounted for by different CO_2-fixing enzymes in I and II. However, measured values of Michaelis const. (*Km*) (cf. Forrester *et al.*, *Pl. Physiol.*, 1966, **41**, 428) for photosynthesis of all three species are similar and approx. equiv. to the natural CO_2 concn. in the air. Thus, the apparent lack of photorespiration in I and II may be due to true deficiency in this process rather than to the possession of an abnormally efficient photosynthetic mechanism. W. J. BAKER.

Action spectrum for an enhancement of endogenous respiration by light in *Chlorella*. W. Kowallik (*Pl. Physiol.*, 1967, **42**, 672–676).—The O_2 consumption of starved, chlorophyll-free yellow mutants of *C. vulgaris* was increased by exposure to very small amounts of blue light (λ 450 mμ). Flavins and *cis*-carotinoids are possibly concerned in this effect. A. G. POLLARD.

Growth and respiration patterns of snap bean fruits. A. E. Watada and L. L. Morris (*Pl. Physiol.*, 1967, **42**, 757–761).—Respiration rates and the growth of the seed pericarp of the beans are examined. The whole fruit showed a climacteric type of respiration, the increase in CO_2 production by the expanding seed being followed by a rapid decrease in that from the pericarp tissue, without simultaneous increase in ethylene production. The apparent climacteric type of respiration is not a typical respiratory effect but results from the differing rates of production of CO_2 from seed and pericarp at different stages of development. A. G. POLLARD.

Heat transfer from a leaf. E. T. Linacre (*Pl. physiol.*, 1967, **42**, 651–658; cf. *Idem, ibid.*, 1964, **39**, 687).—A mathematical interpretation of the resistance to diffusion of heat and water vapour, external to a leaf, based on measurements of leaf temp. changes following a sudden alteration in the intensity of radiation. A. G. POLLARD.

Phloem transport and nutrient intake. H. Fischer (*Z. PflErnähr. Bodenk.*, 1967, **118**, 100–111).—A critical review of the literature with 64 references (cf. *Idem, Handb. Pflphysiol.*, 1967, **13**, 200). A. G. POLLARD.

Net uptake of sodium and potassium by maize and sugar-beet plants. H. Marschner and W. Schafarczyk (*Z. PflErnähr. Bodenk.*, 1967, **118**, 172–186).—The plants were grown in a Na-free culture solution for 10 days (maize) or 21 days (beet) to produce plants of high K and low Na contents. Addition of KCl to the nutrient lowered the uptake of Na by both species, whereas addition of $CaCl_2$ decreased the uptake of Na by maize plants which had a high K content initially. When the initial K content was low the action of $CaCl_2$ was much more limited. With sugar-beet plants the Na content could be increased by addition of $CaCl_2$ to the nutrient. With nutrient solutions containing equiv. proportions of Na and K, additions of Ca^{2+} increased the intake of K and diminished that of Na by both plant species, but the net uptake of Na by beet exceeded that of K. Translocation of Na from roots to shoots in sugar-beet was considerable even when the $[Na^+]$ in the nutrient was only 0·1 mequiv./l. Translocation of Na in maize occurred only when $[Na^+]$ of nutrient was ≮10 mequiv./l. With high concn. of Na and K in the nutrient translocation of Na was increased more than that of K by high transpiration rates. 2,4-Dinitrophenol lowered the accumulation of Na and K in roots of both species but increased translocation to the shoots. Rates of uptake and translocation of Na were much greater in sugar-beet than in maize. (30 references.) A. G. POLLARD.

Influx and efflux of sodium and potassium in maize and sugar-beet. H. Marschner and W. Schafarczyk (*Z. PflErnähr. Bodenk.*, 1967, **118**, 187–201).—Seedlings of maize and sugar-beet having high Na and K contents initially, were placed in nutrient solutions having equiv. concn. of Na and K but the total concn. was varied in the range 0·1–50·0 mequiv./l. In experiments lasting 4 h the efflux of Na and K was considerable. In maize the exchange of Na and K between plants and nutrient solution occurred almost exclusively in the root whereas in sugar-beet the exchange was largely restricted to the shoot. With both species addition of Ca^{2+} to the nutrient decreased the influx of Na and increased that of K, the effect being shown largely in roots and very little in shoots. The influx selectivity was influenced by the relative $[Na^+]$ and $[K^+]$ in the nutrient and by the $[Na^+]$ in the plants; the lower the concn. in the nutrient and the greater the Na concn. in the plants, the greater was the relative influx of K, this being more marked in maize than in beet. In absence of Ca the influx of Na exceeded

that in sugar-beet. The turnover of cations, particularly of Na was more rapid in beet than in maize. (30 references.)
A. G. POLLARD.

Inhibition of oxidative phosphorylation and respiration by ozone in tobacco mitochondria. T. T. Lee (*Pl. Physiol.*, 1967, **42**, 691–696).—The mechanism of O_3 injury to tobacco plants causing inhibition of O_2 intake is examined. The phosphorylative activity increased with the period of exposure to O_3 and was more sensitive to O_3 than was the respiratory system. The mechanism of these effects is discussed. When fed to detached tobacco leaves before contact with O_3, sucrose and glucose increased the phosphorylative activity of mitochondria to a greater extent than did mannitol or lactose. A. G. POLLARD.

Relative permeabilities of plastic films to water and to carbon dioxide. J. T. Woolley (*Pl. Physiol.*, 1967, **42**, 641–643).—In all plastic films examined permeability to water exceeded that to CO_2. Data obtained failed to indicate any synthetic material which might be applied to leaves to prevent or restrict transpiration.
A. G. POLLARD.

Determination of tyrosinase activity. M. L. Weaver and H. A. Steen (*Am. Potato J.*, 1967, **44**, 387–392).—A paper-disc technique for measuring tyrosinase activity in crude enzyme extracts of potato tubers is described. A. H. CORNFIELD.

Formation of a soluble amylopectin-like polysaccharide in potato tubers. R. B. Frydman and C. E. Cardini (*Pl. Physiol.*, 1967, **42**, 628–630).—Incubation of potato sprouts or tuber slices with 0·1 M-glucose 1-phosphate (**I**) at room temp. in a moist chamber was followed by the appearance of a polysaccharide resembling amylopectin. The polysaccharide was a good primer for phosphorylase but was much less effective for starch synthetase (**II**). A second, intracellular, polysaccharide having a more branched structure, was isolated; it was an effective primer for **II**. Neither fructose-6-, glucose-6-phosphate, nor fructose-1,6-diphosphate could replace **I** in these activities. Excretion of the polysaccharide was unaffected by 2,4-dinitrophenol. A. G. POLLARD.

Relation between calcium and strontium transport rates as determined simultaneously in isolated segments of the primary root of *Zea mays*. M. E. Hutchin and B. E. Vaughan (*Pl. Physiol.*, 1967, **42**, 644–650).—The segments (55 mm long) were grown in glass tubes through which were passed nutrient solutions containing ^{85}Sr and ^{45}Ca. Translocation of ^{45}Ca from solutions containing 0·25 mM-Ca was little influenced by [Ca]; that from 0·25–5·0 mM-Ca was considerably slower. When conditions simulating Ca deficiency were established, the transport of Sr increased. The ratio Sr/Ca transported equalled the ratio in the nutrient solution, no discrimination between the two cations being apparent. Dinitrophenol reduced the rates of transport of Ca and Sr to similar extents. A. G. POLLARD.

Effects of Phosfon-S on nucleic acid metabolism in *Pisum sativum*, Alaska. J. Brook, S. H. West and D. S. Anthony (*Pl. Physiol.*, 1967, **42**, 785–790).—Pea seeds were soaked in solutions of different concn. of Phosfon-S (2,4-dichlorobenzyltributylammonium chloride) (**I**), before planting. Metabolic effects on the resulting plants were examined by fractionation of ^{32}P-labelled nucleic acid on columns of methylated albumin–kieselguhr. Treated plants showed increased proportions of ribosomal ribonucleic acid and smaller proportions of sol. ribonucleic acid. Specific activities of the various nucleic acid fractions were lowered. Nucleic acids from treated plants were more resistant to enzymic degradation and the activity of endogenous enzymes was lowered. Experimental data indicate the formation of a Phosfon-S–nucleic acid complex. Relationships between nucleic acid metabolism and growth retardation are discussed. A. G. POLLARD.

Darkening of leaves in phosphorus deficiency. C. Hecht-Buchholz (*Z. PflErnähr. Bodenk.*, 1967, **118**, 12–22).—P-deficient plants of tomato and oats were examined. No increase in chlorophyll *a* or *b* per plant was associated with the deficiency, but in deficient plants inhibition of chlorophyll production was less marked than was the inhibition of leaf growth. Spectrophotometric determination of chlorophyll *a* and *b* showed that leaf darkening was not related to any change in the chloroplast pigments. Anthocyanins influenced the darkening process in tomatoes only when P deficiency was severe. (17 references.) A. G. POLLARD.

Relationships between ripening, pectin methylesterase activity and phenolic compounds in banana fruit, *Musa sapientum*, L. G. H. de Swardt (*Diss. Abstr.*, B, 1967, **27**, 2984–2985).—Relationships between the respiratory climacteric and changes in skin colour, pulp dry matter content, firmness, polygalacturonase (*PG*) and pectin methylesterase (*PME*) activity, pH, ethylene evolution and sol. solids were studied, employing single fruits. When individual bananas were used to follow the respiratory climacteric and several phenomena associated with it, there was a striking similarity of values obtained for all attributes at any given point on the climacteric curve, thus emphasising the significance of the climacteric sequence as a reference for biochemical and physiological phenomena associated with ripening. There was no change in *PG* and *PME* activity in homogenates prepared at different stages of ripeness of the banana fruit if polyvinylpyrrolidone was used as a tannin complexing agent in the extraction media of samples analysed prior to the climacteric peak. Low mol. wt. leucoanthocyanins were probably the inhibitory compounds which inactivated *PME* in preclimacteric fruits, but polymerised forms were not active inhibitors of the enzyme. F. C. SUTTON.

Pectin methylesterase in ripening banana. G. H. de Swardt and E. C. Maxie (*S. Afr. J. agric. Sci.*, 1967, **10**, 501–506).—Extracts prepared without any tannin complexing agent at various stages of ripening showed an apparent max. concn. of the enzyme (*PME*) at the climacteric stage, whereas extracts prepared with a medium containing polyvinylpyrrolidone (**I**) showed the same *PME* activity at the different stages. The optimum concn. of **I** to ensure max. activity at the different stages were determined. Accumulation of *PME* plays no part during ripening. Further experiments indicated the presence in the tissues of a natural enzymic inhibitor that was removed with the cell debris during the prep. of the extracts. (Cf. preceding abstract.) (20 references.) P. S. ARUP.

Influence of cobalt on the development of nitrogen-fixing mycobacteria during utilisation of molecular and bound nitrogen. T. K. Il'ina (*Microbiology*, [*USSR*], 1967, **36**, 525–529).—Mo must be present in the culture medium for mycobacteria to assimilate N_2; Co serves as a supplementary co-factor stimulating this process under certain conditions. N-fixation by both types of mycobacteria studied was more effective in the presence of Mo, Co, Zn, Mn and B. Utilisation of NH_4Cl-N does not depend on whether the medium contains Mo or Co. C.V.

Utilisation of different carbon sources by nitrogen-fixing bacterial associations. T. A. Kaliniskaya (*Microbiology*, [*USSR*], 1967, **36**, 520–524). C.V.

Some factors influencing growth and survival of root nodule bacteria in peat culture. R. J. Roughley (*J. appl. Bact.*, 1968, **31**, 259–265).—Growth and survival of *Rhizobium* spp. (*R*) from three inoculation groups was markedly affected by the gas exchange properties of the container. Complete restriction of aeration caused rapid reduction in numbers after only 1 week. Low moisture content restricted growth but higher levels up to 60% (wet wt.) favoured survival. Size of inoculum appeared unimportant in determining the number of *R* in sterilised peat provided sufficient time had been allowed for multiplication. Storage temp., 4–26°, affected growth rate but not survival of *R* in pure culture for periods of >26 weeks provided moisture was not limiting. C.V.

Abscisic acid: a new name for abscisin II ('dormin'). F. T. Addicott *et al.* (*Science, N.Y.*, 1968, **159**, 1493).—Abscisin-II (**I**) was the name given to the second of two abscission-accelerating compounds isolated from cotton fruit. The same substance was also isolated from sycamore leaves during the search for 'dormin', an endogenous substance inducing dormancy. Further isolations and identifications have been reported. The structure of **I** has been determined and the compound has been synthesised; the name abscisic acid is proposed. C.V.

Possible rôle of proteins containing hydroxyprolines in the cessation of cell elongation. R. Cleland and A. M. Karlsnes (*Pl. Physiol.*, 1967, **42**, 669–671).—In the cell walls of Alaska pea epicotyls, the proportion of proteins containing hydroxyproline increased, notably at the period when rapidly elongating tissue was being replaced by non-elongating mature tissue. A. G. POLLARD.

Absence of a causal relationship between auxin-induced growth and changes in the content of ascorbic and dehydroascorbic acids in excised plant tissues. C. Y. Lin and J. L. Key (*Pl. Physiol.*, 1967, **42**, 742–746).—Data presented indicate no causal relation between the oxidation-reduction balance of ascorbic acid and the growth-promoting activity of auxin in various plant tissues.
A. G. POLLARD.

Antagonism between cytokinins and germination inhibitors. A. A. Khan (*Nature, Lond.*, 1967, **216**, 166–167).—Observed interactions in germination of lettuce seed, between zeatin (**I**) and dormin (**II**), kinetin (**III**) and **II**, **I** and coumarin (**IV**), and **III** and **IV** are reported. Results (graphical) show that **II** (25–50 mg per l) strongly inhibits germination and that **I** and **III** (50 mg of each per l) almost completely reverse this inhibition. The mode of

action of **I** and **III** appears to be similar. Antagonism between **III** and **IV** is max. at ~20° rather than at lower temp. The nearly complete inhibitory effect of **IV** (100 mg per l) is almost completely reversed by 50 mg of **I** or **III** per l. Red light experiments show that **II** blocks the photoreversible system and that this is prevented by **I** or **III**. There is probably an interaction between the phytochrome system, natural inhibitors and cytokinins which function to regulate germination and growth. It is possible that cytokinins and biogenous inhibitors participate in controlling nucleic acid metabolism during seed germination. W. J. BAKER.

Kinetics of growth retardant and hormone interactions in affecting cucumber hypocotyl elongation. T. C. Moore (*Pl. Physiol.*, 1967, **42**, 677–684).—The abilities of indolylacetic acid and gibberellin-A_3 to counteract the inhibitory effects on hypocotyl elongation of 2-chloroethyltrimethylammonium chloride (CCC), of 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride (Amo 1618) and of *N,N*-dimethylaminosuccinamic acid (B-995) are examined. Evidence obtained suggests that the retardants may act upon different processes within the plant system. A. G. POLLARD.

Translocation of radioactive kinetin. H. B. Lagerstedt and R. G. Langston (*Pl. Physiol.*, 1967, **42**, 611–622).—Kinetin, 8-^{14}C (**I**), applied to plants via leaf veins, petioles or roots, but not that to leaf lamina, moved freely in the vascular system, apparently with the transpiration stream. The mobile **I** was detected in vernal tissues and retarded senescence and chlorophyll breakdown. The use of **I** in the qual. determination of kinetin and similar substances is described. Cotton seedlings did not take up **I** but tobacco seedlings absorbed and also translocated the isotope. A. G. POLLARD.

Plant growth and metabolism. III. Metabolic changes accompanying inhibition of longitudinal growth of stem and root by kinetin. D. Banerji and M. M. Laloraya (*Pl. Physiol.*, 1967, **42**, 623–627).—The expansion of cotyledons and the inhibition of root growth produced by kinetin (**I**) in lettuce seedlings were accompanied by associated changes in protein-N. In *Pisum sativum*, during the inhibition of longitudinal growth and water intake caused by **I**, protein synthesis was accelerated. Water uptake and protein synthesis were not necessarily related. Unlike gibberellic acid **I** restricted the mobilisation of N materials from lettuce cotyledons and the resulting growth was characterised by a higher ratio of protein-N : sol. N than that in control plants. A. G. POLLARD.

Induction of desirable characters in wheat by radiation. M. P. Singh (*Indian J. exp. Biol.*, 1966, **4**, 234–236).—Dry seeds of three wheat varieties have been exposed to different dosages of *X*-rays, γ-rays, fast neutrons and radioisotopes to induce awning in tipped varieties and remove hard awns and tough glumes in others. Some desirable mutants have been isolated in the second generation; promising selections have been separated in the third generation. The results show that certain improvements can be made by continuous mutation breeding if the desirable character is conditioned by one or two genes. (10 references.) S. A. BROOKS.

Isotopes in plant nutrition and physiology. (*Proc. symp. Pl. Nutr. Physiol.* 1966, *IAEA*, *Vienna*, 1967, 596 pp).—Some 46 papers were submitted, divided into **Soil chemistry and fertility** (5); **Experimental techniques** (5); **Assimilation and metabolism** (6); **Transport phenomena** (4); **Ion absorption, accumulation and transport** (12); **Genetical aspects of plant nutrition** (10); **Nature, genetics and screening of nutritional genotypes and mutants of higher plants** (4). C.V.

Fluorene-9-carboxylic acid derivatives, preparation and compositions containing them. E. Merck A.-G. (B.P. 1,078,347, 10.11.65. Ger., 27.11.64 and 13.3.65).—Compounds (**I**) claimed have excellent plant morphology-regulating properties (and can also be used in combination with herbicides for control of unwanted vegetation). **I** are fluorenes substituted in the 9-position by COZ and optionally in the 2- and 7-position by Cl, Br, or I and further in the 9-position by OH or Cl; Z is OR, NH·NH_2, or NH·$C_6H_3R^IR^{II}$; R is straight- or branched mono- or poly-unsaturated alkenyl or alkynyl of up to 6 C (optionally containing OH and/or halogen), C_{1-12}-alkyl (which may be interrupted by 1–4 non-adjacent O and/or S and may contain 1–3 halogen or OH, or Ph or naphthyl optionally substituted by 1–3 OH, halogen, or methylenedioxy), aromatic or cycloaliphatic radical of 5–10 C which may contain 1–3 halogen, OH, or methylenedioxy, or is substituted NH_4 cation derived from a (cyclo) aliphatic amine of 1–22 C, the hydrocarbon chain being optionally interrupted by O and/or NH and substituted by OH or NH_2; R^I and R^{II} are H, OH, or Cl, there being at least 1 substituent in the benzene nuclei. E.g. a mixture of 9-hydroxyfluorene-9-carboxylic acid, methallyl alcohol, *p*-$C_6H_4Me·SO_3H$, and toluene is boiled during 45 min., with azeotropic removal of water, then the org. liquor is worked up to give 2-methallyl 9-hydroxyfluorene-9-carboxylate, m.p. 139–141° (toluene). Many more compounds (105) are tabulated. F. R. BASFORD.

Crops and Cropping

Yield of maize grain as affected by fertiliser rates and environmental factors. R. Voss and J. Pesek (*Agron. J.*, 1967, **59**, 567–572).—The statistical treatment of a complex experiment involving the effects of soil test values, cultural methods, and weather factors on the response of maize on different soils to N, P, and K applications is presented. A. H. CORNFIELD.

Response of weed-free maize to post-planting cultivation. S. S. Prihar and D. M. van Doren, jun. (*Agron. J.*, 1967, **59**, 513–516).—Post-planting cultivation to break the crust of a silt loam (free of weeds) increased maize yields by 25% compared with no cultivation. Increased water infiltration due to cultivation accounted for about half of the increased yields. O_2 diffusion rates at a 10-cm depth were unaffected by cultivation. Cultivation reduced short-term evaporation from the soil surface 30–45% and increased root wt. A. H. CORNFIELD.

Herbicide *versus* cultivation for maize with two methods of seedbed preparation. T. D. Hinesly, E. L. Knake and R. D. Seif (*Agron. J.*, 1967, **59**, 509–512).—One or three diskings before sowing produced similar yields of maize on seven soil types over 3 years. Cultivation alone or in addition to pre-emergence application of atrazine (1·4 kg per ha) usually had little or no effect on yields or plant population when compared with atrazine treatment alone. The only exception was on a silt loam, where cultivation increased yields in 2 of 3 years. A. H. CORNFIELD.

Winter cover crops for sod-planted maize. W. W. Moschler, G. M. Shear, D. L. Hallock, R. D. Sears and G. D. Jones (*Agron. J.*, 1967, **59**, 547–551).—Rye was the most satisfactory winter cover crop for use with maize planted in the spring after killing the cover crop with atrazine + paraquat. Rye produced larger amounts of mulch than did wheat and oats, whilst barley was the least satisfactory because of resistance to herbicides. In general the highest maize yields occurred with the largest amounts of cover crop mulch. The additional mulch produced by late versus early killing of the cover crop tended to increase maize yields, but not always significantly. In 4 of 13 comparisons maize yields under this system averaged 44% higher than where the cover crop was removed and the soil tilled conventionally before sowing maize; there were no differences between the two systems in the other 9 comparisons. Moisture content in the 0–15 cm soil layer was higher, particularly early in the season, under sod-planted than under conventionally tilled maize. A. H. CORNFIELD.

Effect of nitrogen on yield and quality of rainfed barley. O. P. Gautam and P. P. Singh (*Fertil. News*, 1967, **12**, No. 12; 38–40).—Extensive field studies are described; it is concluded that economic yields of good quality barley under rainfed conditions can be obtained if 27 kg N/ha is applied, one half of this at a depth of 10 cm and the other half at 18 cm. I. DICKINSON.

Diastatic activity during the ripening period in Swedish rye. C. E. Albertsson (*Getreide Mehl*, 1967, **17**, 109–113).—Diagrams are presented and discussed showing daily rainfall and changes in moisture content and diastatic activity of ripening rye during August in the years 1957 to 1964 at four localities in Sweden. In general, diastatic activity gradually rises while the grain moisture content is in excess of 30%, and the risk of excessive activity is greatest with late harvest and field drying, and lowest with early harvest and warm-air drying. (13 references.) E. C. APLING.

Mineral content of sorghum cultivars and effect of application of sulphur and trace elements on yields and mineral composition. D. F. Owen and R. D. Furr (*Agron. J.*, 1967, **59**, 611–612).—There were significant differences in the contents of N, P, K, S, Ca, Zn, and Mn in five sorghum cultivars grown on a silty clay loam (pH 7·2). Application of S (44·8 kg per ha) and chelated Zn + Mn + Fe + Cu + Mg (2·24 kg of each metal per ha) alone or in combination had no effect on yields or mineral composition of sorghum. Values in all the cultivars were below the optimum beef cattle requirements for P, Ca, S, and Zn. A. H. CORNFIELD.

Efficiency of nitrophosphate and other nitrogenous and phosphatic fertilisers. I. Response of potato in field trials. A. Wing (*Technology, Q. Bull. Fertil. Corp., India*, 1966, **3**, 210–216).—Field experiments involving twelve complex trials in Government farms and twelve simple trials in cultivators' fields conducted in six

potato-growing districts of W. Bengal are reported. Results clearly indicate that for potatoes there is no significant difference in efficiency between sulphonitric nitrophosphate (50% water-sol. and 50% citrate-sol. P_2O_5) and other N/P fertiliser combinations. E. C. APLING.

Influence of N-P-K manuring and soil moisture on the sugar content of potatoes. A. Moll (*Z. PflErnähr. Bodenk.*, 1967, **118**, 35–43).—In pot experiments, varied proportions of N, P, and K fertilisers were added to the soil in which potatoes were planted. The water content of the soil was maintained at 50, 75 or 100% of the water capacity. The reducing sugar content of the tubers was lowered by increasing applications of K and increased by N and P; the effect of P may be due, in part, to the Ca added with the P. Neither N nor P affected the saccharase content of the tubers. The monosaccharide content of the potatoes was inversely related to the K content. The effects of the fertiliser treatments were influenced by the soil moisture contents, monosaccharide changes being greatest in soils of the highest water content. (17 references.) A. G. POLLARD.

Influence of preharvest treatments upon turgor of Katahdin potatoes. E. E. Finney, jun. and H. Findlen (*Am. Potato J.*, 1967, **44**, 383–386).—Mechanical bruising of tubers was correlated with turgor (determined by measuring Young's modulus using a mechanical resonance method). Turgor was increased by irrigation prior to harvesting, decreased by pruning the roots, but unaffected by removing vines 4 days before harvest. A. H. CORNFIELD.

Different methods of storing potatoes. C. P. Hampson (*Res. Rep. Potato Mktg Bd*, 1967, No. 1; 11 pp).—Moisture losses are minimised and quality is best maintained by storage at 4° with judicious intermittent forced-draught ventilation. Continuous convective ventilation gives undue moisture losses. The temp. of the potatoes should be reduced to 4° as soon as possible. Sprouting is inhibited at 4°; at higher temp. nonanol or CIPC/IPC vapour blown through the store have given good results if applied before the 'eyes' of the tubers open. Potatoes stored at 4° could be made suitable for crisping if kept at 18° for 14 days before removal from the store. P. S. ARUP.

Time course of low temperature inhibition of sucrose translocation in sugar-beet. C. A. Swanson and D. R. Geiger (*Pl. Physiol.*, 1967, **42**, 751–756).—Maintenance of a 2 cm zone of sugar-beet petiole at temp. normally inhibiting physiological processes had little effect on the translocation of sucrose from the leaf when normal temp. was restored for a comparatively short time, e.g., 1 h at 25°. A. G. POLLARD.

Influence of ploughing depth and subsoiling on yield of sugar-beet. O. J. Furrer (*Schweiz. landw. Forsch.*, 1967, **6**, 201–212).—Ploughing to 28 cm as compared with 18 cm depth had no effect on the average yield of eight trials. Subsoiling had no significant effect on yields. The incidence of forked beets was reduced by deep ploughing but not by subsoiling. Deep ploughing during autumn did not affect the moisture content of the soil during spring, but improved soil-porosity. (23 references.) P. S. ARUP.

Manganese nutrition of sweetpotatoes, *Ipomea batatas*, in relation to manganese content, deficiency symptoms, and growth. U. N. Mishra and J. D. Kelley (*Agron. J.*, 1967, **59**, 578–581).—When sweetpotato slips were grown in nutrient solution containing 0·002–0·25 ppm Mn, dry wt. of shoots and roots after 3 weeks were at a max. with 0·031 ppm Mn in the nutrient. Max. growth occurred in plants containing 25–60 ppm Mn in the leaf blades and 20–30 ppm Mn in the petioles. Growth was best correlated with Mn% in the blades. Visual deficiency symptoms occurred where blades contained < 25 ppm and petioles contained < 18 ppm Mn. Tissue K, Ca, and Mg levels were not affected by varying Mn supply, but tissue P level was at a max. with 0·004 ppm Mn in the nutrient. A. H. CORNFIELD.

Preparing sweetpotatoes for market. L. J. Kushman (*Mktg Bull. U.S. Dep. Agric.*, 1967, No. 38, 14 pp).—Describes the washing, sorting, treatment against rot, waxing, colouring, storing, packaging and shipping of sweetpotatoes. P.P.R.

