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THE CHARACTERIZATION OF PHYTIN IN PEAS

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Phytin has been extracted from peas by a method depending upon its insolubility in hot 8% acetic acid. It has been characterized on the paper chromatogram and found to be a pure compound. Micro-analysis of this compound gives a carbon–phosphorus ratio compatible with that of a hexaphosphate. Comparison of pea phytin with commercial and synthetic calcium phytate (prepared by Posternak's method) using paper partition chromatography has shown that commercial phytin is composed of two organic phosphates (in addition to orthophosphate), one of which resembles pea phytin. The various compounds formed in addition to phytic acid in Posternak's synthesis have been examined and some have been tentatively identified.

Introduction

Phytin, a mixed calcium–magnesium salt of inositol hexaphosphoric acid, first described by Palladin,[1] was later shown to be an inositol compound by Winterstein,[2] after its extraction from the seeds of Indian mustard, *Sinapis nigra*, by acetic acid and precipitation by ammonia. Although it is universally present in the plant kingdom, especially in seeds, where it is thought to act as a phosphorus reserve, it has rarely been found in animals. It has however assumed considerable importance in human nutrition because phytic acid especially in cereals is thought to render calcium unavailable and therefore to be one of the causes of rickets in infants.[3]

The chief interest in this compound for the purpose of this investigation is centred upon its supposed action in softening peas during cooking by acting as a calcium absorbent. The calcium is thus prevented from combining with the pectin of the middle lamellae of the cells; it would otherwise form an insoluble calcium pectate and hence a hard cooking pea. This view has been expressed by Mattson,[4] and some preliminary work on the estimation of phytin phosphorus in peas was carried out at this Station by Holt.[5]

Holt's method which depends upon the diminution in colour of ferric thiocyanate by the phytate ion suffers from certain disadvantages as a method for phytin determination. The most important is that a number of other compounds can cause a diminution in the colour of ferric thiocyanate, many of which may be present in peas. Holt himself mentions orthophosphate, which from work done here is thought to preponderate in the immature pea. This, therefore, renders the method of doubtful validity except when peas are mature and the orthophosphate content appears to be low. [In this paper the terms 'orthophosphate' and 'pyrophosphate' refer to free or inorganic phosphate.]

It seemed desirable in view of these criticisms to extract phytin from peas, to examine its composition and purity by paper chromatography, and to compare it with commercial phytin used as a standard by Holt, and synthetic calcium phytate prepared by Posternak's method.[6]

Experimental

An improved method of extraction based on that of Clarke[7] also described by Boutwell[8] was used as follows:

500 g. of pea flour were mixed with 2 litres of 2% v/v hydrochloric acid and left in a cold room for 16 hours. After removal of large particles by means of a sieve, the mixture was treated with 800 ml. of 12·5% trichloroacetic acid and stirred for a short while. The proteins were then precipitated as a solid whitish mass. After keeping for about an hour, the mixture was separated on a large Buchner funnel containing no filter paper and without application of vacuum. The resultant liquid was clear and light brown in colour. It was heated to boiling, 10% aqueous ammonia added until the reaction was slightly alkaline to litmus, and then boiled again for a short time. A flocculent precipitate was formed and the solution changed to greenish brown. These changes also take place when ammonia is added in the cold. After keeping the mixture overnight in a cold room, the precipitate was filtered off on a Buchner funnel through two layers of No. 54 Whatman paper, washed with hot water and then dissolved in 400 ml. of 8% v/v acetic acid. On heating this solution, a white precipitate formed, part of which redissolved on cooling. The undissolved portion was filtered off, and to the solution was added a slight excess of ammonia

solution and 95% alcohol. After boiling for a few minutes, the precipitate formed was filtered on a Buchner funnel through Whatman No. 540 paper, well washed with hot water, with 70% alcohol and finally with ether and dried. The yield was 1·5 g. of an intensely white solid. A duplicate micro-analysis of the dried compound by Drs. Weiler and Strauss gave C = 8·21, 8·58 and H = 2·84, 3·07 and P (single determination) = 17·0%. The carbon, hydrogen and phosphorus ratios in this compound are 1 : 4 : 1 which is consistent with a compound having the formula of a hexaphosphate.

(a) Chromatographic examination of pea phytin

This compound in dilute acetic acid solution was found to run as a single spot on paper chromatograms, and was completely free from orthophosphate. The technique used was that of Longenecker[9] for ascending chromatography modified by the author[10] using 1-litre jars and Whatman No. 541 paper. The R_F values obtained in the solvent mixtures found to be most suitable are shown in Table I, together with the corresponding values for orthophosphate and pyrophosphate which will be referred to later. As the R_F values are known to vary under certain conditions, some of which are mentioned below, the average for a large number of estimations has been given where possible. In all cases acidic solvents were preferred to basic as they gave better movement and did not necessitate the pre-washing of the filter paper