Growing egg plant, *Solanum melongena*. Anon. (*Leafl. U.S. Dep. Agric.*, 1967, No. 351, 4 pp).—Discusses the varieties, preferred soil, climate and fertilisers, cultivation, pest control and harvesting. P.P.R.

Nature of ice-sheet injury to lucerne. S. Freyman and V. C. Brink (*Agron. J.*, 1967, **59**, 557–560).—Experiments with simulated ice-sheets on the surface of otherwise unfrozen soils and where the CO_2, O_2, and N_2 contents of the soil atm. were artificially altered indicated that CO_2 accumulation in the soil was the main factor causing death of lucerne. Intermittent thawing of the ice sheet reduced the extent of injury, but stubble protruding through the sheet did not prevent injury. A. H. CORNFIELD.

Effects of temperature and soil moisture on single *versus* split nitrogen applications to rye forage. D. G. Cummins, R. E. Burns and W. W. G. Smart, jun. (*Agron. J.*, 1967, **59**, 497–499).—In pot tests where either temp. was low (10° compared with 21°) or soil moisture was low (water applied to field capacity when soil moisture reached 15% field capacity compared with application at 60% field capacity) top yields were greater where N (50–100 lb per acre) was applied as a single dose than when split into two doses. Only when both temp. and soil moisture were high was it advantageous to split the N application. Soil moisture was more limiting to growth in the first, and temp. was more limiting in the second, harvest. Plant N% at 21° was higher with single N application but at 10° was higher with split application. Plant N% was generally higher at low than at high soil moisture. A. H. CORNFIELD.

Birdsfoot trefoil, *Lotus corniculatus*, in pasture improvement. W. F. Wedin, R. L. Vetter, J. M. Scholl and W. R. Woods (*Agron. J.*, 1967, **59**, 525–528).—Wt. gains of yearling steers and number of grazing days were significantly higher on pastures renovated with birdsfoot trefoil, but still containing white clover, than on Kentucky bluegrass–white clover pastures receiving N and P. Birdsfoot trefoil maintained a higher proportion of economic legumes in the sward than did fertilisation with N and P. A. H. CORNFIELD.

Effect of soil conditions on growth and survival of crown vetch, *Coronilla varia*. (*Agron. J.*, 1967, **59**, 533–536).—Crown vetch, which is widely used for ground cover and slope stabilisation, was found, through field observations and greenhouse studies, to require good drainage, pH of 6·5 to 7·0 and high levels of P, K, Ca, and Mg for rapid seedling growth and stand persistence. Areas of poor growth on slopes were more closely associated with limiting physical conditions, poor drainage, or extreme desiccation than with inadequate fertility or low pH. A. H. CORNFIELD.

Effect of paraquat on the nitrogen content and regrowth of coastal Bermuda-grass, *Cynodon dactylon*. S. C. O. Agbakoba and J. R. Goodin (*Agron. J.*, 1967, **59**, 605–607).—When paraquat (0·45 kg per ha) was applied 5 weeks after application of NH_4NO_3 (73·7 kg per ha) the N% of the forage was maintained at a higher level than where paraquat was not applied. The number of shoots which had been produced 7 weeks after paraquat treatment was only about half that of controls, but fresh wt. of regrowth was not affected. A. H. CORNFIELD.

Effect of nitrogen on winter root growth of bentgrass, *Agrostis palustris*. A. J. Powell, R. E. Blaser and R. E. Schmidt (*Agron. J.*, 1967, **39**, 529–530).—Root growth of bentgrass during the winter was increased by application of N (1 kg per ha) in Oct., Oct. and Dec., and Oct. and Dec. and Feb. but was decreased when 4–8 kg was applied spread over Oct. to April. A. H. CORNFIELD.

Phytotoxic phenolic compounds in sericea lespedeza residues. G. W. Langdale and J. E. Giddens (*Agron. J.*, 1967, **59**, 581–584).—*Lespedeza sericea* stem residues incorporated in soils depressed maize growth. Protocatechuic acid (**I**), in particular, and vanillin, ferulic acid, and *p*-coumaric acid were detected in sericea residues. Stem material was particularly high in these compounds. These phenolic compounds were inhibitory to the auxin effect of indole-acetic acid on *Avena* coleoptiles, with **I** being the least effective. A. H. CORNFIELD.

Pathways of nitrogen transfer in tropical legume-grass associations. A. S. Whitney and Y. Kanehiro (*Agron. J.*, 1967, **59**, 585–588).—The mechanism of transfer of N compounds from legumes to grasses when grown in association was studied. Significant transfer of N compounds occurred from the roots of legumes to grasses, particularly when the legumes were cut back. Release of sol. N from legume leaves was small, but expanding leaves, yellowing leaves and shaded leaves contained more extractable NH_2-N than did other leaves. Leaf fall accounted for significant N losses from legumes which shed mature leaves over a period of time. A combination of these three mechanisms accounted for the extent of transfer of N from legume to grass in a small plot test. A. H. CORNFIELD.

Effect of cobalt applications on soyabean yields. H. G. Smith, jun., T. G. Sherbeck, M. E. Bauer and A. J. Ohlrogge (*Agron. J.*, 1967, **59**, 564–566).—Greenhouse and field trials over 2 years on a number of soil types showed that treatment of soyabean seed with $CoSO_4$ at Co rates > 0·037 g per kg of seed resulted in chlorosis and stunted growth of seedlings. Mild, but not severe, chlorosis

was cured by foliar sprays of Fe^{2+} or application of Fe-chelates to the soil. Seed treatment with 0·018 g Co^{2+} per kg of seed increased yields in 3 of 5 locations, but increases were not significant. Foliar-applied $^{60}Co^{2+}$ was translocated to the nodules.
A. H. CORNFIELD.

Ammonium nutrition and flowering of apple trees. V. O. Grasmanis and G. W. Leeper (*Aust. J. biol. Sci.*, 1967, **20**, 761–767).—In water-culture experiments NH_4^+-N favoured flowering whilst NO_3^--N favoured vegetative growth. The best overall results were obtained by supplying NO_3^--N during the season except during Dec. and Jan., when it was replaced by NH_4NO_3.
P. S. ARUP.

Partial forcing with plastic tunnels in the open field cultivation of strawberries. E. Zioni (*Materie plast.*, 1967, **33**, 1055–1062).—Results of cultivation of four varieties of strawberries with mulching in the open field, covered by continuous polyethylene cloches for differing periods, are described in detail. Differing behaviour on forcing of the varieties is noted and compared. C. A. FINCH.

Fructification in the olive. Factors producing abortion and non-fructification. J. J. Vidal and N. Padlog (*Revta Fac. Agron. Univ. nac., La Plata*, 1966, **42**, 221–238).—Observations on six cultivars are reported. In most cases non-fructification was due to the presence of flowers with aborted pistils, the cause of which requires further study. Non-development of an apparently normal ovary may be due to atmospheric conditions, but genetic factors are also involved. In all trials, cross-pollination resulted in more fruit than self-pollination. (18 references.) E. C. APLING.

Differential tolerance of dry and snap bean (*Phaseolus vulgaris*) and lima bean (*Phaseolus lunatus*) varieties to an acid soil high in exchangeable aluminium. C. D. Foy, W. H. Armiger, A. L. Fleming and W. J. Zaumeyer (*Agron. J.*, 1967, **59**, 561–563).—There were considerable differences due to variety in top and root growth and in the response of growth to liming of dry, snap, and lima beans on a clay loam (original pH 4·4) high in exchangeable Al. Varieties originating in the south and east (U.S.A.) showed greater tolerance to acid soils than did those originating from the midwest or west. A. H. CORNFIELD.

Molybdenum deficiency of cauliflower as influenced by soil moisture and fertiliser rate. L. D. Allen and W. A. Laughlin (*Agron. J.*, 1967, **59**, 505–506).—Cauliflower, growing on a non-irrigated loessial silt loam (pH 6·0), developed visual Mo deficiency symptoms. Spraying with 0·02% Na molybdate on 12 and 21 July resulted in normal growth by Aug. and the treatment resulted in 5–7-fold increase in yields of marketable heads. Response to Mo application was greater where the higher than where the lower level of NPK had been added. On irrigated soil no visual Mo deficiency symptoms appeared. A. H. CORNFIELD.

Sucrose levels and enzyme activity in relation to nutritional stress in sugar-cane. A. G. Alexander (*J. Agric. Univ. P. Rico*, 1967, **51**, 325–333).—Sugar-cane plants gradually subjected to nutritional stress of N, P, K or Ca showed higher leaf sucrose % than did normal plants even before nutrient deficiency levels were sufficiently low to reduce cane yields. Amylase activity was reduced and peroxidase was greatly stimulated by stress of all nutrients. Phosphatase was decreased by NO_3^- and PO_4^{3-} stress, but not by K^+ and Ca^{2+} stress. Amylase activity may be useful in indicating approaching deficiency, of major elements. A. H. CORNFIELD.

Tumour and teratoma induction in tobacco plants by de-budding. J. Skok (*Pl. Physiol.*, 1967, **42**, 767–773).—The formation of tumours and teratoma may occur in totally disbudded tobacco plants but not in intact or in decapitated plants or in those in which apical or axillary development was prevented by total disbudding or exposure to ionising radiation. A. G. POLLARD.

Wood quality of loblolly pine after thinning. D. M. Smith (*Res. Pap. Forest Serv. U.S.*, 1968, No. FPL-89; 12 pp).—Loblolly pine wood produced 4 years after heavy thinning and pruning of 9-year-old stands was compared with wood of unthinned stands of the same age; a three-fold increase in radial growth was accompanied by a significant increase in sp. gr. and % of latewood in response to heavy thinning and pruning. Further work should be carried out to determine whether this increased % of latewood is due to the formation of compression wood. P.P.R.

Pest Control

Contribution of herbicides to food production in the next three decades. C. J. Lewis (*Chemy Ind.*, 1968, 860–863).—A general discussion is presented of probable trends in chemical weed control to improve the technical efficiency of agriculture by designing and integrating cropping programmes and field operations involving use of new herbicides. Examples cited for the Western world are (*i*) greatly increased soyabean yields by use of soil-acting herbicides, (*ii*) weed-control of fallows during spring and summer to ensure adequate soil-water for succeeding crop, (*iii*) obtaining good crop yields by drilling seed directly into uncultivated land made weed-free with herbicides. The application of similar measures to bring rough land into cultivation and to increase food production in the 'developing' and backward countries is described, with emphasis on the economic aspects. (22 references.)
W. J. BAKER.

Allethrin and related pyrethroids. W. F. Barthel (*Wld Rev. Pest Control*, 1967, **6**, 59–64).—A brief review. (41 references.)
C.V.

Reaction of dialkylphosphorous acids with aldehydes and ketones. XXXV. Esters of α-hydroxychloroisopropylphosphonic acids. V. S. Abramov, A. Shalman and A. P. Bulgakova (*Zh. obshch. Khim.*, 1968, **38**, 1315–1320).—Dialkyl-, dialkoxyethyl- and dihaloalkyl-phosphorous acids react easily with chloro deriv. of acetone to give the corresponding esters. These reactions occur energetically with increased number of Cl atoms in the chloroacetone. An example describes the reaction of βββ-trichloroacetone with di-β-chloroethylphosphorous acid to give di-β-chloroethyl α-hydroxy-βββ-trichloroethylphosphonate. Tests on many compounds showed high insecticidal properties. R.J.M.

Structures and pesticidal activities of derivatives of dinitrophenols. VII. Formation of 3,3′,5,5′-tetra-t-butyl-4,4′-diphenoquinone from potassium 2,6-di-t-butyl-4-nitrophenoxide and chloroformates. M. Pianka (*J. Chem. Soc.* C, 1967, **24**, 2618–2619).—Treatment of 2,6-di-t-butyl-4-nitrophenol (**I**) in attempts to prepare the potentially miticidal carbonate was carried out (*a*) by treating its K salt in acetone and (*b*) treating its pyridinium salt in ether with a chloroformate (**II**). Method (*a*) gave 3,3′,5,5′-tetra-t-butyl-4,4′-diphenoquinone m.p. 246–247° in 45–50% yield; method (*b*) gave the original phenol **I**. **II** used were Et, Pr^i, benzyl and cyclohexyl. Replacement of **II** by chlorothioloformates or by ethylene bis-chloroformates or by chlorides of conjugated acids e.g. *p*-chlorobenzoyl chloride, lowered yields considerably. J. I. M. JONES.

Synthesis of new organophosphorus compounds with insecticidal and acaricidal activity. F. Wolf and S. Heidenreich (*Dt. Lebensmittt Rdsch.*, 1968, **64**, 171–177).—A new group of thiophosphoric acid *O,O,S*-triesters of the 1,3,5-tri(*O,O*-dialkylphosphorylmercaptoacyl)hexahydro-*s*-triazine and *N,N′*-methylene-bis(*O,O*-dialkylphosphorylmercaptoacylamine) type have been prepared, together with the analogous dithiophosphoric acid triesters. Methods of prep. and analyses are given. The biological activity (against e.g. *Musca domestica*) in relation to structure of these compounds is discussed. (13 references.) J. B. WOOF.

Agricultural fungicides. III. Preparation of 2- and 4-thiocyanato-3,5-dinitrobenzoates. A. L. Black and L. A. Summers (*Aust. J. Chem.*, 1967, **20**, 2293–2296).—Methyl 3,5-dinitro-2-thiocyanatobenzoate is obtained (75% yield) by diazotising Me 2-amino-3,5-dinitrobenzoate in conc. H_2SO_4 followed by reaction with aq. KCNO at <5°; a Sandmeyer catalyst is unnecessary. The corresponding Et ester and the Me and Et 3,5-dinitro-4-thiocyanatobenzoates are prepared similarly in 52–70% yields. The SCN groups in these esters are very reactive, being eliminated from the two Me esters by $PhNH_2$ in boiling EtOH to yield the corresponding anilinodinitrobenzoates. The four compounds have m.p. between 79° and 138°; their fungitoxicity to plant pathogens is to be reported elsewhere. W. J. BAKER.

The chemistry, herbicidal and fungicidal activities of 2,1,3-benzothiadiazoles. J. J. van Daalen, J. Daams, H. Koopman and A. Tempel (*Recl. Trav. chim. Pays-Bas*, 1967, **86**, 1159–1181).—The herbicidal and fungicidal activity of some 65 2,1,3-benzothiadiazoles are studied with special reference to the 4,5,7-trisubstituted deriv. The findings are tabulated. Inhibition of germination (white mustard, chickweed, millet, wild oats, garden cress), phytotoxicity (bean, tomato, oats, chickweed, beet), fungicidal activity (spore germination of *Fusarium culmorum*, *Venturia inaequalis*) and leaf infestation (*Phytophthora infestans*, *Podosphaera leucotricha* and *Sphaerotheca fuliginea*) are recorded together with methods of testing. The synthesis of the compounds is also described. (19 references.) E. J. H. BIRCH.

Novel *N*-heterocyclic fungicides. H. Tolkmith and D. R. Mussell (*Wld Rev. Pest Control*, 1967, **6**, 74–79).—Dialkylphthal-

imido-*N*-phosphonothionates possess high fungicidal properties with low mammalian toxicity, the diethyl ester giving excellent field results. Certain imidazol-1-yl-phosphinamidothionates also show a broad spectrum of fungitoxicity. The fungicidal action would not appear to be related to the degree of phosphorylation ability. This is quite low but it does seem to be involved in the low mammalian toxicity. (19 references.) C.V.

Synthesis of new fungicides. 2-(4′-Arylthiazolyl-2′-imino)-3-aryl-4-thiazolidones. S. C. Sharma (*Bull. Chem. Soc., Japan*, 1967, **40**, 2422–2424).—*N*-Aryl-*N*′-2-(4′-arylthiazolyl)thioureas are prepared (cf. Bhargava and Sharma, *ibid.*, 1965, **38**, 905) and condensed with monochloroacetic acid and Na acetate in ethanol to give 2-(4′-arylthiazolyl-2′-imino)-3-aryl-4-thiazolidones; the first aryl group is Ph·, *p*-Br·C_6H_4·, *p*-Me·C_6H_4· or *p*-MeO·C_6H_4·, and the second is Ph·., *m*- or *p*-Cl·C_6H_4·, *o*-, *m*- or *p*-Me·C_6H_4·, *o*-, *m*- or *p*-EtO·C_6H_4·. The most fungitoxic of these compounds are **2-(4′-phenylthiazolyl-2′-imino)-3-*o*-methoxyphenyl-4-thiazolidone** and -3-*p*-methoxyphenyl-4-thiazolidone. (28 references.)
E. J. H. BIRCH.

Thermal analysis of the organic systems present during preparation of the fungicides captan and Faltan. I. Thermal analysis of mixtures of maleic- and tetrahydrophthalic-anhydrides. T. Flóra and A. Almásy (*Acta Chim. hung.*, 1967, **54**, 189–201).—The starting materials and intermediates maleic- and tetrahydrophthalic-anhydrides in pure form and as mixtures were submitted to differential thermoanalysis and thermogravimetry and phase diagrams of the binary mixtures of varying composition were also measured. An eutectic mixture of the two anhydrides forms on warming, the eutectic point being at 36° with 31% tetrahydrophthalic anhydride. Calibration curves are used to determine quant. the two components when present in mechanical mixtures or in melts. (20 references.) (In German.) M. SULZBACHER.

Relationship between structure and herbicidal activity of substituted benzothiadiazoles-(2,1,3). H. Koopman, J. J. van Daalen and J. Daams (*Weed Res.*, 1967, **7**, 200–207).—A very large no. of these compounds was synthesised with special reference to the halo- and alkyl-substituted deriv. Experimentally it was found that at least one of the 5- or 6-positions in the mol. must remain unsubstituted. Some di- and tri-substituted deriv. possessed excellent herbicidal activity in contrast to the tetra-substituted compounds. (10 references.) C.V.

New chemical attractants for yellow jackets (*Vespula* spp.). H. G. Davis, T. P. McGovern, G. W. Eddy, T. E. Nelson, K. M. R. Bertun, M. Beroza and J. C. Ingangi (*J. econ. Ent.*, 1968, **61**, 459–462).—Of 102 compounds tested in Oregon, 82 caught one or more specimens and seven were more attractive than 2,4-hexadienyl butyrate. The most attractive compounds were hexyl or heptyl α, β-unsaturated esters. The most abundant species was *Vespula pensylvanica*. No other insect was caught in large numbers.
C. M. HARDWICK.

Synthesis of ^{35}S-[(methylthio)aryl]-phosphorothioates. S. Y. Young, A. Begum and R. S. Berger (*J. econ. Ent.*, 1968, **61**, 569–570).—The synthesis of radioactive fenthion (**I**) and Bayer 9017 (*O,O*-diethyl *O*-[4-(methylthio)-3,5-xylyl]phosphorothioate) (**II**) is described. The crude compounds were purified by chromatography on Florisil. The specific activities of the ^{35}S-insecticides depended on the materials contributing the radionuclide (^{35}S-Me_2S_2 for **II** and ^{35}S-MeSH for **I**). C. M. HARDWICK.

Phototactic response of first-instar larvae of pink bollworm to light of different wavelengths. C. L. Mangum and W. O. Ridgway (*J. econ. Ent.*, 1968, **61**, 396–398).—First instar larvae of *Pectinophora gossypiella* were attracted to light of the shortest wavelength. They went towards near u.v. and were repelled by i.r. in combination with visible light. C. M. HARDWICK.

Influence of near ultra-violet output of attractant lamps on catches of insects by light traps. J. P. Hollingsworth, A. W. Hartstack, jun. and D. A. Lindquist (*J. econ. Ent.*, 1968, **61**, 515–521).—There was a direct relationship between catches of *Heliothis zea* and *Trichoplusia ni* and the milliwattage of near u.v. emission by different black light fluorescent lamps. The effect of trap funnel dia. was also investigated. C. M. HARDWICK.

Relative efficiency of aerial application of ultra low-volume and emulsifiable concentrate formulations of insecticides. J. R. Brazzel, W. W. Watson, J. S. Hursh and M. H. Adair (*J. econ. Ent.*, 1968, **61**, 408–413).—Evaluation of drift and efficiency of deposit was by droplet count and chemical analysis. Application to a precise target area was less efficient at 20 ft than at 5 ft. At 20 ft, droplet count was lower for 100 ft downwind but similar for 300–1200 ft. Ultra low-vol. formulations with droplet size of 100–200 μ, drifted less than emulsifiable concentrate, water-diluted formulations. (13 references.) C. M. HARDWICK.

Low-volume application of malathion by helicopter for controlling larch casebearer. R. E. Denton and S. Tunnock (*J. econ. Ent.*, 1968, **61**, 582–583).—Low-vol. application of 8 oz/acre technical malathion gave ~95% control of *Coleophora laricella*. This was nearly as effective as 0·5 lb malathion mixed with fuel oil and applied at 1 gal/acre. C. M. HARDWICK.

Structure of organophosphorus compounds in relation to control of southern chinch bug. S. H. Kerr (*J. econ. Ent.*, 1968, **61**, 523–525).—In field tests *Blissus insularis* was more susceptible to organophosphorus compounds with ethyl or isopropyl side groups than those with methyl side groups. Aliphatic compounds with 2- and 3-C side chains gave excellent control. The most effective heterocyclics are diethyl compounds while aryl deriv. are mostly more effective than heterocyclic or aliphatic deriv. (13 references.)
C. M. HARDWICK.

Residual activity of diazinon and lindane for control of *Loxosceles reclusa*. B. R. Norment and T. L. Pate (*J. econ. Ent.*, 1968, **61**, 574–575).—Lindane was more active than diazinon at each of five dosages. At the highest dosage, >70% mortality was obtained after a 24 h exposure. C. M. HARDWICK.

Laboratory tests with dichlorvos applied as a wheat protectant against rice weevils. R. L. Kirkpatrick, P. K. Harein and C. V. Cooper (*J. econ. Ent.*, 1968, **61**, 356–358).—After 14 days exposure to 0·8–2·25 ppm dichlorvos ≮90% mortality of adult *Sitophilus oryzae* was found. Reproduction was reduced by ≮89%. With high dosages, a 3-day exposure was as effective as 14 days.
C. M. HARDWICK.

Elimination of root-knot nematodes from plants by chemical bare-root dips or soil drenches. D. P. Harlan and L. Jenkins (*Pl. Dis. Reptr.*, 1967, **51**, 103–107).—In bare-root dip treatments 750–1150 ppm Cynem (*O,O*-diethyl-*O*-2-pyrazinyl phosphorothioate) was the only chemical tested that consistently eliminated nematodes from red leaf barberry, *Berberis thunbergii*, blue mist, *Caryopteris incana*, peony, *Palonia albiflora*, multiflora rose, *Rosa multiflora*, and tomato. Low concn. soil drenches with Cynem, B-25141 [*O,O*-diethyl *O*-(*p*-methylsulphinylphenyl)-phosphorothioate] and N-9227 (*O,O*-diethyl *O*-2-oxo-2*H*-benzopyran-3-yl phosphorothioate) gave complete control of nematodes, but there was some phytotoxicity. A. H. CORNFIELD.

Use of chemical defoliants on peach trees in integrated programme to suppress populations of green peach aphids. G. Tamaki and R. E. Weeks (*J. econ. Ent.*, 1968, **61**, 431–435).—Peach trees were sprayed with DEF (*S,S,S*-tributyl phosphorotrithioate) or Shed-a-leaf ($NaClO_3$) in autumn. Although there was a decrease in the total number of *Myzus persicae*, there was a higher density on the remaining foliage. This, in turn, was associated with an increase in syrphid eggs per leaf. Decreased egg laying in 1966 compared with 1965 was probably due to population trends and physical factors. C. M. HARDWICK.

Initial soil penetration by insecticide emulsions used for subterranean termite control. R. H. Beal and F. L. Carter (*J. econ. Ent.*, 1968, **61**, 380–383).—Seven soils of different types were selected from different areas of U.S.A. The penetration of dieldrin and chlordane was greatest when soil moisture was highest. In general >50% of the insecticide was in the upper ¾ in. after 24 h.
C. M. HARDWICK.

Degradation of diazinon (*O,O*-diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidyl)-phosphorothioate) in soils. J. G. Konrad, D. E. Armstrong and G. Chesters (*Agron. J.*, 1967, **59**, 591–594).—Diazinon was partially hydrolysed chemically in soil with formation of 2-isopropyl-4-methyl-6-hydroxypyrimidine and diethyl thiophosphoric acid. A. H. CORNFIELD.

Soil fumigation with D-D (1,3-dichloropropene–1,2-dichloropropane) for control of *Pratylenchus penetrans* in maize. J. E. Edmunds, C. W. Boothroyd and W. F. Mai (*Pl. Dis. Reptr*, 1967, **51**, 15–19).—Soil injection with D-D (32 gal per acre) two weeks before planting maize gave good control of *Pratylenchus penetrans* and other nematodes associated with the roots. The treatment caused initial stunting of seedlings and produced white stripes running the whole length of the leaf, but had no effect on yields. Six weeks after treatment soil NO_3^- was lower and NH_4^+ was higher in treated than in control plots. The treatment increased Fe and Al and reduced Mn and NO_3^- contents of the leaves of 1-month-old seedlings. A. H. CORNFIELD.

Feeding by *Reticulitermes* spp. on radiation-processed wood-plastic combinations. C. E. Affeltranger (*J. econ. Ent.*, 1968, **61**, 398–410).—Blocks of maple, white pine and birch were impregnated with polymethyl methacrylate, or a co-polymer thereof with polymethacrylic acid. In laboratory tests, *Reticulitermes flavipes* damaged samples treated with polyvinyl acetate but did not increase on impregnated samples. Their reproduction rate increased when fed untreated wood and again after irradiated wood was given. Field tests gave similar results. C. M. HARDWICK.

Effects of potassium and chloride on root necrosis, stalk rot, and pith condition in maize. J. W. Martens and D. C. Arny (*Agron. J.*, 1967, **59**, 499–502).—Application of KCl (168 kg K per ha) decreased root necrosis and delayed death of the plants, but increased stalk rot due to *Diplodia maydis*. The treatment increased pith density (no. of living cells) in two of the three lines studied. Application of NH_4Cl (158 kg Cl per ha) increased pith density in one line and in general had effects similar to, though smaller than, the KCl treatment. A. H. CORNFIELD.

Effect of insecticidal sprays on stunt and mosaic virus diseases of corn [maize] in small field plots in Mississippi. H. N. Pitre (*J. econ, Ent.*, 1968, **61**, 585–587).—Five applications of Azodrin, Baygon. methyl parathion or carbaryl spray, were given at weekly intervals. There was no significant reduction in incidence of mosaic or maize stunt in treated plots. There was a reduction in numbers of *Graminella nigrifrons* following Azodrin and carbaryl sprays. (13 references.) C. M. HARDWICK.

Stem applications of systemic insecticides to corn [maize]. R. L. Ridgway and N. M. Randolph (*J. econ. Ent.*, 1968, **61**, 581–582).—Five systemic insecticides were applied to the stems of maize and new and old growth was bioassayed with *Rhopalosiphum maidis*, 3–21 days after application. Azodrin, Bidrin and oxydemeton-methyl caused greater mortality than phosphamidon and CL-47031 [cyclic ethylene (diethoxyphosphinyl)dithioimidocarbonate]. Application to the stems rather than the leaf sheaths resulted in greater uptake. C. M. HARDWICK.

Five-year study of potential western corn rootworm resistance to diazinon and phorate in Nebraska. H. J. Ball (*J. econ. Ent.*, 1968, **61**, 496–498).—*Diabrotica virgifera* were collected from 18 sites in Nebraska from 1963–1967 and their LD_{50} to diazinon and phorate were determined by topical application. There was a steady increase in LD_{50} values to give 66·1% and 61·7% increases for diazinon and phorate respectively. Field control is at present unaffected. (12 references.) C. M. HARDWICK.

Effects of soil fertility on abundance of green peach aphids on Maryland tobacco. A. W. Wooldridge and F. P. Harrison (*J. econ. Ent.*, 1968, **61**, 387–391).—When *Myzus persicae* were caged individually on tobacco leaves, increased amounts of N and K increased aphid numbers, but had little effect on longevity. In field experiments, a balanced fertiliser containing N, P and K increased fecundity with increased fertiliser application. C. M. HARDWICK.

Reduction of corn [maize] virus disease incidence and control of southwestern corn borer with systemic insecticides. A. J. Keaster and M. L. Fairchild (*J. econ. Ent.*, 1968, **61**, 367–369).—Of six systemic insecticides applied in the furrow at planting time, NIA 10242 (2,3-dihydro-2,-2-dimethyl 1-7-benzofuranyl methylcarbamate) gave the greatest reduction in incidence of maize virus disease and also of *Zeadiatraea grandiosella*. C. M. HARDWICK.

Vertical-pull technique for evaluating tolerance of corn [maize] root systems to northern and western corn rootworms. E. E. Ortman, D. C. Peters and P. J. Fitzgerald (*J. econ. Ent.*, 1968, **61**, 373–375).—As *Diabrotica virgifera* and *D. longicornis* cause extensive root damage, an evaluation of the state of the root system would be a measure of rootworm damage. Statistical studies showed that the no. of pounds pull required to remove a plant (tested for resistance to *Ostrinia nubilalis*) from the soil was related to the various characteristics of the root system. C. M. HARDWICK.

Insecticidal application for integrated control of the brown plant hopper in paddy rice. M. J. MacQuillan (*J. econ. Ent.*, 1968, **61**, 568–569).—Mecarbam larvicidal oil coated on 2–4 mm gravel was broadcast on paddies of 3 month-old rice. Three days after the second application numbers of *Nilaparvata lugens* were significantly reduced; mirid predators were unaffected. C. M. HARDWICK.

Respiratory environments of grain-infesting weevils. I. Comparison of culture-jar and laboratory rearing-room atmospheres. J. H. Williams and D. A. Wilbur (*J. econ. Ent.*, 1968, **61**, 345–348).—O_2, CO_2 and N_2 concn. were measured by gas chromatography in a laboratory rearing room and at three levels in open culture jars. The variation in [CO_2] with the life cycle of *Sitophilus zeamais* is described; [O_2] showed a reverse pattern. Laboratory atm. contained less CO_2 and more O_2 than that in rearing rooms and both had less CO_2 and more O_2 than culture jar atm. C. M. HARDWICK.

Tetrachloroisophthalonitrile (DAC 2787) for control of early blight of potato. M. D. Harrison, C. H. Livingston and R. G. Walter (*Pl. Dis. Reptr*, 1967, **51**, 9–11).—Although application of DAC 2787 (1·0–1·5 lb per acre sprayed four times at 14-day intervals beginning July 15) considerably reduced the incidence of early blight, due to *Alternaria solani*, on potatoes it was no more effective than maneb (1·5 lb per acre) in this respect. Potato yields were not significantly affected by any of the treatments. A. H. CORNFIELD.

Plant pathogens as a possible factor in unexpected pre-plant herbicide damage in sugar-beets. J. Altman and M. Ross (*Pl. Dis. Reptr*, 1967, **51**, 86–88).—Pre-plant soil treatment with Pebulate (*S*-propyl butylethylthiocarbamate) at a normally safe rate (4·5 lb per acre) resulted in greater damping off, due to *Rhizoctonia solani*, of sugar-beet seedlings than where the herbicide was not applied. Pebulate also increased the count of sugar-beet nematode cysts and nematode larvae. A. H. CORNFIELD.

Residual effectiveness of foliar sprays against the oriental fruit fly, melon fly and Mediterranean fruit fly. I. Keiser (*J. econ. Ent.*, 1968, **61**, 438–443).—Many insecticides in 105 doses and formulations were tested in Hawaii over 8 years. Mortalities were compared after 1–16 days weathering. Only six compounds showed interspecific differences. Different compounds were effective during dry and wet periods. C. M. HARDWICK.

Control of the aphid *Amphorophora agathonica* in raspberry nursery stock. G. A. Schaefers (*J. econ. Ent.*, 1968, **61**, 384–387).—Of 15 foliar sprays, Niagara 10242 (2,3-dihydro-2,2-dimethyl 7-benzofuranyl methylcarbamate) was the most effective. Granular systemic insecticides broadcast over the row were superior to sprays. Temik at 6 lb/acre in mid-May and early Aug. gave season-long control. C. M. HARDWICK.

Thiabendazole in resin–solvent and wax-emulsion coatings for control of *Penicillium digitatum* in navel oranges. P. R. Harding, jun. and J. E. Schade (*Pl. Dis. Reptr*, 1967, **51**, 51–53).—Thiabendazole (**I**) showed promise as a preventive of *Penicillium* decay, particularly in resin–solvent treatments. The PhMe in the PhMe–EtOH solvent caused burning and pitting of the orange rind. A. H. CORNFIELD.

Thiabendazole as a postharvest fungicide for Florida citrus fruit. G. E. Brown, A. A. McCornack and J. J. Smoot (*Pl. Dis. Reptr*, 1967, **51**, 95–98).—When tested *in vitro* thiabendazole prevented the growth of *Phomopsis citri* and *Penicillium digitatum* at 2·5 ppm and *Diplodia natalensis* at 20 ppm. At 200 ppm the chemical controlled decay in non-degreened and ethylene-degreened Valencia oranges, comparable with that obtained with carbonated 2-aminobutane at 10,000 ppm. A. H. CORNFIELD.

Control of scab and other diseases of pecan in Alabama. U. L. Diener and F. E. Garrett (*Pl. Dis. Reptr*, 1967, **51**, 185–188).—Application of 50% Ph_3SnOH (0·4 lb), Ph_3SnOAc (0·4 lb) and 65% dodine (1 lb per 100 gal) at 3-weekly intervals gave excellent control of scab, *Fusicladium effusum*, and other diseases of pecan. A. H. CORNFIELD.

Control of root rot, due to *Aphanomyces euteiches*, of peas. G. C. Papavizas (*Pl. Dis. Reptr*, 1967, **51**, 125–129).—The best control of *Aphanomyces* rot of peas was obtained by application of 140 ppm (soil basis) *dl*-β-methylaspartic acid or *dl*-β-aminobutyric acid as a side dressing one week after planting or by Dexon [Na *p*-(dimethylamino)benzenediazosulphonate], at 0·02–0·07 g per kg of soil at planting. A. H. CORNFIELD.

Ozone and soil moisture in relation to incidence of weather fleck on Florida cigar-wrapped tobacco in 1966. C. E. Dean and D. R. Davis (*Pl. Dis. Reptr*, 1967, **51**, 72–75).—Weather fleck on shade-grown tobacco occurred with above-normal concn. of atm. O_3 during two periods of the growing season. Irrigation during periods of elevated O_3 concn. resulted in increased incidence of weather fleck, regardless of previous water treatment. A. H. CORNFIELD.

Toxicity of five carbamate insecticides to the two-spotted spider mite and larvae of the southern armyworm and tobacco budworm. D. A. Wolfenbarger and R. E. Redfern (*J. econ. Ent.*, 1968, **61**, 580–581).—The five carbamates were applied topically, used as foliar sprays against *Heliothis virescens* and *Prodenia eridania*, and

as contact sprays against *Anthonomus grandis* adults and as foliar sprays and systemics against adults and nymphs of *Tetranychus urticae*. C. M. HARDWICK.

Effect of ultra-violet irradiation on egg hatch, subsequent larval development and adult longevity of tobacco budworm and bollworm. A. A. Guerra, M. T. Ouye and H. R. Bullock (*J. econ. Ent.*, 1968, **61**, 541–546).—There was a decreased hatch of *Heliothis virescens* and *H. zea* with exposure to u.v. radiation from 1–20 min. They were unaffected by exposure to long wave radiation. Surviving eggs developed into normal adults. C. M. HARDWICK.

Evaluation of experimental insecticides for control of hornworms on tobacco. C. B. Dominick (*J. econ. Ent.*, 1968, **61**, 483–484).—Nine experimental compounds were sprayed on tobacco and the effect on numbers of *Manduca sexta* and *M. quinquemaculata* was tabulated over 21 days. Initial control was good. There was no phytotoxicity. C. M. HARDWICK.

Fungistatic effect of chemicals on hyphal growth of *Phymatotrichum omnivorum*, the cotton root-rot fungus. B. Sleeth (*Pl. Dis. Reptr*, 1967, **51**, 138–142).—Of 25 chemicals tested at 500 ppm in suspension or solution 18 had little fungistatic effect on hyphal growth after 7–14 days. Pentachloronitrobenzene, thiram, 93% nabam, and Van. 51 (2-benzenethiazolethiol-Na deriv.) did not move in the soil and retained their fungitoxic properties for more than 14 days. NIA 5961 (1-chloro-2-nitropropane), SMDC (Na methyldithiocarbamate), and DMTT (tetrahydro-2*H*-3,5-dimethyl-1,3,5-thiadiazine-2-thione) penetrated the soil 9–10 cm and inhibited hyphal growth for 8–12 days. HCHO penetrated the soil 3–4 cm and retarded hyphal growth for 4–5 days. A. H. CORNFIELD.

Control of tarnished plant bug on cotton with several insecticides. T. C. Cleveland and G. L. Smith (*J. econ. Ent.*, 1968, **61**, 566–567).—The degree of control of *Lygus lineolaris* 1 day after treatment with various sprays and dusts, over 4 seasons, is shown. Control measures were necessary for ~3 weeks per season. C. M. HARDWICK.

Compatibility of arthropod repellents with certain functional finishes of cotton uniform fabric. H. Markarian, J. J. Pratt, jun., C. G. De Marco and H. K. Gouck (*J. econ. Ent.*, 1968, **61**, 464–467).—A length of cloth was treated with various insect repellents, M-1960, benzyl benzoate and deet, and with two standard water repellents, a fire retardant or a gas detoxicant, and their interaction studied. Water repellency was removed when insect repellents were added. The effect of the fire-retardant varied with the insecticide concerned. C. M. HARDWICK.

Control of winter rhizome rot, due to *Botrytis convoluta*, of iris. H. S. MacWhitney (*Pl. Dis. Reptr*, 1967, **51**, 83–86).—Dipping iris rhizomes in Semesan [2-chloro-4-(hydroxymercuri)phenol, 3 lb per 100 gal] for 30 min. or in Ceresan (ethylmercury chloride, 12·5 lb per 100 gal) for 5–10 min. before planting reduced losses from *Botrytis* winter rhizome rot disease. The treatments were effective in preventing infection of wound tissue on the parent rhizome and the spread of inoculum from suberised lesions in contact with the soil, but were not effective in eradicating suberised *Botrytis* infections in planting stock or in reducing infection of new divisions. A. H. CORNFIELD.

Sclerotinia dollar spot, due to *Sclerotinia homoeocarpa*, on bentgrass: varietal susceptibility to infection and influence of variety on fungicide effectiveness. H. Cole, A. T. Perkins and J. Duich (*Pl. Dis. Reptr*, 1967, **51**, 40–42).—Colonial bentgrass varieties were partially resistant, whilst creeping varieties were very susceptible, to infection by *S. homoeocarpa*. Tersan OM [45% thiram + 10% 2-chloro-4-(hydroxymercuri)phenol, 6 oz] and Calo-Clor (60% HgCl – 30% $HgCl_2$, 2 oz per 1000 sq. ft) gave effective control for at least 14 days on all varieties. Dithane M-45 (80% Zn-maneb coordination product, 8 oz per 1000 sq. ft) was effective for 14 days on all varieties, but after 14 days was not effective on the susceptible creeping bent types and was marginally effective on the resistant colonial types. A. H. CORNFIELD.

Control of the white coffee-borer, *Anthores leuconotus*. D. N. McNutt (*E. Afr. agric. For. J.*, 1967, **32**, 469–473).—The pest was controlled by two applications of 0·8% dieldrin to the lower 2 ft of the coffee tree stem with a 10 month interval between applications. A. H. CORNFIELD.

Oligomycin treatment of oak wilt in Northern pin oaks, *Quercus ellipsoidalis*. W. R. Phelps and J. E. Kuntz (*Pl. Dis. Reptr*, 1967, **51**, 160–163).—Trunk injection of 400–800 ppm oligomycin effectively controlled wilting of pin oaks inoculated with the wilt organism just after treatment. Soil drenches and foliar sprays were ineffective. Treatment of trees in the early stage of wilt delayed disease development for a few months, but did not prevent eventual death. Treatment of moderately to severely wilted trees had no effect on the course of the disease. A. H. CORNFIELD.

Gamma irradiation of tobacco budworm: sterilisation, competitiveness, and observations on reproductive biology. H. M. Flint and E. L. Kressin (*J. econ. Ent.*, 1968, **61**, 477–483).—Irradiation with 45 krad sterilised both sexes and longevity was reduced by 0–10%. Sterilisation did not significantly affect the mating ability of either sex. The effect of mating by sterile and untreated males on fertilisation is discussed. (19 references.) C. M. HARDWICK.

Chemosterilisation of male pear psylla with Tepa. G. H. Kaloostian (*J. econ. Ent.*, 1968, **61**, 573–574).—Male *Psylla pyricola* were caged with pear seedlings dipped in 3% Tepa and then caged with normal females. There was high mortality amongst males but the survivors prevented females laying viable eggs. C. M. HARDWICK.

Synthetic chemical sex stimulants for the codling moth. B. A. Butt, M. Beroza, T. P. McGovern and S. K. Freeman (*J. econ. Ent.*, 1968, **61**, 570–572).—Numerous aldehydes and nitriles were screened as attractants for *Carpocapsa pomonella*. The only common factor amongst the active compounds was the presence of a nitrile group. C. M. HARDWICK.

Effects of chemosterilants and growth regulators on the pea aphid fed on artificial diet. O. P. Bhalla and A. G. Robinson (*J. econ. Ent.*, 1968, **61**, 552–555).—Nymphs of *Acyrthosiphon pisum* were fed synthetic diets containing 27 chemosterilants and 3 growth regulators. Six of these caused permanent sterility, two temporary sterility and 21 decreased fecundity. Of the three growth regulators, maleic hydrazide and a synthetic queen substance were very toxic and Cycocel reduced fertility. In most tests, immature aphids were more affected by chemosterilants than were adults. C. M. HARDWICK.

Histochemical effects of Apholate on the reproductive organs of southern corn rootworm. C. E. Mendoza and D. C. Peters (*J. econ. Ent.*, 1968, **61**, 416–420).—The effect of Apholate on alkaline phosphatase was determined by using Gomori's technique to precipitate Ca phosphate. The enzyme was shown to be specific to DNA substrate. The injection of Apholate into *Diabrotica undecimpunctata howardi*, produced clear sites in both male and female reproductive tissue showing the inactivation of alkaline phosphatase. (29 references.) C. M. HARDWICK.

Fertility of eggs of Mexican bean beetles from females mated alternately with normal and Apholate-treated males. R. E. Webb and F. F. Smith (*J. econ. Ent.*, 1968, **61**, 521–523).—Eggs of untreated females were almost all nonviable after the first mating with treated males. When subsequently mated with untreated males the egg hatch was almost normal. A second mating with sterile males reduced viability to a lesser degree. The most recent mating had the greatest influence on viability but earlier ones had some effect. C. M. HARDWICK.

Isolation of a sex pheromone of the European corn borer. J. A. Klun (*J. econ. Ent.*, 1968, **61**, 484–487).—The extraction of sex pheromone from homogenised female *Ostrinia nubilalis* by pptn. and chromatography on silica gel columns, is described. It is a strong electron captor that is sol. in polar org. solvents and is unsaponifiable. It was active for >1 year. It was most active at 20–23°. C. M. HARDWICK.

Sulphonic acid esters as chemosterilants of screw-worm flies with particular reference fo methanediol dimethanesulphonate. M. M. Crystal (*J. econ. Ent.*, 1968, **61**, 446–449).—Of 11 esters of alkyl- and arylsulphonic acids administered to *Cochliomyia hominivorax*, seven were 95% effective when administered orally. By topical application, only methanediol dimethanesulphonate was effective. The number of functional groups of alkanesulphonates was unrelated to the chemisterilant action. Male aggressiveness was not affected. (18 references.) C. M. HARDWICK.

Potential chemosterilants for boll weevils. W. Klassen, J. F. Norland and A. B. Bořkovec (*J. econ. Ent.*, 1968, **61**, 401–407).—Many compounds, made up of methanesulphonates, N-mustards, aziridines, phosphoramides and *s*-triazines were evaluated by feeding to male *Anthonomus grandis* for 3 days. The % hatch was recorded, based on ⩽150 eggs, in order to estimate sterility. C. M. HARDWICK.

Suppression of populations of *Drosophila melanogaster* in tomato field plots with chemosterilant baits. H. C. Mason and F. F. Smith (*J. econ. Ent.*, 1968, **61**, 362–367).—Jars containing fermented bait and sprayed with 2% Apholate gave up to 52% suppression of

oviposition for 1 week. Sprayed boxes of tomatoes were less effective. Diazinon granules at 1 lb/acre gave 93% control. Interplot movement minimised chemosterilant treatments but 50% fewer adults were collected than on control plots. Max. female sterility was 94%. C. M. HARDWICK.

Some effects of three triphenyltin compounds on the fertility and longevity of Japanese beetles. T. L. Ladd, jun. (*J. econ. Ent.*, 1968, **61**, 577–578).—Triphenyltin-chloride, -acetate and -hydroxide were applied to adult *Popillia japonica*. Increased egg infertility, decreased fecundity and a decline in longevity with both sexes was apparent with increasing doses. C. M. HARDWICK.

Boll weevil sex attractant: isolation studies. J. H. Tumlinson, D. D. Hardee, J. P. Minyard, A. C. Thompson, R. T. Gast and P. A. Hedin (*J. econ. Ent.*, 1968, **61**, 470–474).—The most effective method of obtaining attractant was by steam distillation of dichloroethane extracts of male weevils or mixed sex weevils or of faeces. Column chromatography with Florisil or Carbowax coated on silica gel, steam distillation and aeration techniques are described. (15 references.) C. M. HARDWICK.

Specificity of the cabbage looper sex attractant. R. S. Berger and T. D. Canerday (*J. econ. Ent.*, 1968, **61**, 452–454).—The synthesis of *cis*-7-dodecenyl acetate and its *trans* isomer is described. Evaluation of the *cis* isomer and related compounds with *Trichoplusia ni* showed that any alteration from the original structure, reduced activity. All Plusiinae tested exhibited responses to extracts from all other species. C. M. HARDWICK.

Control of *Ernobius mollis* Linn. (Coleoptera: Anobiidae) with dieldrin dips. R. H. Milligan (*N.Z. Jl Sci.*, 1967, **10**, 1012–1019).—Dipping of test-billets with 0·1% dieldrin (**I**) emulsion prevented oviposition by *E. mollis*; embryonic development and hatching of eggs on billets treated with 0·005–0·05% **I** were scarcely affected, but all first instar larvae were killed under these conditions. **I**-films did not repel ovipositing females, but adult mortality led to fewer eggs being laid on the pretreated billets. Results confirm the specific susceptibility of first instar larvae, a fact which could be applied to the bioassay of dil. **I**-films. W. J. BAKER.

[Determination of] organothiophosphate pesticide residues on fruits and vegetables, using microcoulometric gas chromatography. R. C. Nelson (*J. Ass. off. analyt. Chem.*, 1967, **50**, 922–926).—The extract (Mills, Oxley and Gaither, *ibid.*, 1963, **46**, 188) is cleaned up on an activated Florisil column; sulfotep, Thimet, diazinon, Di-Syston, methyl parathion, parathion, ethion, Trithion and EPN are then determined by microcoulometric gas chromatography, employing a titration cell sensitive to SO_2. Recoveries ranged from 73 to 109%. A. A. ELDRIDGE.

Thin layer and gas chromatography of pyrethrum-based insecticides. E. Stahl and J. Pfeifle (*Pyrethrum Post*, 1966, **8**, No. 4, 8–9).—Using special solvent mixtures, pyrethrum extract can be separated by TLC into six individual components (cinerin I & II, jasmolin I & II, pyrethrin I & II); this can be done quant. by employing a Zeiss Special Photometer, measuring the absorption at 288 mμ. A combination of TLC and GLC is particularly valuable; an initial separation by TLC is followed by gas chromatography using a silicone elastomer column swept by He. J. L. WALPOLE.

Determination of terbacil residues using microcoulometric gas chromatography. H. L. J. Pease (*J. agric. Fd Chem.*, 1968, **16**, 54–56).—Terbacil (**I**) is extracted from plant or animal tissues or soil with 1% aq. NaOH. A cleanup procedure involving solvent partition and the use of a Florisil column, is described. The nitromethane–hexane solvent cleanup method (cf. Pease, *ibid.*, 1966, **14**, 94) can also be used. The determination with selective microcoulometric detection is sensitive to 0·04 ppm of **I**, with recoveries of 90–100%. The method is also applicable to the determination of bromacil. P. S. ARUP.

Enzymatic detection of ten organophosphorus pesticides and carbaryl on thin-layer chromatograms: evaluation of indoxyl, substituted indoxyl and 1-naphthyl acetates as substrates of esterases. C. E. Mendoza, P. J. Wales, H. A. McLeod and W. P. McKinley (*Analyst, Lond.*, 1968, **93**, 34–38).—Reproducible detection of ng amounts of the pesticides is achieved on SiO_2-gel (450 μ thick) with indoxyl, 5-bromoindoxyl-, 5-bromo-4-chloroindoxyl- or 5-bromo-6-chloroindoxyl-acetate as substrate for steer-liver esterase, using esterase and substrate spray-solutions at pH <8. Coloured products of enzymatic hydrolysis of substrates are stable and intense; white spots locate pesticides that inhibit the enzyme. Spots persist for days when concn. are: parathion <1 ng; carbophenothion (**I**) <2 ng; azinphos-methyl, diazinon, ethion, malathion and parathion-methyl <5 ng; carbaryl (**II**), Trithion-methyl and mevinphos <20 ng; disulfoton (**III**) <100 ng. **I** and **III** are detectable at ~1 ng and **II** at ~5 ng, but the spots are stable only for ~2 h. Bovine- and sheep-sera are unsatisfactory as esterase source, and 1-naphthyl acetate as substrate. W. J. BAKER.

Separation of dieldrin from pentachlorophenol. J. N. Wilson, M. C. Franks and D. R. Sherlock (*Analyst, Lond.*, 1967, **92**, 782).—The dieldrin (**I**) (~0·5%) is separated from pentachlorophenol (**II**) (5%) by percolation of the sample (diluted with dry MeOH) through De-Acidite K or Amberlyst 29 (OH^- form), whereby **II** is retained on the resin. **I** in the solvent-free percolate is assayed for Cl by the O_2-flask method, the HCl being determined by titration with aq. ethanolic $Hg(NO_3)_2$ (Cf. White, *Mikrochim. Acta*, 1961, 449). Method is applicable to samples of varying size and concn. and also, with minor modifications, to determination of BHC and DDT in combination with **II**. W. J. BAKER.

Modification of Miles' method for determining azinphos-methyl residues in crops [apples, pears, cucumbers, tomatoes, Brussels sprouts, peas]. N. A. Smart (*Analyst, Lond.*, 1967, **92**, 779–781).—Limitations of Meagher's, Bluman's, and Miles' methods were overcome by a modification of Miles' method. The macerated sample is extracted twice with $COMe_2$, an aliquot of the combined filtrates is extracted with $CHCl_3$, the solvent is evaporated, and the residue dissolved in 10 ml of $COMe_2$ for chromatographic treatment (Cf. Miles, *J. Ass. off. agric. Chem.*, 1964, **47**, 882 and Bates, *Analyst, Lond.*, 1962, **87**, 786). Accuracy and sensitivity equal those of Meagher's method; recovery of insecticide is ~74% and standard deviation is $\pm 0{\cdot}03$ to $\pm 0{\cdot}04$ (5 determinations) for 1 ppm concn. A result is obtained in 3 h. (12 references.) W. J. BAKER.

Tetrafluorocyclobutane derivatives. Farbwerke Hoechst A.-G. (B.P. 1,065,541, 15.10.63. Ger., 18.10.62).—Compounds claimed are insecticides and comprise bicyclo[2,2,1]hept-2-enes substituted in the 5-position by 2,2,3,3-tetrafluorocyclobutyl and elsewhere by <4 halogen. An example is 1,2,3,4,7,7-hexachloro-5-(2,2,3,3-tetrafluorocyclobutyl)bicyclo[2,2,1]cycloheptene-2-ene, b.p. 120–130°/0·2 mm, m.p. 77–78° (MeOH), prepared by heating a mixture of hexachlorocyclopentadiene and 1,1,2,2-tetrafluoro-3-vinylcyclobutane at 150° for 24 h. It is lethal to white grubs, wireworms, grain weevils, and African hut tick. F. R. BASFORD.

***N*-Substituted fluoroacetamides.** Nippon Soda K.K. (B.P. 1,065,801, 22.1.65. Jap., 19.2, 22.5 and 4.11.64).—Used as insecticides, the title compounds have the formula $Ar{\cdot}N(R)CO{\cdot}CH_2F$, where Ar includes Ph, $C_6H_4{\cdot}Cl$, $C_6H_2{\cdot}Cl_3$, $C_6H_4{\cdot}F$, $C_6H_4{\cdot}NO_2$ and (substituted) thiazolyl radicals and R is alkyl with >4 C, a hydroxyalkyl with 2–4 C, $Ph{\cdot}CH_2$ or a naphthylmethyl radical. One method of prep., consists of reacting a s-amine hydrochloride, $Ar{\cdot}N(R)H{\cdot}HCl$ with $FCH_2{\cdot}COOH$ in presence of a P-chloride. Thus, $FCH_2{\cdot}COOH$ is added to *N*-methyl-α-naphthylamine hydrochloride in anhyd. xylene and the mixture heated at 90–100°, with addition of PCl_3 over 0·5–1 h. After further heating at 135–140° for 1–2 h., the liquid layer is distilled to remove xylene and the residue is washed and dried to give *N*-methyl-*N*-(1-naphthyl) fluoroacetamide, m.p. 87–88°. Mortality tables for housefly, weevil, the peach red aphid, the citrus red mite, the two spotted spider mite, white peach scale and arrowhead scale are given, together with toxicity tests on mice. S. D. HUGGINS.

Cyclopropanecarboxylic acid esters. Sumitomo Chemical Co. Ltd. (B.P. 1,067,939, 10.12.64; Japan 17 & 28.12.63).—The esters, which have excellent knock-down and killing properties against housefly, mosquito, cockroach etc., are prepared by reacting either (*i*) an *N*-(hydroxymethyl)glutarimide with a cyclopropanecarboxylic acid (or ester, halide or anhydride thereof) or (*ii*) an *N*-(chloromethyl)glutarimide with a salt (alkali metal or NH_4) of such an acid. Thus, *N*-(hydroxymethyl)glutarimide is reacted with chrysanthemoyl chloride in a mixture of toluene and pyridine to give an 84% yield of *N*-(chrysanthemoxymethyl)glutarimide, m.p. 85–90°. E. ENOS JONES.

Pesticidal compositions. Montecatini Societa Generale per l'Industria Mineraria e Chimica (B.P. 1,070,026, 15.6.64. It., 20.6.63).—Particularly suitable for application on walls, ceilings, floors and other absorbent surfaces, the pesticidal agent has the formula $RO(R^1O){\cdot}P(S)S{\cdot}CH_2{\cdot}CONH{\cdot}R^2$, where R and R^1 are saturated linear- or branched-chain alkyl radicals of 1–4 C and R^2 is H or R/R^1, and is mixed with a phenolic, terpenic, terpenphenolic, cumaronic, cumaronindenic resin or modified colofonium resin. Thus, the ethylamide of *O*,*O*-dimethyldithiophosphorylacetic acid is mixed with terpenic resin, softening at 92–100°. The mixture is heated to 85–90° until complete melting and homogenisation takes

place, followed by cooling to give a solid, easily breakable mass. The pulverised mixture is mixed with diatomaceous earth and Na oleylmethyltaurinate and the total mixture ground to granules of < 50 micron dia. Activity of the compositions against *Musca domestica* L. DDT-resisting stock is tabulated. S. D. HUGGINS.

[Preparation of] pesticidally-active, phosphorus-containing derivatives of hexahydro-s-triazine. VEB Farbenfabrik Wolfen (Inventors: F. Wolf, S. Heidenreich and M. Born) (B.P. 1,070,689, 16.8.65).—Compounds $[(R^1O)(R^2O)P(:X)SCH_2CH(R^3)CO]_3 \cdot A$, where A is the $\equiv N_3C_3H_6$ triazinyl radical, each R^1 and R^2, which may be the same or different, is a lower alkyl radical, each R^3 which may be the same or different, is a lower alkyl radical or H, and X is O or S, are prepared by reaction, at 20–150°, in the presence of a solvent, e.g., $CHCl_3$, of 1 mol. of a 1,3,5-tris(acryloyl)-hexahydro-*s*-triazine (**I**) (which is readily obtainable from e.g. acrylonitrile and formaldehyde) with 3 mol. of the same or different phosphoric acid esters. Preferably a tertiary amine catalyst and a polymerisation inhibitor, e.g., hydroquinone, are added. The products, e.g., that obtained from 1,3,5-tris(acryloyl)hexahydro-*s*-triazine and *O,O*-Me_2 dithiophosphate, have a good insecticidal activity, e.g., against houseflies (*Musca domestica*), grain weevils (*Sitophilus granarias*), etc., and a good plant compatibility while, at the same time, having a low toxicity for warm-blooded animals. J. M. JACOBS.

Halogeno-substituted diphenylcyclopropanes and their use in combating pests. Monsanto Chemicals (Australia) Ltd. (B.P. 1,070,950, 20.10.64. Austral., 24.10.63).—The 1-(*p*-halophenyl)-1-(*p'*-halophenyl)-2,2-dichlorocyclopropanes (where the halo substituents are Cl, Br or F and may be the same or different) are active against DDT-resistant flies. In an example, a solution of $HgPh(CCl_3)$, $CH_2{:}C(C_6H_4 \cdot Cl\text{-}p)_2$, and benzene is boiled during 36 h, then solid is filtered off. The filtrate is evaporated to give 2,2-dichloro-1,1-di-(*p*-chlorophenyl)cyclopropane, m.p. 131–132° (light petroleum) in 96·4% yield. F. R. BASFORD.

Compounds having insecticidal activity and their preparation. Monsanto Chemicals (Australia) Ltd. (B.P. 1,070,959, 20.10.64. Austral., 24.10.63).—Compounds claimed comprise 2,2-dichlorocyclopropanes substituted in the 1-position by two C_6H_4R-*p* groups wherein R (which may be different) is alkyl of 1–4 C, alkoxy of 1–3 C, or SMe. They are highly effective against *Aedes aegypti*, and *Musca domestica*. In an example, a solution of $HgPh \cdot CBrCl_2$, $CH_2{:}C(C_6H_4OMe\text{-}p) \cdot C_6H_4OEt\text{-}p'$, and benzene is boiled during 10 h, then evaporation of the filtered liquor leaves 1(-*p*-anisyl)-1-(*p'*-phenethyl)-2,2-dichlorocyclopropane, m.p. 114–117°. At a concn. of 0·2 ppm it is 80% lethal to larvae of *A. aegypti*; 1 μg per insect gave 100% kill of *M. domestica*. F. R. BASFORD.

Tetrahalogenoethyl sulphenyl halides. Hooker Chemical Corp. (B.P. 1,072,737, 18.10.63, U.S., 29.10.62. Addn. to B.P. 949,375, 18.3.60).—1,1,2,2-Tetrachloro (**I**)- and 1,1,2,2-tetrabromo-ethylsulphenyl chloride are claimed and are useful as nematocides and as intermediates. As an example of method of prep., $(S \cdot CCl_2 \cdot CHCl_2)_2$ is gassed at −20° with Cl_2, then the mixture is allowed to attain room temp. Distillation affords an isomeric mixture, b.p. 53–57°/0·5 mm, from which 17% of **I** may be recovered by gas chromatographic separation. It is an intermediate for *O,O*-Et_2 *S*-tetrachloroethyl phosphite which at 1% concn. is 100% lethal to *Musca domestica*. F. R. BASFORD.

Phosphorus-containing esters. Farbenfabriken Bayer A.-G. (Inventors: W. Lorenz, C. Fest, I. Hamman, M. Federmann, W. Flucke and W. Stendel) (B.P. 1,072,979, 23.3.66. Ger., 26.6 and 20.10.65).—Esters $R^1O(R^2)P(X)ON{:}C(CN)R^3$ [where R^1 is a straight or branched chain alkyl of 1–6 C that may be mono- or poly-substituted by halogen; R^2 is alkyl, alkoxy, haloalkoxy, or alkylamino of 1–4 C, or a di-(2–8 C)alkylamino, Ph, OPh, cyclohexyloxy or cyclohexyl radical; R^3 is Ph that may be substituted by up to 3 halogen, alkyl, alkoxy or alkylmercapto groups (each of 1–4 C), or NO_2; or is naphthyl or pyridyl; X is O or S] are described which are highly active biocidal agents of low toxicity to warm blooded animals, and are especially useful as insecticides in agriculture. They are made by reacting an ester halide $R^1O(R^2)P(X)Y$ (Y = halogen) with a nitrile $HO \cdot N{:}C(CN)R^3$, either in form of a salt or in presence of an acid binding agent; e.g. by reacting *O,O*-Me_2-thionophosphoric acid ester chloride with the Na salt of α-hydroxyiminophenylacetic acid nitrile to obtain α-*O,O*-Me_2-thionophosphoryl-α-oximino-phenylacetic acid nitrile (n_D^{22} 1·5528). The compounds are effective against plant pests and animal ecto- and endo-parasites. H. L. WHITEHEAD.

Insecticidal and acaricidal compositions. Farbenfabriken Bayer A.-G. (B.P. 1,077,286, 4.3.66. Ger., 4.3.65).—The active compounds in the compositions have the formula $FCH_2CONHCH(CCl_3)OCONR^1R^2$, wherein R^1 is H or an alkyl (1–4 C), optionally substituted by an alkoxy (1–4 C) and/or halogen and R^2 is an alkyl (1–4 C) or Ph, optionally substituted by alkoxy (1–4 C), halogen, NO_2 and/or CN. These compounds possess low phytotoxicity and their action develops rapidly and is long-lasting against aphids and mites. They are prepared as in B.P. 1,077,285 by reacting chloral fluoroacetamide with an isocyanate or a carbamic acid chloride. S. D. HUGGINS.

[Thiocarbonate] acaricides. Stauffer Chemical Co. (B.P. 1,078,241, 26.1.66. U.S., 26.2.65).—Compounds claimed have the formula $2,4,6\text{-}(NO_2)_2 \cdot R^1 \cdot C_6H_2 \cdot O \cdot COSR$ wherein R is alkyl, cycloalkyl, CH_2Ph, or Ph which may contain halogen; R^1 is alkyl of 1–6 C. In an example, NEt_3 is added to a solution of $4,6,2\text{-}(NO_2)_2 \cdot Bu^s \cdot C_6H_2 \cdot OH$ in acetone, followed by $Cl \cdot COSCH_2Ph$, then after 2 h at the boil the cooled mixture is poured into water. Product is extracted with CH_2Cl_2, washed and freed from solvent, to leave 4,6-dinitro-2-s-butylphenyl *S*-benzylthiocarbonate, m.p. 81–85°. F. R. BASFORD.

Phenylmercapto-methanesulphonamide. Farbenfabriken Bayer A.-G. (B.P. 1,068,983, 2.3.66. Ger., 12.3.65).—$CH_2Cl \cdot SO_2NH_2$ is added to a mixture of PhSH, water, and 40% aq. NaOH (at 60°), then after 1 h at the boil the cooled mixture is filtered. Recrystallisation of filter-cake from benzene (+10% EtOH)–light petroleum affords (phenylthio)methanesulphonamide, m.p. 101–103° (50–52% yield), a product with fungitoxic properties which is active against *Phytophthora infestans*, and other phytotoxic fungi. F. R. BASFORD.

Thiuram disulphides. Imperial Chemical Industries Ltd. (Inventors: H. M. Fox and R. Ghosh) (B.P. 1,069,911, 30.1.63).—The claimed fungicides (I) have the formula $R^1R^2N \cdot NR^5 \cdot CS \cdot S \cdot CS \cdot NR^6 \cdot NR^3R^4$, where R^1–R^4 are each alkyl of 1–6 C or R^1 and R^2 and/or R^3 and R^4, together with the adjacent N constitute a 6-membered heterocyclic ring; R^5 and R^6 are each H or alkyl of 1–6 C, provided that when R^5 and R^6 are both H, R^1, R^2, R^3 and R^4 are not all Me. Oxidation of one or more compounds of formula $R^1R^2 \cdot N \cdot NR^5 \cdot CS \cdot SH$ give **I**. Thus, 1,1-Et_2-hydrazine and CS_2 are stirred into cooled water, and heated to 40°, before adding aq. NaOH. The mixture is stirred for 2·5 h at 40°, cooled to 0 to −5° and reacted with aq. NH_4 persulphate, added slowly over 2·5 h. Ice-water is added, stirring continued for 0·5 h and the ppt. filtered, and washed to give *N,N'*-bis(diethylamino)thiuram disulphide, m.p. 145° (MeOH). The **I** are active against e.g. *Piricularia oryzae*, *Phytophthora infestans* and *Botrytis fabae*. S. D. HUGGINS.

Imidazolyl phosphorus compounds. Dow Chemical Co. (B.P. 1,069,240, 28.4.66. U.S., 29.4.65).—Possessing high fungitoxicity and low mammalian toxicity, the claimed compounds are obtained by reacting a phosphorothioic chloride (**I**) $(R)R^1 \cdot PS \cdot Cl$, [where R can be Ph, styryl, an N-heterocyclic saturated radical (e.g. morpholino), an N-heteroaromatic or substituted amino radical; R^1 = R or is dialkylamido, or R + R^1 form an N-heterocyclic group] with an imidazole compound in an HCl acceptor, (a t-org. amine) at 25–60°. Thus, imidazole is added, over 4·5 h, to Ph_2-**I** in benzene at 25–28° and the mixture stirred at 24–26° for 12 h. The imidazole hydrochloride is filtered off, washed with benzene and the filtrate and washings concentrated to give imidazol-1-yl diphenylphosphine sulphide, m.p. 98–103°, in 89·5% yield. The compounds are active against e.g., cherry leaf spot, apple scab, rice blast, powdery mildew. S. D. HUGGINS.

Trichlorodinitrophenyl-heterocyclic ring compounds. Fisons Pest Control Ltd. (Inventors: A. J. Lambie, G. T. Newbold and M. B. Purdew) (B.P. 1,069,991, 11.1.64).—The claimed fungicides are prepared by reacting 1,2,3,4-tetrachloro-5,6-dinitrobenzene (**I**) with a cyclic s-amine (other than pyrrolidine or ethyleneimine) at > 50°. Thus, morpholine is added to a suspension of **I** in EtOH, the mixture is heated on a steam bath until clear, and after standing for 2–3 h, a bright orange solid crystallises out to give a 56·1% yield of impure 4-(trichloro-*o*-dinitrophenyl)morpholine which melts at 123–124° after recrystallisation from EtOH. The compounds are active against e.g. *Alternaria solani* and *Botrytis fabae*. S. D. HUGGINS.

1,1,2,2-Tetrahaloethyl alkanethiolsulphonates. Chevron Research Co. (B.P. 1,070,640, 1.4.66. U.S., 1.4.65).—Used as fungicides, the title compounds have the formula $RSO_2SCCl_2CCl_2H$ where R is an alkyl of 1–4 C. As an example of prep., a mixture of Me disulphide and glacial AcOH is cooled to 23–32°F and stirred continuously while Cl_2 is added over 1·5 h, stirred for 0·5 h at 32°F, warmed to room temp. and stirred for another 0·5 h. The

mixture is then cooled to 23°F and 1,1,2,2-tetrachloroethyl sulphenyl chloride added very rapidly at 23°F. Water is added, dropwise, over 0·5 h at 23–32°F, followed by 0·75 h stirring at 32°F and 3 h stirring as the temp. rises to room temp. The mixture is then stripped under vac. to 160–170°F, leaving a residue of crude 1,1,2,2-tetrachloroethyl methanethiolsulphonate (**I**) (99·4% yield). This is purified by dissolving in mixed hexane–$Pr^{i}OH$, stirring at 50° and cooling to −15°, adding a seed crystal at 20°, and filtering to give pure **I**, m.p. 44·5–46°, b.p. 110–113°C. S. D. HUGGINS.

[A] **2,6-Dinitro-4-alkyl phenols and their carbonates.** [B] **Dinitrophenol carbonates.** Murphy Chemical Co. Ltd. (Inventors: M. Pianka and J. D. Edwards) (B.P. 1,070,755–6, 7.8.64 and [A] 14.7.65).—[A] Used as pesticides, the claimed compounds of formula $4,2,6\text{-}R^{1}(NO_2)_2\cdot C_6H_2\cdot OCO_2R$ (where R^1 is 1-Et-n-hexyl or other C_7-C_{11} alkylidene group and R is lower alkyl) are prepared from the corresponding phenol or phenate (also claimed) and a haloformate (see [B] for similar method of prep. of the *o*-alkyl isomers).

[B] The claimed pesticides have the formula $6,2,4\text{-}R^{1}(NO_2)_2\cdot C_6H_2OCOOR$ (where R is alkyl of 1–6 C and R^1 is 1-Et-n-hexyl or 1-Pr^{n}-n-pentyl) and are prepared from the corresponding phenol or phenate (also claimed) and a haloformate in presence of an acid binding agent and in a (ketone) solvent. Thus, 2-(1-ethyl-n-hexyl)-4,6-dinitrophenol, K_2CO_3 and Me_2CO are refluxed for 0·5 h and $Cl\cdot CO\cdot OEt$ then added and refluxed for 2·75 h. The KCl ppt. is filtered off, Me_2CO is removed and the residue dissolved in benzene and worked up to give 2-(1-ethyl-n-hexyl)-4,6-dinitrophenyl ethyl carbonate as a yellow oil. It is found to have a 100% kill against *Tetranychus telarius* at 300 and 100 ppm, a 97% kill at 30 ppm and a 77% kill at 10 ppm, while a 95% cucumber mildew eradication occurs at 50 ppm. S. D. HUGGINS.

Combating soil-dwelling fungi and nematodes. Nihon Nohyaku K.K. (B.P. 1,074,136, 7.7.64. Jap., 9.7.63).—The claimed composition contains 1 pt. by wt. of 2,3-dibromopropionitrile (**I**) and 0·1–10 pt. by wt. of 1,2,2-trichloronitroethylene (**II**) or 95–1 pt. of **I** and 5–99 pt. of **II**, together with an org. solvent or solid carrier and an emulsifying agent. Thus **I** (2), **II** (4), diatomaceous earth (10), and clay (84 pt.) are ground together to make a dust. The first example describes an azeotropic mixture, b.p. 65–80°/33 mm Hg, containing **II**, and prepared by nitrating C_2HCl_3, which is useful on its own as a soil disinfectant. S. D. HUGGINS.

Aryl 2-cyanoallyl-dithiocarbamates. Shell Internationale Research Mij N.V. (B.P. 1,074,764, 5.8.65. U.S., 8.8.64).—Fungicidal compositions contain the active compounds of formula $CH_2\cdot C\cdot CN\cdot CH_2\cdot NH\cdot C(S)SR$, where R is an aryl group of 6–14 C, optionally substituted with one or more Br or Cl, alkyl or alkoxy groups of 1–5 C, OH, NO_2, NH_2, monoalkylamino groups of 1–4 C or dialkylamino groups of 2–8 C. A 2-cyanoallylisothiocyanate is reacted with the appropriate (optionally substituted) aryl mercaptan; thus in the prep. of *p*-chlorophenyl (2-cyanoallyl)-dithiocarbamate (**I**), m.p. 146–147°, 2-cyanoallylisothiocyanate is added to *p*-chlorobenzenethiol in Et_2O and C_5H_5N, and stirred at 20–35° for 1 h. The mixture is filtered and **I** recrystallised from MeOH in 80% yield. S. D. HUGGINS.

Fungicidal compositions. Imperial Chemical Industries Ltd. (Inventors: J. T. Braunholtz and P. F. H. Freeman) (B.P. 1,074,803, 19.2.63).—The claimed 3-substituted-4-ylidene-isoxazol-5-ones are obtained by reaction of an isoxazol-5-one with the appropriate aldehyde or ketone. As an example, wheat-seeds are dusted at the rate of 1000 ppm with a composition containing 25% by wt. of 4-furfurylidene-3-methylisoxazol-5-one and 75% China clay. The seeds are then sown in soil inoculated with 2% by wt. of a soil/cornmeal culture of *Fusarium culmorum*. After 7 days the seedlings emerging are compared with untreated seeds to show that 84% of the treated seeds germinated compared with 45% in the untreated seeds. After 18 days, 30% of the treated seeds gave healthy seedlings compared with 3% of untreated seeds. S. D. HUGGINS.

Salicylaldimino derivatives. Pechiney-Progil (B.P. 1,075,582, 16.2.65. Fr., 18.2. and 29.12.64).—Fungicidal compositions contain the Schiff's bases and their chelates, of formula $3,5,2\text{-}X_2\cdot OH\cdot C_6H_2\cdot CH{:}NROH$ (X is halogen, R is alkylene, hydroxyalkylene or dihydroxyalkylene of 2–10 C and the chelates contain Cu, Zn, Fe, Mn or Ni). In an example, 3,5-dichlorosalicylic aldehyde (prepared by chlorinating salicyclic aldehyde in AcOH at 100°) is dissolved in boiling EtOH, $NH_2\cdot CH_2CH(OH)CH_3$ is added and the mixture boiled for 2 h. After removing EtOH and cooling, a 65% yield of *N*-(2-hydroxy-3,5-dichlorobenzylidene)-1-aminopropan-2-ol, m.p. 82° (EtOH) is recovered. The Cu chelate (m.p. 222%) can be obtained in 98% yield by adding conc. aq. $Cu(OAc)_2$ to the base in anhyd. EtOH, the ppt. being dried, without heating, and washed with water and with EtOH. The compounds are active against e.g. *Alternaria solani* and *Erysiphe graminis*. S. D. HUGGINS.

***N*-(Thiocarbamoylthio)-imides.** Roussel-Uclaf (B.P. 1,077,180, 22.12.65. Fr., 22.12.64 and 1.10.65).—Antifungal properties are exhibited by the cyclic $Z(CO)_2{:}N\cdot SC(S)NR^{1}R^{2}$ wherein Z [which together with $(CO)_2$ and :N forms an imide ring] is an org. divalent aliphatic group, $-(CH_2)_{n}-$, *n* being 1–4, an alicyclic group $(CH_2)_n{:}(CH\cdot)_2$, *n* being 2–6, a divalent alicyclic group of the bicyclo[2,2,1] heptane or bicyclo[2,2,1] heptene structure, an *o*-phenylene deriv. or a divalent heterocyclic radical of *o*-configuration, all divalent groups being optionally substituted; R^1 and R^2 are lower alkyl, alkenyl, aralkyl or aryl radicals or with the amino-N may form a heterocyclic ring optionally containing a further hetero atom. In an example, Na dimethyldithiocarbamate in Me_2CO is mixed with *N*-bromosuccinimide and stirred for 2 h at 20°; the mixture is worked up to give *N*-(dimethylthiocarbamoylthio)-succinimide, m.p. 166–167° (Me_2CO, then MeOH). The products are active against e.g. *Alternaria oleracea*. S. D. HUGGINS.

Pyridyl amides. Mobil Oil Corp. (B.P. 1,078,288, 22.3.66. U.S., 29.3.65).—Compounds with systemic fungicidal activity have the formula $R^{1}NRC(:Z)A$, wherein A is NR_2 or non-aromatic branched-chain hydrocarbon radical; R is H or non-aromatic hydrocarbon radical; Z is O or S; and R^1 is pyridyl which is substituted (on a C not adjacent to the amide group) by 1–3 halogen, or alkoxy of 1–8 C and optionally further by 1–2 OH, halogen, NO_2, alkyl of 1–4 C, alkenyl of 2–4 C, alkoxy of 1–8 C, NR''_2 (R'' is H or alkyl), $CONH_2$, SR'', CN, CNS or SCN. In an example, a mixture of 5-chloro-2-aminopyridine, MeNCO and toluene is kept at room temp. during several days in presence of $SnBu_2(OAc)_2$, then solvent is removed/<1 atm. The residue is slurried with boiling benzene, then filtered off, washed, and dried, to give 1-(5-chloropyrid-2-yl)-3-methylurea, m.p. 195–197° from MeOH. Its effectiveness in the control of *Puccinia rubigo-vera* (leaf rust) on wheat is described. F. R. BASFORD.

Preparation of *N*-aryl substituted amides. Chemical Investors, S.A. (B.P., 1,065,532, 9.3.64. U.S., 15.3. and 8.5.63).—The title compounds are produced by heating $R^{I}\cdot CO_2R^{II}$ with NH_2R in presence of alkali metal alkoxide in a mutual solvent (R is aryl or alkaryl which may contain alkoxy or halogen, R^1 is alkyl, cycloalkyl, alkenyl, aryl, alkaryl, or aralkyl; and R^{II} is alkyl of 1–3 C). E.g., a mixture of $Cl[CH_2]_2\cdot CH(OMe)\cdot CO_2Me$, benzene, and NaOMe (freshly prepared) is boiled during 36 h, cooled, and water added to dissolve salts. The org. phase is filtered, treated with Br_2 in $CHCl_3$ (to remove olefinic material), then distilled, to give Me 1-methoxycyclopropane-1-carboxylate, b.p. 77–80°/43 mm. This is then reacted with $1,3,4\text{-}NH_2\cdot C_6H_3Cl_2$ in benzene in presence of NaOMe during 12 h at the boil, to give 1-methoxycyclopropane-1-carb-3′,4′-dichloroanilide, m.p. 93–94°. Some of the products are herbicides; others are polymer intermediates. F. R. BASFORD.

[Herbicidal] 4-hydroxybenzonitrile derivatives. May and Baker Ltd. (Inventors: B. J. Heywood and W. G. Leeds) (B.P. 1,067,031–3, 24.9.62, [A] 9.1.63, [A and C] 28.3.63).—[A] The claimed compounds are benzonitriles substituted in the 2- and 6-positions by H or halogen, in the 3-position by halogen, in the 4-position by RO, where R is halogen, an acyl or an alkoxycarbonyl group, and in the 5-position by H, halogen, or $-NO_2$. Thus, an aq. suspension is prepared by grinding 3-chloro-5-bromo-4-hydroxy benzonitrile (**II**), Texofor FX170 and water in a ball mill. The diluted concentrate is applied to wheat crops in the 4-leaf stage at a rate of 0·56 kg of **II**/ha to control e.g. *Sinapis arvensis*, *Chenopodium album*, and other broad-leafed weeds. There are 93 claims and 27 examples.

[B] Benzonitriles, substituted in the 3- and 5-position by identical halogen and in the 4-position by OR, where R is H or Ac (e.g. 3,5-dibromo-4-acetoxybenzonitrile) are claimed as herbicides against broad-leafed weeds, as in [A]. (71 claims, 19 examples).

[C] Benzonitriles of formula $3,5,4\text{-}(X_2)RO\cdot C_6H_2\cdot CN$, where each X is identical halogen and R is alkoxycarbonyl, or acyl other than Ac [e.g. 3,5-di-iodo-4-(α,α-dichloropropionyloxy)benzonitrile] are claimed as herbicides, as in [A] and [B]. (72 claims, 38 examples). S. D. HUGGINS.

Halogenated alkoxyalkoxybenzonitriles. May and Baker Ltd. (Inventors: R. F. Collins and B. J. Heywood) (B.P. 1,067,034, 23.9.64).—Useful as selective herbicides, the title compounds are halogenated benzonitriles of formula $R^{1}OCHR^{2}O\cdot C_6H_2\cdot(X^{1})(X^{2})Cl$, where R^1 is a straight or branched chain alkyl or alkenyl group

with 1–18 C, R^2 is H or as R^1, X^1 is halogen and X^2 is halogen or $-NO_2$. The metallic salt of a phenol of formula $MO \cdot C_6H_2 \cdot (X^1)(X^2) \cdot CN$ (M is an equiv. of a metal) is reacted with an alkoxyalkyl halide R^1OCHR^2Y (Y=halogen). Thus, monochlorodimethyl ether is added to the Na salt of 3,5-di-iodo-4-hydroxybenzonitrile dissolved in tetramethylenesulphone at 50° and the mixture heated for 1 h at 100° to yield pure 3,5-di-iodo-4-methoxymethoxybenzonitrile, m.p. 143–145° (light petroleum).

S. D. HUGGINS.

Preparation of herbicide mixtures from urea, biuret or their chemical equivalents. Ministerul Industriei Petrolului si Chimiei (B.P. 1,069,736, 12.10.64. Rumania, 12.10.63).—Urea or biuret (or chemical equiv. thereof) is heated at <400° in an inert solvent while simultaneously chlorinating (with HCl), then the product is treated under alkaline conditions at 0–70° in presence of a surface-active agent (alkylaryl sulphonate) with an alkylamine, to give a herbicidal product. E.g., HCl is passed during 3 h at 170° into a suspension of urea, petroleum, b.p. >220°, and 2% of $ZnCl_2$ to 30% Cl content. The resulting solid is dispersed in water in presence of Na butylnaphthalene sulphonate (**I**), then a 50% solution of NH_2Et (enough to react with all the Cl) is added; the pH is kept at 10–11 with 40% aq. NaOH. After 2 h at 40° product (**II**) is filtered off, washed with water, then dried at 105°, and admixed with **I** and $CH_2(C_{10}H_6 \cdot SO_3Na)_2$, to give a composition containing 50% of **II**, characterised by high activity against *Avena fatua* and *Sinapis arvensis*. F. R. BASFORD.

Derivatives of 1,10-phenanthroline. Monsanto Chemicals (Australia) Ltd. (B.P. 1,072,099, 10.9.64. Australia, 12.9.63).—Compounds claimed include 3,4-dimethyl-1,2,3,4-dihydro-1,10-phenanthroline and deriv. in which the 5- to 9-positions are substituted by Me or Et. They are formed by a variety of methods and are useful as selective herbicides or as intermediates in the prep. of the parent 1,10-phenanthroline (**I**). E.g., a solution of 8-amino-3,4-dimethylquinoline, AcOH, and $Ac \cdot CHMe \cdot CH_2OH$ is stirred at 95–100° during 1 h, then diluted with water and extracted with benzene. The extract is chromatographed on Al_2O_3, and the recovered crude oily product is further purified via its picrate, to give 3,4,7,8-tetramethyl-1,2,3,4-tetrahydro-1,10-phenanthroline, m.p. 72–72·4° (from MeOH). This is converted into **I** in 97% yield by boiling it with conc. HCl and 80% arsenic acid for 7·5 h.

F. R. BASFORD.

Herbicidal mixtures containing half esters of substituted ureas [and their use]. Badische Anilin- & Soda-Fabrik A.-G. (Inventors G. Steinbrunn, A. Fischer, G. Scheuerer and H. Stummyer) (B.P. 1,074,333, 13.10.64. Ger., 18.10.63).—Improved agents for effecting the selective control of unwanted vegetation between crop plants (especially cotton plants) contain, as active ingredient, one or more half esters formed by reacting a di- or tetra-carboxylic acid anhydride (maleic or succinic anhydride) with a urea $X_n \cdot C_6H_{5-n} \cdot N[CON(R)Me] \cdot Y$, where X = H, halogen or alkyl (1–4 C); $n = 1$ or 2 (where $n = 2$, the X may be identical or different); Y = β-hydroxypropyl; R = Me, MeO or isobutinyl. The half esters can be used as such, or as their salts, together with diluent, emulsifier, etc. H. L. WHITEHEAD.

[Herbicidal] aromatic N-containing compounds. Monsanto Co. (B.P. 1,078,071, 20.8.64; U.S., 22.8 and 9.12.63; Addition to B.P. 1,008,851).—The compounds **I** have the formula: $R \cdot N(COR^5)\text{-}CH(X^1)R^4$. R is an aromatic radical substituted in both *o*-positions and in one of these by a t-alkyl group, R^4 is H or a hydrocarbon radical which may contain OH or a halogen, R^5 is a hydrocarbon with 1–18 C which may contain a halogen and X^1 is a halogen. **I** are produced by reacting an aromatic imine with an acyl halide. The compounds are useful as herbicides. An example is 2-bromo-*N*-bromomethyl-2′-t-butyl-6′-methyl acetanilide, m.p. 103–107°, prepared by adding 2-t-butyl-6-methyl-*N*-methyleneaniline to a mixture of bromoacetyl bromide and n-heptane over 15 min. and boiling the resulting mixture for 5 min.

E. ENOS JONES.

[Production of] urea derivatives and herbicidal mixtures containing them. Badische Anilin- & Soda-Fabrik A.-G. (Inventors: G. Steinbrunn, E. Flickinger and A. Fischer) (B.P. 1,078,209, 24.11.64. Ger., 27.11.63).—Compounds $X_n \cdot C_6H_{5-n} \cdot NHCON(Me)OCH(Me)C{\equiv}CH$ in which X is chlorine or alkyl, n is 0, 1 or 2 (when n is 2 the X may be identical or different) have excellent selective herbicidal activity and are prepared by conversion of the corresponding isocyanates with MeNHOH to hydroxyureas and reaction of these with halogenobutyne-1. E.g., $PhNHCON(Me)OCH(Me)C{\equiv}CH$ is obtained by treating PhNCO with MeNHOH, separating the resulting *N*-phenyl-*N*′-methyl-*N*′-hydroxyurea and reacting it with 2-chloro-butyne-1 in alcoholic NaOH solution. J. M. JACOBS.

[Preparation of] herbicidal products derived from urea. Pechiney-Progil (B.P. 1,078,537, 23.10.64. Fr., 25.10.63).—The herbicides comprise a benzoyl (**I**)-urea of which the N atom carrying the **I**-group also carries an optionally substituted phenyl radical, while one or both H on the second N atom is replaced by an alkyl, alkoxy, alkenyl or alkynyl group $C_{<5}$, the ring of the **I**-group being itself optionally substituted by one or more halogens, alkyl, alkoxy, alkenyl, alkynyl, CN, NO_2, or amino groups e.g., an *N*-(3,4-dichlorophenyl)- *N*′,*N*′-dimethyl- (or diethyl-) urea carrying a **I** or a chloro,- nitro-, methoxy-, ethoxy-, dimethoxy-, trimethoxy-, 2-methyl-4-methoxy- or 3-methyl-4-methoxy-**I**-group on the N atom. This is prepared by condensing an amine with a phenyl isocyanate to form a substituted phenylurea, which is then treated with a **I**-chloride (carrying the desired substitutions) in presence of an HCl acceptor (e.g., dimethyl-, trimethyl- or diethylamine, morpholine or pyridine). The **I**-urea is applied at the rate of 0·5–20 kg per ha. J. M. JACOBS.

[Preparation of] *N*, *N*′, *N*′-trisubstituted urea derivatives and herbicidal compositions containing them. Badische Anilin- & Soda-Fabrik A.-G. (Inventors: G. Steinbrunn, A. Fischer and E. Flickinger) (B.P. 1,079,205, 3.12.64. Ger., 3.12.63 and 4.7.64).—Selective herbicides which have a good effect against broad-leaved weeds and grasses in beet, wheat, barley, maize, rice, etc., have the formula $R^2SCH_2 \cdot X \cdot C_6H_3NHCON(Me)R^1$ in which R^1 is Me, MeO or isobutynyl, R^2 is an alkyl of 1–4 C, optionally OH-substituted, an alkenyl radical C_{3-4} bearing 2 or 3 Cl atoms as substituents or the 2, 3, 6-trichlorobenzyl radical, X is H or Cl and the R^2SCH_2- and X-groups are in the *m*- or *p*-positions, relative to the amido group, e.g., *N*-[4-(2′3′3′-trichloroallylthiomethyl-3-chlorophenyl]-*N*′-methyl-*N*′-isobutynyl (or -methyl or -methoxy)-urea. They are prepared from the appropriate substituted ureas (obtained from the corresponding substituted phenylisocyanates or chlorophenylisocyanates, or their carbamyl chlorides, by reaction with dimethylamine, methylmethoxyamine or methylisobutinylamine) by heating them with an alkyl, hydroxy-alkyl, chloroalkenyl or trichlorobenzylmercaptan (or metal salt) in the presence of an acid-binding substance. J. M. JACOBS.

Animal Husbandry

Production of fodder yeast on molasses mash with regard to colloidal phenomena in foam prevention. D. Jocic and K. Osvatic (*Kemija Ind.*, 1967, **16**, 127–130).—The influence of colloidal particles of molasses on foam formation and the colouring of yeast, as well as their influence on the growth of biomass in the course of fermentation of natural mash is described. The growth of the yeast biomass in a continuous process, by addition of mineral salts and molasses as stimulants for the growth is also described. (12 references.) T. M. BARZYKOWSKI.

Nutritive value of nitrogen-fertilised orchard-grass pasture at different periods of the year. R. L. Reid, G. A. Jung and C. M. Kinsey (*Agron. J.*, 1967, **59**, 519–525).—In trials with cut orchard-grass herbage fed *ad lib.* to sheep indoors, the level of N treatment of the sward (56–504 kg per ha) in March had significant effects on protein digestibility, but little effect on intake and digestibility of dry matter and cellulose. Consumption of the harvested grass was higher in June than in May or Sept., whilst consumption of frost-killed grass cut in March was markedly the lowest. Consumption of orchard-grass by grazing sheep was related to the level of N fertilisation of the sward, increased consumption due to applied N being most evident in the March and May growth periods. N fertilisation also improved the relative acceptance of herbage.

A. H. CORNFIELD.

Applicability of chromogen and nitrogen as internal indicators of forage digestibility. L. E. Davis, G. C. Marten and R. M. Jordan (*Agron. J.*, 1967, **59**, 544–546).—The digestibility values of the org. matter of three species of forage by sheep were compared using the conventional total collection method as control, the chromogen ratio method, the chromogen-fecal index method, and the N fecal index method. None of the indicator methods were useful for predicting absolute values of org. matter digestibility of lucerne, bromegrass, and reed canary-grass. The chromogen methods gave low values for lucerne, and the N fecal index method showed low values for reed canary-grass. A. H. CORNFIELD.

Insecticides for control of cattle tick and southern cattle tick on cattle. R. O. Drummond, S. E. Ernst, J. L. Trevino and O. H. Graham (*J. econ. Ent.*, 1968, **61**, 467–470).—Over half the 30 insecticides tested as sprays against *Boophilus* spp. gave >99%

control of viable egg production. Of the three used as dips, Dursban gave good control throughout 42 weeks.
C. M. HARDWICK.

Effect of topdressed nitrogen and potassium on the feeding value of orchard-grass hay for lactating dairy cows. F. A. Martz, J. R. Brown, B. K. Das and D. D. Padgitt (*Agron. J.*, 1967, **59**, 599–602).—Daily intake of orchard-grass hay fed *ad lib.* to dairy cows was significantly less where N (112 kg) + P (99 kg) + K (186 kg per ha) had been applied to an established sward than where (N or P) + K or N + P had been applied. There were no significant differences in fat-corrected milk production due to the hays from the four fertiliser treatments. Top-dressing with NPK nearly tripled hay yield and quadrupled crude protein yield compared with the P treatment. A. H. CORNFIELD.

Rearing of dairy calves on pasture with or without whole oats. A. D. L. Gorrill (*Can. J. Anim. Sci.*, 1967, **47**, 211–216).—Groups of bull calves weaned at 55 kg body wt., were put on pasture at age 2 weeks or at 15 weeks old. Half of each group received whole oats (max. 1·4 kg/day) while on pasture. At 21 weeks of age the calves were taken off pasture and fed hay and grain until 52 weeks old. Calves put on pasture after weaning gained 223 g/day until 15 weeks old; those put on pasture at 15 weeks old gained 177 g/day on average from weaning to 21 weeks. Feeding oats to calves on pasture did not increase growth until weaning or at 15 weeks of age. Average body wt. at 52 weeks of age were 251, 228 and 242 kg for groups started on pasture at 2 weeks, after weaning and at 15 weeks of age, respectively. A. G. POLLARD.

Subsequent performance of bull calves wintered on two planes of nutrition and two feeding methods. J. E. Lawson (*Can. J. anim. Sci.*, 1967, **47**, 181–186).—Bull calves given a high-plane ration (*HP*) (grain : hay, 2 : 1) for 168 days were compared with those on a low-plane (*LP*) ration (hay only) for a similar time. Each group was further divided according to the manner of feeding, (*i*) individually or (*ii*) as a group. After 168 days the animals fed individually showed average daily gains in wt. (*DG*) 2·1-times greater than did those of corresponding group-fed animals. After a further period of 168 days at pasture the *DG* of the calves previously given the *LP* ration was 1·7 times greater than those previously given the *HP* ration individually and 1·9-times greater than when group-fed. The lower gains during the initial period on *LP* than on *HP* rations were not off-set by the greater gains during the subsequent grazing. The consumption of total digestible nutrients per unit gain in wt. of calves fed the *HP* ration was ~75% of that in those fed the *LP* ration. A. G. POLLARD.

Effect (nil) of weekly omission of one meal on performance of fattening calves. J. Ladrat and W. Joussellin (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1968, **54**, 376–379).—The omission had no significant effect on performance. P. S. ARUP.

Algebraic model of the lactation curve in cattle. P. D. P. Wood (*Nature, Lond.*, 1967, **216**, 164–165).—The general equation for the usual γ-type curve is converted algebraically into a simple linear regression model, from which various properties can be quickly deduced, e.g., relations between persistency (maintenance of peak yield), total yield and level of production. The regression curve fitted to a single Friesian lactation is developed and results are applied to random sampling of 524 Friesian heifer lactations. Such a statistical analysis permits differentiation between progeny groups and between herds, although differences in persistency are less apparent. Lactation curves of eight bull progeny groups are also shown and discussed briefly. W. J. BAKER.

Moisture content as a basis for estimating fatness of boneless beef samples. R. J. Forrest (*Can. J. Anim. Sci.*, 1967, **47**, 153–159).—Data obtained from boneless, specific carcass cuts (rib, chuck and round sections) from Holstein–Friesian steer carcasses were were utilised to establish the relationship:

$$\% \text{ Fat} = 100 - \left[\frac{100}{\% \text{ fat-free moisture}}\right] \times \% \text{ moisture}$$

The values obtained by this means agreed very closely with those determined by ether extraction (Soxhlet). A. G. POLLARD.

Consumption and digestibility of crown vetch, *Coronilla varia*, forage by sheep. P. J. Reynolds, C. Jackson, jun., I. L. Lindahl and P. R. Henson (*Agron. J.*, 1967, **59**, 589–591).—Crown vetch soilage cut in the early bloom stage was consumed as readily by sheep as was regrowth ladino clover–orchard-grass soilage. Ground, pelleted, crown vetch cut in July in the seed stage was poorly digested. Gross energy digestibility and voluntary consumption of chopped, frozen crown vetch cut in early bloom were similar to published values for dehydrated early bloom lucerne, birdsfoot trefoil, and red clover. A. H. CORNFIELD.

Energy metabolism and body composition of weanling pigs. G. M. Jenkinson, L. G. Young and G. C. Ashton (*Can. J. Anim. Sci.*, 1967, **47**, 217–226).—In two trials weanling pigs were fed a practical diet in different proportions varying from maintenance level to *ad lib.* The relationship between energy retention and energy intake was linear above maintenance level. Digestible energy was the best measure of dietary energy utilisation owing to its low variability and its ease of determination. The crude fat and moisture contents of carcasses showed a negative linear relationship. The daily gain in wt. at energy equilibrium by both groups of pigs was 0·09 kg. Pigs required 166·83 $W_{kg}^{0.75}$ kcal. and 137·41 $W_{kg}^{0.75}$ kcal of metabolisable energy per day to maintain energy equilibrium in the two groups respectively.
A. G. POLLARD.

Raw soyabeans in swine growing-finishing rations. L. G. Young (*Can. J. anim. Sci.*, 1967, **47**, 227–234).—The effects of replacing the protein from 44% of soyabean meal (**I**) in pig rations by that from raw ground soyabeans (**II**) on the live performance and carcass characteristics are examined. With **II** at 10·8, 16·5 and 22·15% in the growers rations, growth rates and feed efficiency were lower than with the **I** control ration. In the finishing ration replacement of the protein of **I** by that of **II** produced similar though less consistent effects. With a growers ration containing 3·65% of **II** pelletting improved feed efficiency. The area of the loin eye muscle tended to become smaller with increase in the level of **II** in the ration. Other carcass characteristics were unaffected by replacement of **I** by **II**. A. G. POLLARD.

Studies on the diet of the grazing animal. III. Effect of pasture species and pasture structure on the herbage intake of sheep. G. W. Arnold and M. L. Dudzinski (*Aust. J. agric. Res.*, 1967, **18**, 657–666).—In general, where there was a limited range of total dry matter per acre, this variable and *in vitro* diet digestibility, were the major contributors to variation in digestible org. matter intake. Over a wider range, *in vitro* diet digestibility was replaced by green dry matter per acre or mean leaf length of green herbage. (11 references.) E. G. BRICKELL.

Physiological responses in sheep during eating and the energy cost of eating. B. A. Young (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 114).—Increased energy expenditure (*EE*) during the act of eating ranged from 5·3 to 12·4 cal/min/kg. live wt. equivalent to 2% of the daily *EE* of a penned sheep receiving a maintenance ration. There was also a 30 to 70% increase in heart rate which followed a similar pattern to the rate of *EE*. Heart-rate cannot, however, be used as an accurate index of *EE* because of a large standard error associated with individual regression equations. E. G. BRICKELL.

Utilisation of low-quality roughage. J. Z. Foot (*J. Aust. Inst. agric. Sci.*, 1967, **33**, 113).—Three experiments with Corriedale wethers are described. (*i*) Addition of ethanol to an *ad lib.* oat-straw diet had no advantages. (*ii*) The voluntary intake of feed of lucerne hay was increased when the diet was ground and pelletted and the N balance of animals on this diet was higher despite a decrease in digestibility of the roughage. With oat-straw the volatile fatty acid of the rumen contents was higher for pelletted than for chopped feed. (*iii*) Preference was shown for chopped lucerne hay over chopped oat-straw by sheep but not by red kangaroos. The latter maintained wt. on the poor quality diet and appeared to be as well adapted to utilise this type of roughage as were the sheep. E. G. BRICKELL.

Pharmacology and toxicology of carbon tetrachloride in the sheep. IV. Reduction and augmentation of toxicity by selenium. A. C. Kondos and G. L. McClymont (*Aust. J. agric. Res.*, 1967, **18**, 667–682).—Susceptibility to toxic effects of CCl_4 was increased by oral administration of 1 mg Se as $NaSeO_3$ plus 100 I.U. of tocopherol acetate on alternate days for 120 days, 2 mg Se per day for 21 days, and 6 mg Se per day for 6 days, before dosing with CCl_4. Susceptibility was reduced by smaller total amounts of Se, and by oral doses of 6 mg Se per day for 3 days, a single oral or intramuscular dose of 5 or 12 mg about 20 h, or 5 to 12 mg orally 10–20 min., before dosing with CCl_4. Administration of Se to fluke-infested sheep did not protect the liver fluke (*Fasciola hepatica*) against CCl_4. Administration of Se to sheep dosed with CCl_4 and then given a high protein diet greatly reduced mortality. (36 references.) E. G. BRICKELL.

Interaction effect of nitrogen storage with specific strains in the hatchability of turkey eggs. G. W. Friars and J. Singh (*Can. J. anim. Sci.*, 1967, **47**, 186–191).—Fertile eggs from each of three strains of turkeys were subjected to (*a*) refrigerated storage or (*b*) refrigerated storage in N_2 in closed containers. The hatch-

ability of eggs from a specific strain of birds was improved by storage in N_2, to extents which were not consistently related to the general level of hatchability of that strain. The influence of N_2 on the preservation of embryonic activity was greater in the second than in the first week of storage and appeared to be enhanced when other environmental factors tended to restrict the viability of the embryos. A. G. POLLARD.

Identification and quantitative evaluation of antibiotic residues in chickens fed on a spiramycin diet. G. R. Jolles and B. L. Terlain (*J. agric. Fd Chem.*, 1968, **16**, 60–64).—Extracts of chicken tissues were made with benzene, CH_2Cl_2, MeOH and water in succession. The residues obtained after evaporation of solvent were chromatographed on silica gel columns with MeEtCO as the solvent system in which the % of MeOH was gradually increased from 0 to 100. The eluates were examined by TLC. Residues of spiramycin (0·1 ppm) were found only in the liver of chickens fed with 10 g of spiramycin embonate per ton of feed. At a dose level of 100 g/ton, spiramycin and its metabolites appeared in small amounts in all the tissues. P. S. ARUP.

Effects of excess dietary iodine upon pullets and laying hens. L. R. Arrington, R. A. Santa Cruz, R. H. Harms and H. R. Wilson (*J. Nutr.*, 1967, **92**, 325–330).—I_2, as KI, was fed to sexually mature pullets and hens that had completed one year of lay. Five levels of I_2 were used from zero to 5000 ppm. Egg production decreased with increasing I_2 and ceased at the highest level. Decreases were greater for hens than for pullets. Production commenced, and increased rapidly within one week after change to control diets. Moulting did not accompany cessation in laying although some mature hens did moult. Mature ova were present but ovulation did not occur. Wt. of eggs was reduced during I_2 feeding but returned to normal within 3 weeks of cessation; fertility of eggs was not affected but high embryonic death, low hatchability and delayed hatching were found. C.V.

Method of analysing milk and faeces of cows for coumaphos and its oxygen analogue after feeding coumaphos for control of housefly larvae. M. C. Bowman, M. Beroza, C. H. Gordon, R. W. Miller and N. O. Morgan (*J. econ. Ent.*, 1968, **61**, 358–362).—Separation of the residues from faeces and milk was by liquid chromatography on silica gel followed by hexane–acetonitrile partition and gas chromatography. The level of residues was determined by a P-sensitive flame photometric cell, sensitive to ~0·003 ppm for milk and 0·05 ppm for faeces. Typical chromatograms are given. Potosan [*O,O*-diethyl *O*-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)-phosphorothioate] was found in faeces and was probably a metabolite of coumaphos. (34 references.) C. M. HARDWICK.

Dust stations for control of the face fly in South Carolina. J. A. Seawright and T. R. Adkins, jun. (*J. econ. Ent.*, 1968, **61**, 504–505).—In order to provide frequent treatment, self-dusting applicators containing Dimetilan, ronnel [fenchlorphos], coumaphos, malathion and SD 8447 [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate], were installed. All treatments gave 54–85% reduction of *Musca autumnalis*. (21 references.) C. M. HARDWICK.

Evaluation of Shell SD 8447, SD 8448, and SD 8436 as candidate systemic insecticides for control of common and northern cattle grubs. W. M. Rogoff, A. R. Roth, G. H. Gretz, W. S. Bigley and R. Orchard (*J. econ. Ent.*, 1968, **61**, 487–490).—In preliminary tests SD 8447 [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate], SD 8448 [2-chloro-1-(2,4,5-trichlorophenyl)vinyl diethyl phosphate] and SD 8436 [2-chloro-1-(2,4-dibromophenyl)vinyl dimethyl phosphate] gave 89–100% control of *Hypoderma* spp. when applied as pour-on treatments. Later tests produced ~50% control by SD 8447 and ~60% from those treated with SD 8436. There was no cholinesterase depression, no sign of toxicity and no difference in wt. gains. C. M. HARDWICK.

Determination of Dursban in livestock dips and sprays. F. P. Czech (*J. Ass. off. analyt. Chem.*, 1967, **50**, 861–868).—An i.r. spectrophotometric procedure for determining Dursban (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) in livestock dips (emulsions or wettable powders), sprays, hair and skin is described. It is rapid, precise and specific; the u.v. spectrophotometric procedure is more sensitive though slower. The chemical method (described), involving Na biphenyl reduction with coulometric titration, is also precise. A. A. ELDRIDGE.

Acid phytase. International Minerals and Chemical Corp. (B.P. 1,064,304, 27.7.65. U.S., 28.7.64).—Useful in animal foodstuffs to convert phytin P to assimilable phosphate, the acid phytase is obtained by aerobically culturing e.g. *Aspergillus niger* in a medium containing N, C and inorg. salts and maintaining the PO_4^{3-} content at <0·005% by wt. (determined as P). Other suitable micro-organisms are *Saccharomyces cerevisiae* and *Escherichia coli*. S. D. HUGGINS.

Quinoline derivatives. Imperial Chemical Industries Ltd. (Inventors: J. P. Cairns and W. Hepworth) (B.P. 1,070,223, 9.3. and 2.12.65) (43 claims).—Compounds claimed are anticoccidial agents and comprise 3-CO_2R-4-OH-7-OR^1-quinolines (or tautomers thereof) wherein R is alkyl and R^1 is alkyl, aryl, or aralkyl (optionally containing halogen, NO_2, or alkyl). The quinoline nucleus may also be further substituted by halogen, alkyl, alkenyl, or alkoxy—but in the absence of any such substituent then R is Et and R^1 is neither Me nor Ph, and when R and R^1 are both alkyl of 1–4 C then the quinoline nucleus does not bear as sole additional substituent alkoxy of 1–4 C in the 6-position. As an example of method of prep., a mixture of *m*-$NH_2 \cdot C_6H_4 \cdot OC_{12}H_{25}$ and $OEtCH:C(CO_2Et)_2$ is heated at 100° during 2 h, then the crude Et_2(*m*-dodecoxyanilinomethylene)malonate is added slowly to boiling Ph_2O. After a further 30 min. at the boil, the filtered solid is washed with CCl_4, to give Et 4-hydroxy-7-dodecoxyquinoline-3-carboxylate, m.p. 255° ($HCONMe_2$). F. R. BASFORD.

Benzanilide compounds. Parke, Davis and Co. (B.P. 1,070,516, 31.3.66. Austral., 1.4.65).—Useful as antiparasitic agents against *Fasciola hepatica* and a variety of nematodes, the claimed halogen-containing 2-acyloxybenzanilide compounds are non-irritating to the mucosa of warm-blooded animals and are less toxic than the salicylanilide compounds. The appropriate halogen-containing salicylanilide compound is mixed with a reactive alkanoic acid RCO_2H, deriv., where R is H, straight- or branched-chain alkyl of 1–17 C, a cycloalkyl of 3–8 C or cycloalkyl-substituted alkyl radical, in a solvent e.g. PhCl. Thus, a stirred solution of 3,5-dibromo-4′-chlorosalicylanilide in excess Ac_2O and a little 96% H_2SO_4 at 50° is cooled to room temp. and kept for 2 h, before pouring into water and stirring for 0·5 h to give the insol. 2-acetoxy-3,5-dibromo-4′-chlorobenzanilide, m.p. 182–182·5° (EtOH). S. D. HUGGINS.

[A] **Amidines.** [B] **Naphthamidines.** Wellcome Foundation Ltd. (B.P. 1,074,102–3, 22.3. and [A] 19.12.63).—[A] The claimed 4-$R^2O \cdot C_{10}H_6 \cdot C(:NH)NRR^1$ (substituted α-naphthamidines) are used for treating tapeworm in dogs, cats, pigs, sheep and cattle. R and R^1 are the same, each being alkyl, R^2 is alkyl and together with R or R^1 has ≤9 C, and R + R^1 + R^2 have ≥19 C. In an example, *N,N*-dihexyl-4-butoxy-α-naphthamidine hydrochloride (**I**), m.p. 218–219° is obtained by adding dihexylamine to a solution of EtMgBr in anhyd. Et_2O. After 45 min. refluxing, a solution of 4-butoxy-α-naphthonitrile is added, the mixture is refluxed for 24 h and worked up to give **I**.

[B] Compositions containing α-naphthamidines of formula 4-$R^2O \cdot C_{10}H_6 \cdot C(:NH)NRR^1$ are claimed (for treatment of tapeworm) in which R and R^1 are the same and each is alkyl, R^2 is alkyl, and R + R^1 + R^2 have a total of 6–8 C, e.g. *N,N*-di-n-butyl-4-n-butoxy-α-naphthamidine hydrochloride. They are prepared by reacting a halomagnesium dialkylamine with a 4-alkoxy-α-naphthonitrile. S. D. HUGGINS.

Substituted 6-phenyl 2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazoles, their salts and intermediates. American Cyanamid Co. (B.P. 1,076,109, 11.11.65. U.S., 5.10.65).—The title compounds, in which the substituent R in the Ph group is an optional C_{1-6}-alkyl or alkoxy, OPh, Ph, halogen, NO_2 or C_2?$_7$-alkanoylamino, are anthelmintic agents and are prepared by reacting a 2-ethyleneimino-1-phenylethanol (**I**) with HCNS or thiourea and a strong acid, treating the resulting $OH \cdot CHPh \cdot CH_2 \cdot NH[CH_2]_2SY$ [Y is CN or $C(NH_2):NH$] with strong acid, to give a 2-imino-3-(2-hydroxy-2-phenylethyl)thiazolidine which is converted by interaction with $SOCl_2$ into the corresponding 2-chloro-analogue. This is subjected to ring closure with the aid of e.g. K_2CO_3. E.g., EtOH containing HCl is added to a solution of KCNS in EtOH, then heated to 50°, cooled and KCl filtered off. The filtrate is added at 30–35° to a solution of **I** (R = H) (prep. described), then the product, viz., 2-[2-hydroxy-1-phenylethylamino]ethyl thiocyanate, m.p. 199–201° is reacted with a solution of HCl in EtOH to give a 95% yield of 2-imino-3-(2-hydroxy-2-phenylethyl) thiazolidine hydrochloride, m.p. 198–200° (from EtOH). This is boiled in $CHCl_3$ with $SOCl_2$ for 30 min., with formation of 2-imino-3-(2-chloro-2-phenylethyl)thiazolidine hydrochloride, m.p. 245–246°, which is dissolved in $CHCl_3$ and boiled with aq. K_2CO_3 for 1 h. The mixture is cooled; the washed org. layer gives 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole, m.p. 90–92° (from ether). The products are active in sheep, cattle, swine,

chickens etc. against e.g. *Ostertagia circumcincta*, *Cooperia* sp., *Ascaris suum* and *Ascaridia galli* (larvae). F. R. BASFORD.

Benzothiazole compounds. Monsanto Chemicals (Australia) Ltd. (B.P. 1,077,177, 22.4.65. Austral., 23.4.64).—Active against helminthiasis, the 2-trichloromethylbenzothiazoles, the Ph-ring of which is substituted by R^1 and R^2 (H, halogen, OH, alkyl, alkoxy, alkylthio, haloalkyl, NO_2, NH_2, acylamino, alkylamino or dialkylamino) are obtained by reacting an acid addition salt of the appropriate *o*-aminothiophenol with $CCl_3 \cdot CN$. 2-Trichloromethylbenzothiazole is obtained in 65% yield, m.p. 33°, (MeOH) by reacting CCl_3CN with *o*-aminothiophenol hydrochloride in dry EtOH at room temp. S. D. HUGGINS.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Wheat foods as sources of nutrients. W. B. Bradley (*Baker's Dig.*, 1967, **41**, No. 5; 66–71).—Observations made in a comprehensive study by the American Institute of Baking are summarised. 18 Amino-acids and nine vitamins are determined in wheat, flour, bread and other mill products and a table of the mineral composition is presented. The results are interpreted in terms of man's requirements. (10 references.) I. DICKINSON.

Control of cereal damage by irradiation. Th. Grünewald and A. Frank (*Dt. Lebensmitt Rdsch.*, 1968, **64**, 133–138).—After listing the pests likely to cause damage to stored grain it is pointed out that pest control by contact or gaseous insecticides is likely to give rise to undesirable residues. These treatments are not effective against all stages of beetle infestation. Irradiation is effective and at low levels causes no change in the grain. Tests on rodents and dogs have shown that irradiated wheat is perfectly safe and the process is officially approved in the U.S.A. A description of the commercial installation using a ^{60}Co source is given. (26 references.) J. B. WOOF.

Durum-type wheat with high bread-making quality. P. J. Kaltsikes, L. E. Evans and W. Bushak (*Science, N.Y.*, 1968, **159**, 211–213).—This wheat was produced by crossing a durum-wheat variety with a common bread-wheat (*BW*) variety, backcrossing to the *BW* type for three generations and then selecting 28 chromosome plants. The high quality is tentatively attributed to a translocation involving one of the D-genome chromosomes. C.V.

New aspects of the functions and properties of the soluble wheat-flour pentosans. H. Neukom, T. Geissmann and T. J. Painter (*Baker's Dig.*, 1967, **41**, No. 5; 52–55).—Sol. pentosans have an important influence on baking properties which is due not only to their own physical properties, but also to the fact that these properties change pronouncedly upon mild oxidation; pentosans also react with proteins to give macromolecular complexes, the nature of these is, as yet, not quite clear. (23 references.) I. DICKINSON.

Flour improvers: their effects, application and chemical actions. C. C. Tsen and I. Hlynka (*Baker's Dig.*, 1967, **41**, No. 5; 58–64).—A review of recent literature covering bromate, iodate, ascorbic acid, chlorine dioxide, and two relatively new improvers, azodicarbonamide (Maturox) and acetone peroxides (Keetox). The effect of the improvers, the treatment level and the mechanism of action is discussed. (46 references.) I. DICKINSON.

Wheat flour lipids, a minor component of major importance in breadmaking. Y. Pomeranz (*Baker's Dig.*, 1967, **41**, No. 5; 48–50, 170).—A review of the literature covering wheat flour lipids (*L*), free and bound *L*, nonpolar and polar *L*, *L* in breadmaking and in oxidation, and *L* binding and dough structure. (49 references.) I. DICKINSON.

Changes in biochemical and bread-making properties of storage-damaged flour. Y. Pomeranz, R. D. Daftary, M. D. Shogren, R. C. Hoseney, and K. F. Finney (*J. agric. Fd Chem.*, 1968, **16**, 92–96).—The damaged flour contained no free lipins (**I**), and bound **I** were reduced to ~30% of the normal content. Electrophoretic examination of the proteins of the damaged flour showed only slight deviations from the normal composition. The damage, causing loss of loaf-vol. potential, poor crumb-quality and colour, and necessitating long mixing times, could be ascribed entirely to **I** breakdown. Loaf-vol. potential could be restored by addition of polar **I**, but the other defects were unaffected or only partly alleviated. (17 references.) P. S. ARUP.

Better way to process starch. R. D. Carney (*Fd Engng*, 1967, **39**, No. 11, 90–92).—Innovations allowing the highly efficient handling and drying of corn-starch slurry are described, 150,000 gal, 23 Bé being handled in a 24 h day. Starches of precise particle size classification and moisture range 6–13% are produced. The plant is designed with structural simplicity and emphasis is laid upon ease of cleaning. C.V.

Bakers' yeasts. Present developments and future potentials. E. H. Schuldt, jun. (*Baker's Dig.*, 1967, **41**, No. 5; 90–93).—Bread flavour and yeast, bulk yeast, the yeast liquefying system, active dry yeast and inactive dried yeasts (as a protein supplement) are discussed. (12 references.) I. DICKINSON.

Enzyme supplementation in baking. G. Reed (*Baker's Dig.*, 1967, **41**, No. 5; 84–87, 123).—Enzyme usage which has not changed materially since 1961 is reviewed and later developments (e.g. use of lipoxidase with unsaturated fatty acids to produce white bread with a 'nutty' flavour, of lactase to hydrolyse milk sugar and of pentosanases in attempts to remove pentosans from wheat flour) are discussed. Tables of α-amylase (**I**) prep. are shown and comparison is made of **I** of different origin; compressimeter values vs. days' storage of production bread show the effect of bacterial **I** addition on bread firmness. (28 references.) I. DICKINSON.

Recent advance in oxidants for continuous dough mixing. F. F. Barrett and R. R. Joiner (*Baker's Dig.*, **41**, No. 6; 46–48).—Preliminary experiments showed that azodicarbonamide (**I**) made a promising oxidant in continuous dough mixing operations, especially in combination with a slow acting improver such as bromate. Extensive tests are described and a tablet containing **I** (20 ppm) and $KBrO_3$ (40 ppm) sufficient to treat 100 lb of flour was prepared. Commercial scale bakery trials with this additive gave bread of superior quality. I. DICKINSON.

Emulsifiers: a review of their applications in baking. J. G. Endres (*Baker's Dig.*, 1967, **41**, No. 5; 96–98).—Attempts to obtain ideally constituted surfactants specially suitable for baking are briefly discussed; it is concluded that future utilisation of surfactants will probably be based on optimal combinations rather than on single, functionally limited compounds. I. DICKINSON.

Algin products in bakery foods. R. L. Edlin (*Baker's Dig.*, 1967, **41**, No. 6; 49–51).—The approx. mesh size, average solution viscosity (in cP), general properties, suggested rate of use and applications of some commercial forms of algin are presented in a table. These forms include Na-, K-, Ca- and NH_4-, and propylene glycol-alginates. I. DICKINSON.

Bread flavour concepts. J. A. Johnson and A. El-Dash (*Baker's Dig.*, 1967, **41**, No. 5; 74–78).—The factors which modify bread flavour are reviewed and the possibility of estimating consumer reaction to bread flavour is discussed. Several methods for the evaluation of flavour, descriptive tests, objective tests and methods of intensifying bread aroma e.g. by addition of papain are examined. (30 references.) I. DICKINSON.

Continuous production of pie dough. D. L. Preonas, A. I. Nelson and M. P. Steinberg (*Baker's Dig.*, 1967, **41**, No. 6; 34–40).—The literature is reviewed and a flow-diagram of a proposed system for continuous production of pie dough given. Organoleptic evaluation of baked pie crusts was based on a method by Matthews and Dawson (*Cereal Chem.*, 1963, **40**, 291). The conditions necessary for producing a continuous pie dough can be summarised: (1) an automatic weigh feeder should be used for flour metering. (2) Fat should be metered prior to its continuous plasticising. (3) Fat should be piped and extruded so as to assume control of its plasticity. (4) A progressive cavity conveyor lends itself to mixing and conveying the fat and flour mixture. (5) Water addition should be in the form of a fine mist. (6) Gluten development can be achieved with an apparatus similar to a meat grinder if the effects of friction and pressure are minimised. (19 references.) I. DICKINSON.

Influence of rapid dough mixing on the quality of wheat–rye mixture bread, rye bread and wholemeal breads. H. Huber (*Brot. Gebäck*, 1967, **21**, 212–221).—An extensive series of comparative baking trials are reported showing the variation in bread vol., shape, crust and crumb properties and taste with mixing time at two mixing speeds (40 and 120 rpm) for doughs from various flour grades. Fast mixing is suitable and advantageous with all wheat doughs, including those containing rye flour; it is not suitable for rye meal doughs. No increase in water absorption due to fast mixing was observed with any of the mixes tested. E. C. APLING.

Microbiological problems in baking. R. J. Robinson (*Baker's Dig.*, 1967, **41**, No. 5; 80–83, 173–176).—Problems arise from the

ingredients, processing, personnel, equipment-plant condition and distribution. Three groups of micro-organisms are apparent; (1) purified strains of the yeast *Saccharomyces cerevisiae*, (2) desirable bacteria which aid in gluten development and produce certain flavour characteristics and (3) undesirable micro-organisms such as *Bacillus mesentericus*, torulae, moulds, viruses and those bacteria causing food poisoning. Microbiological aspects of baking (e.g. discoloration of products by organisms such as *Serratia marcescens* and *Oidium aurantiacum*; 'rope' infections; mould spoilage) and their control are discussed. (55 references.) I. DICKINSON.

Applications of high frequency energy in the baking field. R. V. Decareau (*Baker's Dig.*, 1967, **41**, No. 6; 52–54, 69).—A review of the literature, dealing with mould inhibition, defrosting, proofing and baking, and the comparative operating costs of a high frequency-thermal and a conventional bread baking system (6000 lb/h). (10 references.) I. DICKINSON.

Sugars and confectionery

Acid catalysed degradation of D-fructose. P. E. Shaw, J. H. Tatum and R. E. Berry (*Carbohydrate Res.*, 1967, **5**, 266–273).—A study of non-enzymic browning in dehydrated foods led to this work. The two main furan deriv. formed in this degradation are 5-(hydroxymethyl)-2-furaldehyde and 2-(2-hydroxyacetyl)furan; minor products included furfural, 5-methyl-2-furaldehyde, isomaltol, 2-(2-hydroxyacetyl)furan formate and 4-hydroxy-2,3,5-hexanetrione. Formic, acetic and levulinic acids were also present. A new hydroxyfuranone was isolated and its structure is discussed. Degradation studies at pH 1·0, 2·15 and 3·5 showed variation in the minor components formed. The mechanism of the formation of the furan deriv. is discussed. (21 references.) C.V.

Characteristics of honey. S. N. Mitra and T. V. Mathew (*J. Instn Chem. India*, 1968, **40**, 26–30).—Data for moisture, ash, reducing sugars, sucrose, dextrose, fructose, fructose : dextrose ratio and formic acid are presented for 30 samples of *Apis Indica* and 14 of *Apis Dorsata* honey with notes on the aniline chloride and Fiehe's tests for detection of adulteration by technical invert sugar, and on the KI-I_2 test for the presence of erythrodextrin (present in commercial glucose). (10 references.) E. G. BRICKELL.

Fermentation and Alcoholic Beverages

Effect of light on metabolism of propionic acid bacteria. L. I. Vorob'eva, L. F. Kozyreva and L. L. Lisenkova (*Microbiology, [USSR]*, 1967, **36**, 335–339).—Growth, fermentation and oxidative ability of *Propionibacterium shermanii* were studied. Light intensity $>60\times10^3$ erg. cm²/sec inhibited growth of this organism while light intensity of 30×10^3 erg. cm²/sec inhibited bacterial growth, decreased oxidative ability and altered the ratio of fermentation products. Vitamin B_{12} and flavine concn. decreased in the presence of light but cytochrome b content was not interfered with. (15 references.) C.V.

Aroma composition of alcoholic beverages. H. Suomalainen and L. Nykanen (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 807–811).—The qual. similarity in the composition of volatile aroma compounds in alcoholic beverages, mainly alcohols, fatty acids, esters and carbonyl compounds, is very striking in different beverages produced from different sources. This has been observed by gas chromatography of beers produced from malt, of wines from grapes and berries, of brandies and cognacs from grapes, of rums from cane molasses and of whisky from grain and malt. The role of yeast in aroma formation is important. The influence of yeast strain, composition of yeast fat and presence of biotin on fatty acid synthesis by yeast has been experimentally tested. (28 references.) (In English.) M. SULZBACHER.

Aseptic brewing. T. C. N. Carroll (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 816–817).—The planning of microbiological control, the design of buildings, vessels, pumps, rousers, couplings, sample cocks and centrifuges are briefly described. Services and piping and wort and beer mains are also discussed. The aseptic process gives an almost indefinite life for the beer without filtration or pasteurisation. (In English.) M. SULZBACHER.

Industrial enzymes used in brewing. L. T. Saletan (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 827–832).—The properties and activities of bacterial α-amylase, fungal α-amylase, amyloglucosidase and of papain are discussed. The α-amylases are used in brewing for liquefaction and dextrinisation of starch from cereal grains. The amyloglucosidase converts the liquified starch quant. to dextrose. Papain maintains the colloidal stability and the brilliant appearance of packaged beer. Data of practical use of these enzymes in the brewing process are given. The detection and quant. determination of papain in the finished beer, following pasteurisation and filtration, are also described. (13 references.) (In English.) M. SULZBACHER.

Stabilisation of beer by casein. R. Vancraenenbroeck, R. Lontie and D. Eyben (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 780–783).—After a discussion of the improvement of the physico-chemical stability of beer by addition of proteins, insoluble in beer, which adsorb polyphenols and particularly anthocyanogens, laboratory experiments on casein (**I**), found to be the most active adsorbant, are reported. Treatment with commercial **I** followed by sieving and washing was very advantageous and addition of **I** solubilised at pH 7·0 prevented turbidity on storage and avoided anomalies of taste which may develop with non-washed commercial **I**. (17 references.) (In French.) M. SULZBACHER.

Nitrogenous matter of beer retained by adsorbants. G. Preaux, P. Holemans, M. van der Vurst and R. Lontie (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 784–788).—The composition of the amino-acids in the nitrogenous matter of beer, retained by bentonite, Stabifix and Stabiquick and filtered off, was determined by cation exchange chromatography. Their solubilities in 60% EtOH or Pr^iOH (±1% thioglycolic acid) and in 60% Pr^iOH with 0·5% $NaBH_4$ were measured. The adsorbants gave different results. Stabifix was the most specific. The beers, before and after treatment with bentonite and Stabifix, were compared by chromatography on Sephadex G-25 and G-50. No differentiation was found by this method. (28 references.) (In French.) M. SULZBACHER.

Control of a [method of] filtration of beer through kieselguhr. J.-P. Beguery (*Brasserie*, 1968, **23**, 269–270, 278–289).—A detailed account is given of the operation of a countercurrent system in which kieselguhr, in regulated amounts, is injected into the beer. Method for supervising the functioning of the plant, and modifications for dealing with various types of beer are described. P. S. ARUP.

Gas chromatographic determination of some volatile substances in beer by direct injection. B. Drews, G. Bärwald and H.-J. Niefind (*Mschr. Brau.*, 1968, **21**, 131–136).—Analysis of acetaldehyde, AcOMe, AcOEt, isoamyl acetate, Pr^nOH, Bu^iOH and isoamyl alcohols has been carried out by direct injection of 10 μl of beer. A 10 cm precolumn of washed sea sand adsorbs non-volatiles whilst fractionation is achieved on a 2 m column packed with 10% Carbowax 1500 on Chromosorb P joined to a 4 m one packed with 10% Igepal on Chromosorb W. Peak area calibration and identification of components is carried out with standard mixtures. Analyses of a number of beers, with average values and standard deviations are given. The sum of higher individual alcohols agreed well with the total obtained colorimetrically. The direct method can also be used for β-phenylethyl alcohol and β-phenylethyl acetate if the oven temp. is raised to 140°. (15 references.) J. B. WOOF.

Fermentation rates of grape juice. V. Biotin content and its effect on alcoholic fermentation rate. C. S. Ough and R. E. Kunkee (*Appl. Microbiol.*, 1968, **16**, 572–576).—Juices from white grapes had less biotin **I** than those from red. There was a significant difference in **I** content between different varieties. **I** in Cabernet Sauvignon increased significantly with maturity; this was not found with the white variety. **I** concn., with total N, can be used to estimate indirectly the yeast growth potential and therefore to assess fermentation rate of the juice. C.V.

Formation of secondary products in the alcohlic fermentation of grape must by successive associations of yeasts. G. Florenzano, W. Balloni and R. Materassi (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32** (III), 791–793).—When two or three different yeast strains e.g. *Saccharomyces rosei*, *S. ellipsoideus*, *S. veronae*, *S. oviformis* or *Candida pulcherrima*, are inoculated over several days instead of one yeast source, the fermentation of grape must gives variations in the formation of secondary products, whilst the alcohol yield remains unaltered. A lower production of AcOH is accompanied by increase of succinic acid, 2,3-butanediol and particularly glycerol. It is assumed that the biochemical activity of the first yeast influences that of the successive yeasts. AcOH and glycerol as secondary products influence the metabolism of the successive yeast associations. (In French.) M. SULZBACHER.

Maturing of 'fino' wines with 'flower' yeasts. I. Introduction and ethanol metabolism. M. D. Cabezudo Ibáñez, C. Llaguno

Marchena and J. M. Garrido Márquez (*Revta Ciencia apl.*, 1967, **21**, 501–512).—In tests on the assimilation of ethanol, the development of various classes of yeasts in a synthetic medium with and without addition of ethanol, biotin and ethanol plus biotin has been followed by transmission measurements. (55 references.)
L. A. O'NEILL.

Homolactic cocci isolated from wines. E. Peynaud and S. Domercq (*Revue Ferment. Ind. aliment.*, 1967, **22**, 133–140).—An investigation of the physiological properties of 34 such strains (numbering 4·6% of the total lactic acid bacteria found in wines) characterises them as members of the species *Pediococcus cerevisiae*. The question as to the rôle of these bacteria in wine is controversial. (18 references.)
P. S. ARUP.

Fruits, Vegetables, etc.

Fruit industries of Latin America. G. K. Maliphaut (*Tropical Sci.*, 1967, **9**, 20–45).—Fruit production in the Latin American countries is reviewed covering 2000 m. hectares, 2×10^7 ha being devoted to bananas, citrus, pineapples, grapes, apples, pears, plums and peaches. (27 references.)
C.V.

Some compounds formed during non-enzymic browning of orange powder. J. H. Tatum, P. E. Shaw and R. E. Berry (*J. agric. Fd Chem.*, 1967, **15**, 773–775).—Compounds extracted from the powders with 50% aq. $COMe_2$ and thence into Et_2O, separated by GLC and identified by spectroscopy include eight furans, three pyrroles, three acids, a lactone, and methylcyclopentenolone. (18 references.)
P. S. ARUP.

Precursors of typical and atypical roasted peanut flavour. K. J. A. Newell, M. E. Mason and R. S. Matlock (*J. agric. Fd Sci.*, 1967, **15**, 767–772).—Analyses of extracts of fresh and roasted, defatted, peanuts made with M-NaCl, 3 N-$HClO_4$, and 80% EtOH, in comparison with organoleptic tests, revealed amino-acids and carbohydrates as presumptive flavour precursors, the content of which decreased on roasting. Comparisons between the amino-acid composition of good (and poor flavoured) or mature, and immature, peanuts and the results obtained after roasting, showed aspartic acid, glutamic acid, glutamine, asparagine, histidine, and phenylalanine to be precursors of typical flavours. Threonine, tyrosine, lysine, and an unknown acid were responsible for atypical flavours. A mechanism for the process of flavour production is suggested. (25 references.)
P. S. ARUP.

Non-alcoholic beverages

Detection of pulp and peel extracts in orange juice by means of pentosan content. E. Benk (*Dt. LebensmittRdsch.*, 1968, **64**, 146–148).—To determine pentosan, the juice sample is distilled with 12% HCl (acid being added to maintain constant vol.). After dilution of distillate, furfural is determined colorimetrically by the orcinol reaction. The pentosan content found, as xylose equiv., was 0·04 to 0·17% for juice from clutter-head presses and in commercial samples 0·1 to 0·19%. Up to 0·63% could be detected in home made extracts. It is concluded that the method is useful in detecting pulp adulteration. (15 references.)
J. B. WOOF.

Milk, Dairy Products, Eggs

Sephadex chromatographic study of the influence of thermal treatment on the proteins of milk serum. R. Lontie and G. Preaux (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 820–826).—The albumin and albumin–globulin fractions of the serum proteins of fresh and heated milk were submitted to chromatography on Sephadex G-100 at pH 4·7–8·2 and to gel electrophoresis, agar at pH 8·6 being used. The pH influences the vol. and elution order of the β-lactoglobulins A and B. Pasteurisation does not alter the chromatogram of the albumin fraction but affects that of the albumin–globulin fraction, in which the globulins are made insol. The effect of sterilisation is still more marked. (87 references.) (In French.)
M. SULZBACHER.

Isolation and physical-chemical characterisation of a glycoprotein from the proteose-peptone fraction of cows' milk. Wesu C. Ng (*Diss. Abstr.* B, 1968, **28**, 3741).—Proteose-peptone fraction of milk contains three major components, separable by acrylamide gel electrophoresis. The slowest moving component (**I**) has been isolated (*i*) from the acid-whey supernatant of heated skimmilk by fractionation with $(NH_4)_2SO_4$ and (*ii*) from unheated skimmilk by fractionation of the crude globulin fraction with $(NH_4)_2SO_4$. The prep. were purified by electrophoresis and the composition and properties of **I** determined.
P.P.R.

Coagulation of milk by rennet. J. Garnier, J. C. Mercier and G. Mocquot (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 857–858).—The properties and problems of the stable suspension of caseins in milk are briefly reviewed. The action of rennet is explained and its proteolytic specificity is illustrated by amino-acid composition tables. (25 references.) (In French.)
M. SULZBACHER.

Distribution of lipids in various fractions of cows' milk. J. Cerbulis (*J. agric. Fd Chem.*, 1967, **15**, 784–786).—Results are given for the distribution in cream, separated milk, casein, whey, and separator slime of 'free' lipids (**I**) (extracted with light petroleum) and 'bound' **I** (subsequently extracted with $CHCl_3$–MeOH (2 : 1).) The fractions were analysed by TLC and by column chromatography; neutral **I** were found in both fractions but phospho-**I** in the 'bound' fraction, only. Di- and mono-glycerides occurred chiefly in the 'bound' neutral fraction. The phospho-**I** composition was essentially the same in all fractions. (11 references.)
P. S. ARUP.

Rapid detection of excess chlorides in milk. M. López Lozano (*Revta Fac. Agron. Univ. nac., La Plata*, 1966, **42**, 105–112).—Use of the colorimetric method of Jacquet and Le Nir (*Le Lait*, 1953, **33**, 16) for milk testing at milk delivery points over a period of 7 months is reported. The efficiency of the test is confirmed, and its inclusion as a routine in the quality evaluation at milk reception is recommended.
E. C. APLING.

Effect of low-frequency ultrasound and elevated temperatures on isolation of bacteria from raw milk. C. N. Huhtanen (*Appl. Microbiol.*, 1968, **16**, 470–475).—This treatment significantly increased total bacterial counts and counts of enterococci, coliforms and staphylococci. Warming diluted milk 12 min. at 30–40° increased the counts of the same organisms but the heat produced by the ultrasonic treatment did not entirely account for this effect which appeared to be related to the energy output of the generator and energy absorbed by the treated materials.
C.V.

***Brucella abortus* in milk supplied to Glasgow.** R. A. Tadayon (*J. appl. Bact.*, 1968, **31**, 145–150).—Bulk milk samples (128) from 114 farms producing certified milk, 78 bulk raw and 50 pasteurised milk samples from 27 pasteurising plants, 73 milk samples from aborting animals and 51 individual samples of certified milk were examined for the presence of Brucella spp. Of the samples, 18 (15·8%), 8 (10·3%), 28 (50·0%) and 5 (9·8%) respectively contained *B. abortus*; 48 cultures of Brucella were typed and four of these behaved like *B. malitensis*. The remainder resembled *B. abortus*; of these, 41 belonged to type 1 and the remainder to type 2. (28 references.)
C.V.

Rapid test to find 'potentially' psychrophilic organisms in pasteurised dairy products. L. Hankin and W. F. Dillman (*J. Milk Fd Technol.*, 1968, **31**, 141–145).—Samples were examined by oxidase test and standard test for psychrophiles. Statistical analysis showed the tests to be positively correlated. The argument is based on the supposition that most psychrophilic problems are traceable to pseudomonads, organisms that are strongly oxidase-positive. The test, which allows the oxidase-positive colonies to develop a blue colour, is described. It is simple and rapid. (11 references.)
C.V.

Presence of spore-forming [bacteria] in milk dessert. J. C. Ocampo (*Revta Fac. Agron. Univ. nac., La Plata*, 1966, **42**, 97–103).—Survival of soil-borne, spore-forming aerobic and anaerobic bacteria, producing gas or H_2S, after heating at 101–105° for 2 h during the manufacturing process, is demonstrated.
E. C. APLING.

Dairy products for bakery utilisation. M. H. Mertens and R. Mykleby (*Baker's Dig.*, 1967, **41**, No. 5; 110–113).—Older and newer types of dairy products available to the baker are outlined. A table of typical analyses of various dried dairy products is presented and manufacture of some of these is described.
I. DICKINSON.

Behaviour of *Salmonella typhimurium* during manufacture and curing of Cheddar cheese. J. M. Goepfert, N. F. Olson and E. H. Marth (*Appl. Microbiol.*, 1968, **16**, 862–866).—Nine vats of stirred-curd granular Cheddar cheese were made with whole milk contaminated after pasteurisation. The organisms multiplied rapidly until salting of the curd; thereafter, and during curing, they declined in number, the fall being dependent on temp. of curing. Evidence is presented to suggest that the production of volatile fatty acids in the curd may be responsible for this decline. (14 references.)
C.V.

Bacteriological hygiene of pasteurised soft cheeses. A. Blumenthal (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1967, **58,** 166–169).—The following standards are proposed: phosphatase test negative; max. permissible counts per g for coliform bacteria 1000, for moulds 100, and for yeasts and Oidia 50,000; tests for Salmonella, Shigella, and coagulase-positive staphylococci, negative. P. S. ARUP.

Alterations in the electrophoretic characteristics of albumen proteins induced by the storage of eggs. G. Croizier and B. Sauveur (*Annls Biol. anim. Biochim. Biophys.*, 1967, **7,** 317–321).—The effect of an atm. of 4% CO_2 on the deterioration of shell egg albumen during 21–22 days storage at 10° was studied by two starch gel electrophoretic methods based on those of (*a*) Lush, (*Nature, Lond.*, 1961, **189,** 981) and (*b*) Kristjansson, (*Genetics*, 1963, **48,** 1059). Both methods showed changes resulting from storage, but method (*b*) was preferred. Results show that deteriorative changes in the albumen are retarded in CO_2. E. C. APLING.

Recent developments in the egg industry. O. J. Kahlenberg (*Baker's Dig.*, 1967, **41,** No. 5; 100–102, 113).—Methods of pasteurisation for liquid whole eggs, for yolk and whites, and the functional performance of these products in baking are reviewed and a number of the egg solid and frozen egg products that have been commercially developed for the baking industry are described. (20 references.) I. DICKINSON.

Edible Oils and Fats

Polycyclic aromatic hydrocarbons in solvents used in extraction of edible oils. J. W. Howard, T. Fazio and R. H. White (*J. agric. Fd Chem.*, 1968, **16,** 72–76).—Recoveries of benzo[*a*]pyrene, dibenz-[*a,h*]anthracene, benz[*a*]anthracene, and benzo[*g,h*]perylene added to hexane solvents at 0·002 ppm were 86–95% as determined by the procedure described by Howard *et al.* (cf. *J. Ass. off. analyt. Chem.*, 1966, **49,** 595). Traces of pyrene, fluoranthene, anthracene, phenanthrene, and substituted phenanthrenes were isolated from 9 of the 15 solvents that were examined. No known carcinogenic hydrocarbons were found. (10 references). P. S. ARUP.

Introduction to chemistry and biochemistry of fatty acids and their glycerides. F. D. Gunstone (*Chapman Hall*, 1967, 2nd Ed., 209 pp).—The natural deriv. of fatty acids (*FA*), structure of the natural *FA*, separation and isolation of *FA*, synthesis, physical properties of *FA* and their esters, chemical properties of these two groups, reduction and hydrogenation and oxidation are covered together with the halogen-containing compounds, polymerisation, stereomutation, double-bond migration, other olefinic reactions and reactions of the carboxylic acid group. Glyceride synthesis, the component acids and glycerides of the natural fats and biosynthesis and metabolism are also reviewed. C.V.

Lipid chromatographic analysis. Vol. I. Ed. G. V. Marinetti [*Edward Arnold (Publishers) Ltd.*, Lond., 1967, 537 pp].—**Paper chromatography of phosphatides and glycolipids on silicic acid-impregnated filter paper;** M. Kates (97 references). **Thin layer chromatography of phosphatides and glycolipids;** O. Renkonen and P. Varo (117 references). **Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids;** G. Rowser, G. Kritchevsky and A. Yamamato (24 references). **Analysis of phosphatides and glycolipids by chromatography of their partial hydrolysis or alcoholysis products;** R. M. C. Dawson (72 references). **Thin layer chromatography of neutral glycerides;** V. Mahadevan (53 references). **Column chromatography of neutral glycerides and fatty acids;** K. K. Carroll and B. Serdarevich (166 references). **Gas chromatography of neutral glycerides;** A. Kuksis (84 references). **Isolation and gas–liquid chromatography of alkoxy lipids;** H. K. Mangold and W. J. Baumann (80 references). **Gas–liquid chromatography of fatty acids and derivatives;** R. A. Stein, V. Slawson and J. F. Mead (377 references). **Gas chromatography of the long-chain aldehydes;** G. M. Gray (52 references). **Thin layer- and gas-chromatography of sphingosine and related compounds;** B. Weiss (63 references). **Gas chromatography of inositol and glycerol;** R. N. Roberts (38 references). **Gas chromatography estimation of carbohydrates and glycolipids;** C. C. Sweeley and D. E. Vance (67 references). **Chromatographic analysis of nitrogen bases derived from lipids;** J. M. McKibbin (42 references). C.V.

Changing the physical properties of fats and oils for specific uses. C. W. Hoerr (*Baker's Dig.*, 1967, **41,** No. 6; 42–45, 71).—Methods used for analysing the composition of fats and oils and for determining their physical properties are reviewed. Processing procedures used to change the properties of fats and oils for specific applications in food products are discussed, i.e. blending, hydrogenation, interesterfication, glycerolysis, fractionation, solidification, and tempering. Further knowledge of the mol. composition of fat and oil in relation to their physical properties and performance in specific applications, will lead to the evolution of 'tailor-made' fats giving optimum results. (15 references.) I. DICKINSON.

Shortening requirements of the continuous mixing process. P. M. Koren (*Baker's Dig.*, 1967, **41,** No. 5; 104–108, 171).—A fluid vegetable shortening, not to be confused with straight hydrogenated oils, is compared with other shorteners in a continuous mixing process. The advantages of the fluid shortening include, uniformity; this is most important for bakers who have not the analytical means to determine the proper hard flake level to use. The fluidity enables the product to be pumped and metered at room temp. without heating or N_2 blanketing of the tank. Since stability is good, there is no separation of solids in storage, therefore agitation is not necessary. Resistance to oxidation is much improved because of the natural antioxidants in the oil and the cool storage. The balanced fluid vegetable shortening bread has all the characteristics of an excellent loaf, good volume, good grain, fine texture, good crumb sheen and good softness. I. DICKINSON.

Meat and Poultry

Effect of chelating agents on post mortem changes in muscle. P. D. Weiner (*Diss. Abstr.*, 1968, **28,** 3742).—Effect of chelating agents (EDTA, CDTA, EGTA and Na oxalate) and of Ca^{2+} and Mg^{2+} upon development of rigor mortis and subsequent quality factors of meat was determined, using pig and rabbit muscle. ATP degradation, pH changes, water-holding capacity, colour, cooking losses and shear values were among the factors investigated. P.P.R.

Age and genetic effects on pH changes in adipose tissue. J. D. Sink (*J. agric. Fd Chem.*, 1967, **15,** 778–780).—Initial post mortem pH values of the outer and inner layers of *panniculus adiposus* from two breeds of pigs increased with the age of the pigs (125–225 days) and decreased for values determined 4 to 24 h later. The changes in pH were most marked in the tissues of the older pigs. Only in the older pigs (225-day) did animal breed affect these changes. (10 references.) P. S. ARUP.

Quantitative determination of various components of commercial meat extracts. H. Sulser, P. Schenk and W. Büchi (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1967, **58,** 157–165).—Analyses of eight samples including determinations of amino-acids and dipeptides, showed that variations in the content of creatinine, β-alanine, histidine, and dipeptides were too wide to permit the use of these data as criteria for adulteration, even when the presence of whale meat is excluded. (20 references.) P. S. ARUP.

Changes in sulphydryl and disulphide content of chicken muscle and effect of *N*-ethylmaleimide. T. H. Gawronski, J. V. Spencer and M. H. Pubols (*J. agric. Fd Chem.*, 1967, **15,** 781–783).—The sulphydryl to disulphide ratio of *pectoralis major* muscle decreased from 1·7 to 1·1 within 2 h post mortem and much more slowly afterwards. In experiments with the excised muscle, the addition to the ageing medium of *N*-ethyl-maleimide (which reacts specifically with sulphydryl groups) prevented the complete tenderisation of the tissue as measured by shear-resistance tests. The importance of these groups in the changes leading to tenderisation is indicated. (13 references.) P. S. ARUP.

Pre-chilling and moisture loss in poultry carcasses. G. A. Maciel, I. S. Schneider and J. C. Panetta (*Archos bras. Nutr.*, 1967, **22,** 7–19).—Poultry carcasses immersed in chilled water (1·5 of water and 1 pt of crushed ice per carcass) and manually agitated at 10 min. interval, attained a temp. of 3·5 to 6·5° in 30 min.; without agitation, the same temp. were reached in 60 min. Max. water absorption was 6%. After thawing the pre-chilled carcasses lost between 1% and 2% of water. There was no difference in the rates of water absorption between broilers and hens. (12 references.) I. DICKINSON.

Fish

Inhibition by EDTA of inosine monophosphate dephosphorylation in refrigerated fishery products. H. S. Groninger and J. Spinelli (*J. agric. Fd Chem.*, 1968, **16,** 97–99).—Treatment of fish muscle with 300 ppm of EDTA inhibited the enzymic action in species of fish having medium or slow rates of dephosphorylation. The ineffectiveness of EDTA in fish with rapid dephosphorylation rates was due to a time-lag between the application and effective action of the EDTA. Successful use of EDTA improved the flavour of the fish. (14 references.) P. S. ARUP.

Denaturation of proteins during frozen storage of pomphrets, mackerel and sardines. S. S. Pawar and N. G. Magar (*J. Fd Sci. Technol.*, 1967, **4**, 74–75).—Various glazes, such as ascorbic acid, $NaNO_2$, NaCl/glucose, citric acid and cold water were applied and the fish was subjected to slow and quick freezing; sol. protein was determined after seven months. The extractability of sol. proteins decreased to different extents in all three fish; use of the glazes retarded the loss of solubility during storage. Quick frozen fish contained slightly more sol. protein than those which were slow-frozen and stored. Sarcoplasmic proteins of the pomphrets (*Stromateus cinereus*) (a non-fatty type of fish) did not change during storage, which led to the conclusion that denaturation is associated with the actomyosin fraction. (13 references.) I. DICKINSON.

Incidence of *Clostridium botulinum* Type E in salmon and other marine fish in the Pacific Northwest. J. M. Craig, S. Hayes and K. S. Pilcher (*Appl. Microbiol.*, 1968, **16**, 553–557).—Of 369 salmon specimens, 48 yielded cultures containing toxin lethal to mice, almost half of these being botulinal toxin (*BT*) type E. Sole and cod, 113 specimens, 22 Dungeness crabs, 16 oysters and 115 clams were studied; of these 18, 4, 5 and 27 respectively, contained *BT*, generally type E although A and B were sometimes found. (16 references.) C.V.

Spices, Flavours, etc.

Flavour concentrates. D. Jacqmain (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 850–852).—Developments in the technical production of (*i*) essential oils by steam distillation, dry and vac. distillation, by pressing and of (*ii*) oleo-resins by extraction are discussed. The use of the flavour concentrates is outlined. (In French.) M. SULZBACHER.

Flavour mixtures. Essential trace compounds and less important main components. P. L. Elberg (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 772–773).—Investigation of flavour concentrates, separation of the components into classes of compounds and organoleptic assessment are reviewed in brief. The importance of trace components with low threshold values but relatively higher effective flavour contribution is stressed. (In English.) M. SULZBACHER.

Thermal decomposition of ferulic acid. W. Fiddler, W. E. Parker, A. E. Wasserman and R. C. Doerr (*J. agric. Fd Chem.*, 1967, **15**, 757–761).—Decomposition of the acid, an intermediate in the degradation of lignin, starts at 245°; products identified by GLC, mass spectrometry, and TLC were (under air or N_2) 4-vinylguaiacol (as the chief product), 4-methyl-, and 4-ethyl-guaiacol, and (under air only) vanillin, acetovanillone (4-hydroxy-3-methoxyacetophenone), and vanillic acid. A probable mechanism is proposed for these changes. A further degradation stage starts at 340°, yielding products of greater number and variety. The phenolic degradation products are probably the partial cause of the smoky flavour of hardwood smoke-cured foods. (13 references.) P. S. ARUP.

Food products or beverages of improved flavour. Abbott Laboratories (B.P. 1,065,369, 15.12.65. U.S., 16.12.64).—Flavour and texture of certain food products [starch product containing $\nless 45$ wt.-% of starch, e.g., white bread dough or cereal; fruit-flavoured gelatin; margarine, skim milk or ice-cream or beverages (fruit concentrate or non-dairy beverage whiteners)] are improved by adding a sub-threshold amount of cyclamic acid. F. R. BASFORD.

Colouring Matters

Carotenoids, natural food colours. O. Isler, M. Montavon and R. Ruegg (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 853–856).—After exposition of the biogenesis and the metabolism of carotenoids, industrial production methods for β-ionone and carotenoids are discussed. The series of β-apo-carotenoids is presented. Recent syntheses of rhodoxanthine, 2,2′-diketospirilloxanthine and analogues are briefly described. Compositions for use, toxicity and metabolism, and medicinal prep. are mentioned. (15 references.) (In French.) M. SULZBACHER.

Technical synthesis of food dyestuffs of the carotene series. H. Freyschlag, W. Reif and H. Pommer (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 42–43).—After a short review of the use of carotenes as food dyestuffs, a technical prep. of β-carotene from vitamin A acetate is outlined. The C_{20}-phosphonium salt, made from vitamin A acetate and Ph_3P is condensed with vitamin A aldehyde (retinene), by the Wittig reaction. A related synthetic method is used for the prep. of the apo-carotinals. The reagent used is the 'C_5-ylenal', which, for instance, gives with retinene the β-apo-12′-carotinal. (In German.). M. SULZBACHER.

Contamination of some food products [by degradation of dyestuffs and preservatives]. A. M. Lolua, I. N. Poutilova and L. E. Chernenko (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 818–819).—Transformation of food dyes of the azo type, e.g. amarant, tropeolin and chrysoidin and of preservatives such as sorbic acid in acid medium during contact with steel, and the influence of these substances on the dissolution rate of Fe in citric, malic, tartaric acids and in HCl have been examined. The combinations chosen inhibited the reaction of Fe with acids. Detrimental substances, e.g., hydro-azo compounds, aromatic amines and caproic acid were, however, formed. (In English.) M. SULZBACHER.

Preservatives

Application of the ring oven technique to the semi-quantitative determination of some antioxidants. S. M. Šibalić, V. M. Adamavić and N. Miletić (*Mikrochim. Acta*, 1967, No. 6; 1028–1030).—The Weisz ring-oven method was applied to the semi-quant. determination of four antioxidants commonly used in food; butylated hydroxyanisole (**I**), butylated hydroxytoluene (**II**), dodecyl gallate (**III**) and nordihydroguaiaretic acid (**IV**). **I**, **III** and **IV** formed stable colours with 0·5% 2,6-dichloroquinonechlorimide solution in ethanol and 0·5 to 2·5 μg were determined with errors of 5, 2 and 4% respectively, but **II** cannot be determined as the colour formed is unstable. G. W. FLINN.

Content of biphenyl in citrus fruits. A. Rajzman (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1967, **58**, 195–206).—Fruit pulps of oranges, lemons, and grapefruit (1201 samples) that had been stored in packings treated with biphenyl (**I**) contained 0·01–0·91 ppm of **I**; samples containing $>0·5$ ppm were $<11\%$. Results for 2179 whole fruits were 0·2–120·7 ppm; samples containing >70 ppm were $<12\%$. P. S. ARUP.

Effectiveness of EDTA as a fish preservative. R. E. Levin (*J. Milk Fd Technol.*, 1967, **30**, 277–283).—Concn. of 0·005% Na_4-EDTA (**I**) markedly inhibited the growth of psychrophiles *Pseudomonas putrefaciens*, *P. fragi* and *P. fluorescens*. *Achromobacter lipolyticum* was the only psychrophilic organism of those tested (20) that was insensitive to **I**. An application of 1% **I** to haddock fillets for 1 min. failed to suppress growth when stored at 3° but this temp. + dip extended the time of satisfactory storage (based on odour and taste) from 5 to 9–10 days. Formation of Me_3N and volatile basic N was markedly suppressed in **I**-dipped fillets. (14 references.) C.V.

Pesticides in Food

Residues in food and feed. R. E. Duggan (*Pestic. monitoring J.*, 1967, **1**, No. 3, 2–8).—Results on 8548 samples of fluid milk, 3598 samples of other dairy products, and 690 samples of manufactured dairy products imported into the U.S.A., are reported with reference to chlorinated pesticide residues. Those of DDT, DDE, TDE, dieldrin, heptachlor-epoxide, BHC, lindane, aldrin, heptachlor, and methoxychlor accounted for 99·3% and on a fat basis about 95% of the values were below 0·51 and 71·5% were below 0·11 ppm. Average levels for DDT and its analogues were 0·134 while those of dieldrin and heptachlor-epoxide were 0·042 and 0·036 ppm respectively. E. G. BRICKELL.

Determination of aldrin residues in vegetables by chemical conversion of aldrin to dieldrin. K. Noren (*Analyst, Lond.*, 1968, **93**, 39–41).—After extraction of pesticides with hexane–Pr^iOH, followed by clean-up with Florisil, the aldrin is oxidised with peroxyacetic acid at 65° and the resulting dieldrin (**I**) determined by GLC on a column of 5% DC-11 on Chromosorb W at 195°. Conversion is ~95% and recovery of **I** ~84% (for carrots, cabbage, and potatoes); other chlorinated pesticides are unaffected and do not interfere. W. J. BAKER.

Removal of DDT, malathion and carbaryl from tomatoes by commercial and home preparative methods. R. P. Farrow, F. C. Lamb, R. W. Cook, J. R. Kimbail and E. R. Elkins (*J. agric. Fd Chem.*, 1968, **16**, 65–71).—Commercial canning and juicing removed practically all of the residues, whilst home cooking removed most of the DDT and malathion, but only ~75% of the carbaryl. (25 references.) P. S. ARUP.

Food Processing, Refrigeration

Effect on weight of mushrooms preserved by blanching. A. Mehlitz and G. Geerds (*Dt. LebensmittRdsch.*, 1968, **64,** 140–142).—Mushrooms were blanched for 1, 2, 3 and 5 min. at temp. of 60°, 80° and 100° and weight changes and weights required to fill standard cans were noted. The condition and texture were also observed. The best products were obtained at 80° for 2 min.
J. B. WOOF.

Survival of aerobic and anaerobic bacteria in chicken meat during freeze-dehydration, rehydration and storage. J. R. Chipley and K. N. May (*Appl. Microbiol.*, 1968, **16,** 445–449).—Total and anaerobic counts were made on boneless cubed and frozen chicken meat. Survival after freeze-drying and rehydration at room temp. for 30 min. and at 50, 85 and 100° for 10 min. was determined. Samples were also inoculated with *Clostridium sporogenes* (**I**), dried and rehydrated at 100° and stored at 37°. Approx. 21% of the aerobes and 37% of the anaerobes survived drying and rehydration at room temp. While many genera of aerobes, anaerobes and facultative anaerobes survived drying and rehydration at 50°, only spore-formers survived this treatment at 80 or 100°. Low temp. (4°) storage gave a shelf-life >20 days; at higher temp. it was <30 h. Approx. 81% **I** survived rehydration at 100° and grew to $>10^7$ cells in 40 h.
C.V.

Preservation of meat by freezing. H. Beck (*Archos. bras. Nutr.*, 1967, **22,** 93–98).—The advantages and disadvantages of freezing at temp. of −17, −40 to 48 and −150° are studied. Damage caused to animal tissue by formation of ice crystals, by increase of osmotic pressure and by denaturisation of the colloidal constituents of the cells is discussed. The use of glucose is suggested for the protection of frozen meat and fish against dehydration and for the protection of fats from rancidity and also to prevent undue penetration of salt from freezing brines.
I. DICKINSON.

Thawing of deep-frozen bakery goods with microwaves. B. Belderok and M. J. M. van't Root (*Brot. Gebäck*, 1967, **21,** 221–224).—A brief explanation of the properties of microwaves, their possibilities and limitations, for use with bakery products.
E. C. APLING.

Packaging

Regulations for the use of plastic materials in food packaging. A. Rodeyns (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32,** (III), 481–484).—After exposition of the purposes and aims of regulations concerning plastic packaging materials, the legal position in France, Germany, Italy, Holland, Belgium, Austria, Spain, Switzerland, Gt. Britain and U.S.A. is shortly explained and agreement and standardisation on an international basis is called for to eliminate the existing differences. (16 references.) (In French.)
M. SULZBACHER.

Determination of plasticiser uptake from plastics [packaging] by foods. IV. Migration of di-(2-ethylhexyl)phthalate and Mesamoll from synthetic rubber into milk. A. Reichle and H. Tengler (*Dt. LebensmittRdsch.*, 1968, **64,** 142–145).—The milk sample, after addition of K oxalate to inhibit coagulation, is extracted with an ether–ethanol mixture. After removal of most of the solvent the residue is re-extracted with light petroleum and the sol. portion hydrolysed with ethanolic KOH. A known amount of triethylphenol is added to the original milk sample and this together with the cresol, phenol and ethylhexanol formed by hydrolysis of the plasticisers is steam-distilled off and determined by gas-chromatography. The column (2 m × 4 mm) packed with 10% P2000 on kieselgur is operated at 130° with He as carrier. The limit of detection is 2 ppm, an amount which was found to be picked up by milk at 38° within 60 min. of contact. (10 references.)
J. B. WOOF.

Determination of residual solvents in packaging materials. R. A. Wilks, jun. and S. G. Gilbert (*Mater. Res. Stand.*, 1968, **8,** 29–32).—The original hot-jar method (Gilbert *et al., Mod. Packaging*, 1965, 167) is modified (mainly to prevent loss of solvent) by use of an evacuated air-tight jar into which the sample is injected by a leak-proof gas-phase sampling and transfer device. After volatilisation of solvent by heating, a 5-ml sample of the headspace in the jar is injected into a 6-ft column of 10% Carbowax 20 M on Anakrom ABS for chromatography. For polymer-coated cellophane, the error was within 6% when either toluene or tetrahydrofuran (in μg concn.) was the solvent. Method is widely applicable, e.g., to evaluate paper and polyethylene wrappers printed and dried under various conditions and also to vinyl-lacquer-coated Al foil. Sensitive foods may pick up residual solvent during storage.
W. J. BAKER.

Variations in the amount of filled goods in pre-packaged foodstuffs. A. Fincke and P. Krauss (*Fette Seifen AnstrMittel*, 1967, **69,** 849–854).—In order to determine the wt. variations which occur in the amounts of packaged foodstuffs, about 120,000 individual weighings have been carried out in investigations over a wide variety of products and industries. From the results obtained, and in order to meet official legislation, methods of recording and determining tolerance limits, based on statistically analysed results, are proposed. Such methods also take into account technical and economic factors.
G. R. WHALLEY.

The influence of vacuum on the stability of bread packs. A. Schultz (*Brot. Gebäck*, 1967, **21,** 208–212).—The effect of reduced pressure in limiting growth of seven common mould types is reported for plate cultures and for rye bread packed in various packaging materials. Practical storage trials of rye bread packed in three different laminates, using either heat sterilisation or evacuation and gas packing in CO_2 showed that heat sterilisation gave a more stable and better quality pack.
E. C. APLING.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Nutritional evaluation of food-protein with micro-organisms. I. Studies of evaluation. Y. Ichinose and E. Matsui (*A. Rep. natn. Inst. Nutr., Tokyo*, 1966, 32–33).—Nutritional assessment of food proteins based on the growth of *Leuconostoc mesenteroides* P-60 are briefly reported. Estimates of biological value obtained for five food proteins were in agreement with literature values.
E. C. APLING.

Plant-protein concentrates as human food. N. W. Pirie (*Chemy Ind.*, 1968, 864–866).—The composition, selection and optimum processing of oil-seeds, leafy vegetables, legumes, flours (wheat, rice, maize) and leaves (natural and by-product) to obtain protein concentrates are discussed. Methods of improving the palatability of the concentrate are indicated, and economics of production are briefly noted.
W. J. BAKER.

Potential of microbiological protein. D. W. Ribbons (*Chemy Ind.*, 1968, 867–870).—Discusses the suitability and versatility of yeasts, algae and certain bacteria to produce protein by microbial fermentation or growth on e.g. paperwaste effluents, petroleum fractions, molasses, CH_4, CO_2, etc. Factors influencing the use of microbial proteins for animal or human food are examined, viz. digestibility and the enzymatic chemistry (as yet little known) of the bacterial cell-walls during the nutritional cycle. Advantages of microbial over agricultural culture are listed.
W. J. BAKER.

Recent developments in fermentation of petroleum fractions. C. Vernet, B. Laine and G. Evans (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32,** (III), 774–779).—A survey of the fermentation of petroleum fractions for the production of protein deals with general aims and gives a description of the process operated by BP in Lavéra (France) and Grangemouth. The fermentation, recovery and washing and final purification are discussed. Composition of the products is presented and evaluated. (In French.)
M. SULZBACHER.

Nutritive value for rats of certain by-products of the corn refining industry. D. A. Christensen, L. E. Lloyd and E. W. Crampton (*J. Nutr.*, 1967, **91,** 137–142).—Growing rats were used to evaluate egg replacement value and growth promoting ability in relation to amino-acid content arising from wet milling of corn. In the first experiment the diets consisted of 12·4% crude protein derived entirely from whole dried egg, corn steep-water solids, corn gluten and germ oilmeal (**I**), corn fine bran (**II**), zein and zein-extracted gluten or reconstituted starch-free corn (**III**). Despite superior chemical potential and essential amino-acid index the protein quality of the corn germ oilmeal (measured by egg replacement value) was lower than that of all the proteins except **II**. In a second experiment protein from the same sources except **II** was fed to weanling rats in diets containing 17% crude protein. Growth and efficiency confirmed the previous work that **I** would not support growth and that **III** produced the most rapid and efficient gains. Growth and efficiency, however, were poorer than with egg protein. (19 references.)
C.V.

[I] Binding of fatty acids by proteins. [II] Denaturation of proteins by fatty acids. H. B. Bull and K. Breese (*Arch. Biochem.*

Biophys., 1967, **120**, 303–308, 309–315).—I. Egg albumin has been titrated with acetic, propionic, n-butyric, i-butyric, n-valeric, n-caproic and n-heptanoic acids; the method of equilibrium dialysis was used. At low concn. of acid, the binding at a given acid concn. is the same for all acids and after a critical concn. is reached, depending on the mol. wt. of the acid, the extent of binding increases geometrically with the length of the C-chain of the acid. It is likely that the unionised acids are bound at higher acid concn. and no limit to the binding is noted. Several binding sites are probably involved; e.g. the peptide bonds.

II. Fatty acids are denaturants of egg albumin and the effectiveness increases with the length of the C-chain. The extent of denaturation is measured by the solubility of the protein at, or near the isoelectric point in the presence of Na_2SO_4. Degree of denaturation depends on pH, acid concn., etc. The energy of activation in the presence of acetic acid is ~33000 cal./mole and it is necessary to bind ~10 moles fatty acid per mole protein before denaturation begins. Changes in viscosity, appearance and tendency to gelation with the onset of denaturation are discussed. C.V.

Gelatin: how it is made. F. Kramer (*Fd Engng*, 1967, **39**, No. 11, 74–77).—The essentials are outlined and a flow sheet is presented. A continuous process (U.S.P. 2,743,265) is also described. C.V.

Availability of amino-acids from selected proteins. A. Bailur (*Diss. Abstr.*, B, 1968, **28**, 3763).—Availability of amino-acids in three proteins, casein-lactalbumin (5 : 1), peanut meal and wheat gluten was studied by (*a*) acid hydrolysis (*b*) *in vitro* digestion with peptic and pancreatic enzymes and (*c*) *in vivo* studies with rats. The studies on rats showed that in weanlings, threonine (**I**) appeared to be available from peanut meal as measured by wt. gain, but with adult, protein-depleted rats, **I** was not completely available from the protein, as indicated by wt. gain and N retention values. P.P.R.

Biological value of foods. J. Ruiz Santaella (*Confcias Inst. nac. Invest. agron.*, 1966–1967, 153–185).—Recent advances in the use of the concept 'biological value' in the evaluation of foodstuffs are reviewed. Data include the influence on 'biological value' of various amino-acid supplementations of maize bran, linseed- and sunflower-cake, and of methionine supplemention of casein. (29 references.) E. C. APLING.

Isomerisation of vitamin A in fish liver oil. H. Baba (*A. Rep. natn. Inst. Nutr., Tokyo*, 1966, 36–38).—Chromatographic separations of the isomers produced by isomerisation of vitamin A (**I**) by reaction with maleic anhydride, and studies of the effects of the globular fraction of liver cell and of phospholipid on the isomerisation of **I** in fish liver oil, are briefly reported. E. C. APLING.

Production of riboflavin by a *Candida utilis* mutant. G. Florenzano, W. Balloni and R. Materassi (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 789–790).—Observations that a Co-induced mutant of *C. utilis* possessed remarkable flavinogenic activity led to tests on the conditions governing the synthesis of riboflavin. It was found that production of riboflavin depends greatly on the presence of Co in the culture medium. Best yields are obtained with Co concn. between 10 and 20 μg/ml. (In French.) M. SULZBACHER.

Thiamine, pantothenic acid and pyridoxine content in three *Candida* species in relation to carbon source. M. R. Borukaeva (*Microbiology*, [*USSR*], 1967, **36**, 345–349).—*Candida lipolytica* (*CL*), *C. utilis* (*CU*) and *C.* sp. F-201 (*CF*) were studied and considerable differences found in the thiamine (**I**), pantothenic acid (**II**) and pyridoxine (**III**) content of the cells, depending upon the nature of the C source. The effect on *CL* of replacement of glucose in the medium by paraffin is a reduction in the contents of **II** and **III**. The reaction of *CU* to a replacement of glucose by acetate is shown by an increase in **I** and decrease in **II** and III. *CF* reacts to a substitution of phenol for glucose by an increase in **I** and **III**; **II** remains unchanged. C.V.

Accumulation of B-group vitamins by *Candida tropicalis* utilising petroleum hydrocarbons. E. I. Kvansikov, D. M. Isakova and V. I. Vaskivnyuk (*Microbiology*, [*USSR*], 1967, **36**, 784–788).—The vitamins can be divided into two groups according to their pattern of accumulation in the cells of *Candida tropicalis* K 41. The first group consists of thiamine and pantothenic acid (**I**) and nicotinic acids (**II**). The second comprises riboflavine (**III**), pyridoxine, biotin and *p*-aminobenzoic acid. The concn. of these remains constant during cultivation of yeasts in batch or continuous flow cultures and appreciable amounts of the second group are released into the medium. The yeast strain studied produced more vitamins, specially **I**, **II** and **III** on media containing hydrocarbons than on a glucose medium. (11 references.) C.V.

Use of ascorbic acid in bread production. P. E. Marston (*Baker's Dig.*, 1967, **41**, No. 6, 30–33, 70).—*l*-Ascorbic acid (**I**) is an effective improver over a wide range of conditions; it is accepted as a safe food additive in many countries and virtually all wheat flours are tolerant to the presence of **I** in doughs at levels well above their probable requirements. The breadmaking processes discussed indicate the changing emphasis in bread technology. In spite of apparent limitations of **I** as a dough maturing agent, it has now become well established as a useful improver and when its mode of action becomes more fully understood further progress may be made. (17 references.) I. DICKINSON.

Rôle of sulphurous acid in peroxidase oxidation of ascorbic acid in presence of hydrogen peroxide. II. Activity of peroxidase enzymes in some sulphited juices and pulps for consumption. E. Monikowski and H. Chmielnicka (*Revue Ferment. Ind. aliment.*, 1967, **22**, 141–144).—Further to Part I (cf. *ibid.*, 1964, **19**, 113) where it was shown that the rate of oxidation of ascorbic acid depends on the (thermoresistant) peroxidase activity of the juice or pulp, experiments were made to determine the losses of peroxidic activity in preserved pulps in presence of H_2SO_3 in concn. of 0–1400 mg of SO_2 per 100 g. Max. inhibition was observed at pH 3·5 to 4·4. Complete inhibition after 8 weeks was attained with ~125 mg per 100 g for apples, and 1400 mg per 100 g for horseradish; intermediate dosages were required for tomatoes and cabbages. P. S. ARUP.

Stability of vitamin E during storage of wheat and various wheat products. V. Hellström and R. Andersson (*Getreide Mehl*, 1967, **17**, 129–131).—Determinations of total tocopherol (**I**) were carried out on 11 samples before and after storage for up to 12 months at 15°, by hydrolysis with MeOH-KOH, extraction and measurement of total Fe (III)-reducing substances with α-α′-dipyridyl. The total **I** content could be separated by TLC on silica gel into two main fractions: (*i*) **α-I** and **ζ$_1$-I** (α-tocotrienol) and (*ii*) **β-I** and **ε-I** (β-tocotrienol). (17 references.) E. C. APLING.

Unclassified

Food science and technology. M. Pyke (*John Murray, Lond.*, 1968, 2nd Ed., 219 pp).—The general principles are reviewed and traditional methods are summarised. The second part deals with practical technology of cereal chemistry (wheat, rice, barley, oats and maize), meats, fish and poultry, milk, cheese and eggs, fats and oils, fruits and vegetables, the technological aspects of canning, quick freezing, accelerated freeze-drying, dehydration and the application of radioactivity, and the use of chemical additives. Training and employment of food technologists is examined and the general trends during the next forty years are adumbrated. Five appendices are included: daily allowances recommended by BMA, nutritive composition of foods, 'development' of dough, sugar, syrup and soft drinks, and aseptic canning. C.V.

Biochemical aspects of the effects of alcohol on the central nervous system. H. Wallgren (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 812–815).—Theories about the mode of action of alcohol are discussed. Its effects on the cerebral metabolism in the intact organism, and on the functioning of excitable cell membranes are considered. The pharmacological activity of aliphatic alcohols is reviewed; it is suggested that the slight polarity and the lipid solubility of alcohols are important factors in their effect on nerve cells. A possible mode of interaction with membrane lipoids is assumed. (34 references.) (In English.) M. SULZBACHER.

Use of enzymes by the food industry. E. Beckhorn (*Industrie chim. belge, Chimie et Civilisation*, 1967, **32**, (III), 833–837).—Recently developed food processing methods utilising enzymes are described. These include use of amyloglucosidase for dextrose production, use of *B. subtilis* α-amylase for starch liquefaction, post mortem tenderisation of beef carcasses by injection of proteolytic enzymes, ham tenderisation by controlled chemical inactivation of a proteolytic enzyme, use of *B. subtilis* α-amylase to retard staling of bread and bakery goods and new methods for recovery and processing of fruit juices, eliminating the need for presses. Characteristics of the enzyme systems and their selection are discussed. (34 references.) (In English.) M. SULZBACHER.

Effect of lipid materials on the production of lipase by *Torulopsis ernobii*. F. Yoshida, H. Motai and E. Ichishima (*Appl. Microbiol.*, 1968, **16**, 845–847).—This yeast produced ~50 units extracellular lipase (glycerol ester hydrolase) per ml when grown in 50-l fermentors

at 33° for 40 h in a medium at pH 5·0. Addition of fats, oils, triglycerides or higher fatty acids to this medium (0·2 to 0·6% concn.) increased production, a two fold increase being obtained with 0·2% olive oil or 0·14% oleic acid + 0·04% palmitic acid. Production of lipase paralleled growth and the rôle of lipid materials in augmenting lipase production appeared to be related to cell growth. C.V.

Lipase activity of *Mucor pusillus*. G. A. Somkuti and F. J. Babel (*Appl. Microbiol.*, 1968, **16**, 617–619).—Two strains of *Mucor pusillus* were examined for their ability to synthesise lipase (**I**) in a complex medium used to produce milk-clotting protease. In both strains max. activity was reached in 6 days incubation under submerged condition at 35°. **I** secreted into the hydrolysed butter fat and vegetable lipids as well as selected synthetic triglycerides; ~50% of the **I**-activity was destroyed at 58° for 45 min. C.V.

Extracellular lipids of yeasts. F. H. Stodola, M. H. Deinema and J. F. T. Spencer (*Bact. Rev.*, 1967, **31**, 194–213).—A general discussion and review. (151 references.) C.V.

Studies on the utilisation of *Chlorella:* [by-product yields]. Kunihiko Shino (*A. Rep. natn. Inst. Nutr., Tokyo*, 1966, 40–42).—Results of a pilot trial of the separation and fractionation of lipid material from 30 kg of mass culture wet algae are reported. E. C. APLING.

Glycerol ester hydrolase activity of lactic acid bacteria. A. Oterholm, Z. J. Ordal and L. D. Witter (*Appl. Microbiol.*, 1968, **16**, 524–527).—17 strains were examined using an improved agar-well technique and eight by determining the activity in cell-free extracts using a pH-stat procedure. All cultures showed activity and hydrolysed tributyrin more actively than tricaproin. The cell extract studies showed that they contained intracellular esterases (*E*) and lipases (*L*). The culture supernatant fluid was without activity. *L* and *E* differed in their relative activity to each other in the different extracts and in the ease with which they could be freed from cellular debris. It is suggested that *L* of these organisms is an endozyme and *E* an ectozyme. C.V.

Growth of micro-organisms at extremes of temperature [a symposium]. *J. appl. Bact.*, 1968, **31**, 1–107).—**Physiology of micro-organisms at low temperatures.** A. H. Rose (48 references). **Physiology of growth at high temperatures.** L. L. Campbell and B. Pace (62 references). **Thermophilic actinomycetes.** T. Cross (97 references). **Occurrence and significance of thermophiles in canned foods.** T. G. Gillespie and R. H. Thorpe (10 references). **Thermophiles in sugar.** M. P. Scarr (21 references). **Low temperature growth characteristics of *Clostridia*.** T. A. Roberts and G. Hobbs (80 references). **Psychrophilic spoilage bacteria of fish.** B. G. Shaw and J. M. Shewan (18 references). **Psychrophilic spoilage bacteria of poultry.** E. M. Barnes (23 references). C.V.

Two improved media for isolating and enumerating enterococci in certain frozen foods. R. V. F. Lachica and P. A. Hartman (*J. appl. Bact.*, 1968, **31**, 151–156).—Direct plating was used with two selective media: Tween-carbonate (*TC*) and thallous acetate–citrate (*TAC*). Four other media were also studied. *TC* and *TAC* were most selective but *TC* agar at pH 6 inhibited enterococceal growth in the absence of bicarbonate. (23 references.) C.V.

***Clostridium bolulinum* type E. Growth and toxin production in food.** M. Ajmal (*J. appl. Bact.*, 1968, **31**, 120–123; 124–132).—Incubation at 30° yielded max. numbers of viable cells but toxicity did not differ from cultures grown at 22°; this indicated that toxin synthesis per cell was greater at the lower temp. Toxin was produced under both aerobic and anaerobic conditions. Irradiated fish was more susceptible to toxin production than unirradiated. Toxic outgrowth was found in two products of ham and one of cured ox tongue with NaCl concn. <3·7% and <3 ppm nitrite. Corned beef containing 4·6% NaCl and 60 ppm nitrite did not permit growth even with an inoculum of 1×10^5 spores/g. (22 references.) C.V.

Use of phenolphthalein diphosphate agar with polymyxin as a selective medium for isolation and ennumeration of coagulase-positive staphylococci from foods. B. C. Hobbs, M. Kendall and R. J. Gilbert (*Appl. Microbiol.*, 1968, **16**, 535).—Eight media, and their use, are described. The countries using these are indicated. C.V.

Food-borne virus: detection in a model system. J. E. Herrmann and D. O. Cliver (*Appl. Microbiol.*, 1968, **16**, 595–602).—A model system based on 25 g samples of cottage cheese was used; this was contaminated with various quantities of coxsackie virus type AG. The technique used is described. C.V.

Yeast product. H. Griffon and G. Tixier (B.P. 1,073,030, 6.11.64. Fr., 6.11.63).—The present claims arise out of B.P. 970,944. Yeast and starch are formed into tablets, from a homogeneous suspension of yeast in water to which starch is added until a soft paste is obtained, this then being granulated and dried at ≯37° until the moisture content is 8–15% by wt. and the composition contains a final wt. of yeast cells of 60–80%. Thus, 100 kg yeast of known water content is added to 30 l purified water and mixed until a homogeneous paste is obtained. A calculated quantity of starch is added depending on the moisture content of the yeast, e.g. 66·6 kg starch for 100 kg yeast of 13% moisture content, and the mixture agitated and then granulated by a 3 mm mesh screen. The grains are dehydrated at 37°, until the moisture content is 8–10% by wt.; a light beige-coloured powder is obtained. Pressures of ~1800 kg/cm² used in prep. of tablets ensured death of >90% of the yeast cells. S. D. HUGGINS.

3.—SANITATION, WATER, etc.

Comparative trial of norbormide and zinc phosphide against *Rattus norvegicus* on farms. B. D. Rennison, L. E. Hammond and G. L. Jones (*J. Hyg., Camb.*, 1968, **66**, 147–158).—Norbormide (**I**) [5-(α-hydroxy-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)norborn-5-ene-2,3-dicarboximide] (1·0 and 0·5%) and Zn phosphide (**II**) (5·0 and 2·5%) were compared in four types of cereal bait after pre-baiting and as direct poisons. Treatments with **II** were significantly more successful than those with **I** irrespective of the cereal bait, concn. of poison or method of treatment. **I** is recommended in such situations where **II** cannot be used on account of risk to livestock. C.V.

Systemic insecticides for control of oriental rat fleas: bait tests with hooded white rats. P. H. Clark and M. M. Cole (*J. econ. Ent.*, 1968, **61**, 505–508).—When added to the diets of rats, diazinon, fenthion, Mirex and trichlorfon, were effective against *Xenopsylla cheopis* at various dosages. Butonate, Dimethoate, menazon and Thiocron [*O,O*-dimethyl phosphorodithioate *S*-ester with 2-mercapto-*N*-(2-methoxyethyl)acetamide] were ineffective. C. M. HARDWICK.

Systemic insecticides for control of oriental rat fleas: tests in guinea pigs. P. H. Clark and M. M. Cole (*J. econ. Ent.*, 1968, **61**, 420–423).—The effect on *Xenopsylla cheopis*, of oral administration of 29 compounds to guinea pig hosts, is given. Fourteen compounds killed all fleas within 5 h. Five others were also fatal to the host. C. M. HARDWICK.

Insecticide resistance of German cockroaches from various areas of Louisiana. G. W. Bennett and W. T. Spink (*J. econ. Ent.*, 1968, **61**, 426–431).—*Blattella germanica* from seven locations were compared with a susceptible strain for resistance to chlordane, malathion, diazinon, Baygon and fenthion, by topical application. Resistance at LD_{50} level was highest to chlordane and at LD_{90} to malathion. (13 references.) C. M. HARDWICK.

Gas chromatographic determination of dimethyl fumarate in malathion extracts and preparations used for control of body lice. F. Acree, jun., R. B. Turner and M. Beroza (*J. econ. Ent.*, 1968, **61**, 457–459).—Analysis by electron capture and flame ionisation, in cloth, dusts and commercial prep., was used to determine the rate of loss of dimethyl fumarate due to volatility. Malathion was not volatile. C. M. HARDWICK.

Metabolism of insecticides by *Culex pipiens quinquefasciatus*. I. *In vivo* metabolism of DDT by larvae. G. H. S. Hooper (*J. econ. Ent.*, 1968, **61**, 490–493).—The metabolism of DDT to DDE and TDE was studied in five susceptible and one DDT-resistant strain of the southern house mosquito, by gas–liquid chromatography and thin layer chromatography. (21 references.) C. M. HARDWICK.

Ovicidal activity of aliphatic amines and petroleum oil against two species of mosquitoes. M. S. Mulla and M. F. B. Chaudhury (*J. econ. Ent.*, 1968, **61**, 510–515).—Most of a homologous series of 19 primary aliphatic amines were highly effective against eggs of *Anopheles albimanus* but only oleylamine was effective against eggs of *Culex pipiens quinquefasciatus*. Younger eggs were more susceptible than older ones. 24-h exposure was necessary to be effective. Petroleum oil at 25 ppm caused high mortality by lowering the surface tension, causing egg rafts to sink. The effect of treatment on embryonic development is discussed. C. M. HARDWICK.

Uptake of ^{32}P by yellow-fever mosquito larvae of equal age, and those pupating synchronously: its loss and transfer during development, mating and oviposition. M. S. Quraishi (*J. econ. Ent.*, 1968,

61, 530–533).—Females took up more radioactive P than did the males. Uptake varied with the stage of development. Little radioactivity was transferred to a normal female during her first copulation. (15 references.) C. M. HARDWICK.

Susceptibility studies on *Anopheles culicifacies* with DDT and dieldrin in Gujarat state, India. A. M. Shalaby (*J. econ. Ent.*, 1968, **61,** 533–541).—DDT and dieldrin resistance showed a >4% rise at the LC_{50} level over an 18 month period. Variations in susceptibility in different parts of the state were related to their record of insecticide application. C. M. HARDWICK.

Effect of post-treatment temperature on toxicity of insecticides to resistant and suceptible houseflies. E. S. Evans, jun. and E. J. Hansens (*J. econ. Ent.*, 1968, **61,** 543–546).—Laboratory and field strains of houseflies were treated topically with diazinon and lindane, and then held at 15° or 30° for varying periods. An interaction was found between post-treatment temp., degree of resistance and the insecticide. C. M. HARDWICK.

Binding of a cationic detergent by yeast cells in relation to germicidal action. T. Fujita and S. Koga (*J. gen. appl. Microbiol., Tokyo*, 1966, 229–237).—Yeast cells suspended in a solution of cetyl-trimethylammonium bromide (**I**), a typical cationic detergent, rapidly bind **I**-ions, this action being complete in <2 min.; germicidal action continues for at least 1 h. The mortality of the **I**-treated cells depends on the wt. ratio of bound **I** to yeast cells, 10 times the cell surface area having to be covered by bound **I** to ensure a 100% kill. C.V.

Ultra-violet bactericidal irradiation of ice. P. A. Ladanyi and S. M. Morrison (*Appl. Microbiol.*, 1968, **16,** 463–467).—Germicidal activity of 2537 Å radiation on bacteria in ice cubes of varying thickness and in aq. suspensions beneath the ice layer was studied, *Escherichia coli*, *Serratia marcescens*, *Bacillus subtilis* and *Sarcina lutea* being used. Details of experimentation are given; u.v. for 1 min. killed 97% gram negative and at least 60% gram positive bacteria in the ice cubes (30 mm thick) and 15 min. exposure of a 19 cm thick block of ice inactivated 98% of bacteria suspended in a buffer solution. Certain selected applications are considered. (15 references.) C.V.

Effect of bacterial cell moisture on the sporicidal activity of β-propiolactone vapour. R. K. Hoffman (*Appl. Microbiol.*, 1968, **16,** 641–644).—The activity of a vapour-phase disinfectant is usually expressed in terms of R.H. With β-propiolactone (**I**) the important factors are the moisture content and location of water in the cell, not necessarily R.H. The experimental work is described. It is shown that dessicated spores must be exposed to **I** at higher humidities than those normally used when dessication has not been resorted to. (10 references). C.V.

Bactericidal activity laboratory test. J. C. Curry (*Soap chem. Spec.*, 1968, **44,** No. 3; 40, 42, 150).—A modification of the method of Barber (*J. Milk Fd Technol.*, 1949, **12,** 257) for determining bactericidal activity in the laboratory, is described. This procedure utilises the bactericidal contact time (*BCT*), and is primarily concerned with product application, and such products are tested at their use concn. against a type and no. of organisms, at a practical temp. To equate the *BCT* test with the Chambers test, the initial count should be 10^8/ml and a 0·01 ml loop employed to an end-point of 99·99% kill. The results are all recorded as a function of time to effect a certain degree of kill. G. R. WHALLEY.

Water re-use in the beet sugar industry. G. W. Crane (*Effluent Wat. Treat. J.*, 1967, **7,** 639–644).—Complete re-use of water can reduce effluent from 1,171 to 20 tons per 100 tons sliced beets, representing a saving of 10^6 gal/day at a factory processing 2,400 tons beet daily. This, perhaps optimum figure, is discussed. The stored water improves in quality by natural purification, the BOD falling from 2000 ppm as produced, to 100 ppm in September and part of this stored water can be used to fill the condenser and transport water systems to start up the following season. C.V.

Carbamic acid esters. Farbenfabriken Bayer A.-G. (Inventors: C. Fest and G. Hermann) (B.P. 1,077,285, 27.10.65. Ger., 29.10.64).—The esters have the formula $FCH_2CONHCH(CCl_3)$-$OCONR^1R^2$, (**I**), wherein R^1 is H or (1–4 C) alkyl or (1–10 C) aryl, R^2 is alkyl (1–4 C) or aryl (1–10 C) and R^1 and R^2 may be substituted by alkoxy (1–4 C), halogen, NO_2 or CN, or may, with the N atom, form a heterocyclic ring. In an example, chloral fluoroacetamide is dissolved in Et_2O and reacted at 15–20° with Me_2NCOCl in presence of pyridine for 12 h, to give the **I** in which R^1 and R^2 are both Me, of m.p. 105°. The compounds are active against short-tailed mice e.g. *Microtus arvalis* and *Arvicola terrestris* but have low toxicity towards other rodents such as white mice. S. D. HUGGINS.

4.—APPARATUS AND UNCLASSIFIED

Steels for the fertiliser industry. J. O. Edström and G. Lindh (*Chem. Age India*, 1967, **18,** 779–788).—The manufacture of intermediate and final fertiliser products results in severe strength and corrosion problems. The commercial quality of high alloyed steels available for use in plants for synthesis gas, hydrogen, ammonia, ammonium sulphate, ammonia oxidation, nitric acid neutralisation, urea, wet and dry superphosphate, are described. K. GRAUPNER.

Statistical evaluation of results of investigations on foodstuffs and [other] necessities carried out by statistical analysis. E. Renner (*Dt. Lebensmitt Rdsch.*, 1968, **64,** 148–150).—A discussion of the statistical handling of results from large scale analyses, especially of foods, tobacco and cosmetics, and the significance of average values and standard deviations. J. B. WOOF.

Vanadium in some biological specimens. R. Soremark (*J. Nutr.*, 1967, **92,** 183–190).—Concn. of V was studied in common fruits and vegetables, animal foods and drinking water. Thermal neutron activation analysis was used. Concn. were generally very low (10^{-4} ppm) but pronounced differences among species were found. Lobster, gelatin, mackerel, parsley, dill, lettuce, radishes, sardines and calves liver were relatively rich. Uptake by vegetables from the soil varied from one vegetable to another. (35 references.) C.V.

Determination of strontium in environmental media. A. B. Strong, G. L. Rehnberg and U. R. Moss (*Talanta*, 1968, **15,** 73–77). —A flame-photometric method for the determination of Sr in milk, composite foods, vegetation and marine biota and bone samples is described. It is rapid and inexpensive and samples can be analysed on a mass scale. The Sr is separated on Dowex 50W-X8 cation exchange resin and the emission line at 460·7 nm is used for analysis. The recovery ranged from 85 to 90%. A. S. BURRIDGE.

Improved method for determination of sulphonamides in milk and tissues. F. Tishler, J. L. Sutter, J. N. Bathish and H. E. Hagman (*J. agric. Fd Chem.*, 1968, **16,** 50–53).—Sulphonamides (**I**) are extracted from milk with $CHCl_3$-$COMe_2$ (2 : 1), and after evaporation of the solvent, dissolved in hexane from which they are extracted into 0·5 N-HCl. The determination is based on the measurement at 545 nm of the colour developed on diazotisation of the **I** and coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride. Procaine, penicillin G, or other amines, if present, are removed by making the 0·5 N HCl solution alkaline and extracting with $CHCl_3$; the solution is then re-acidified for the determination of **I**. The extraction from tissues is carried out in a blender with $CHCl_3$–$COMe_2$ (1 : 1). The method is sensitive to 0·1 ppm; **I** recoveries were 75–100%. P. S. ARUP.

Chemistry and structure of some borate polyol compounds of biochemical interest. V. Weser (*Structure & Bonding*, 1967, **2,** 160–180).—The structure and reactivity of boric acid, boric esters and borate complexes are discussed and methods of determination (potentiometry, electrophoresis, optical rotation, NMR spectroscopy, chromatography) are reviewed. The reaction of borates with saccharides, nucleotides, nucleosides, aromatic polyols etc. are examined. (77 references.) C.V.

[A] **4(4-Oxo-2,6,6-trimethyl-2-cyclohexen-1-yl)-1,3-butadiene in tobacco.** [B] **Preparation of [tobacco improver].** R. J. Reynolds Tobacco Co. (B.P. 1,078,423–4, 6.11.64. U.S., 29.11.63).—[A] Improved aroma and flavour results from the application of a liquid solution containing 0·005–0·03% by wt. of the title compound (**I**).

[B] A 55% yield of **I** is obtained by reduction of 5-oxo-α-ionone (prep. described) with $NaBH_4$ in aq. MeOH to 5-oxo-α-ionol, which is then dehydrated to the **I**, by heating with $KHSO_4$. S. D. HUGGINS.

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ABSTRACTS

OCTOBER 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.

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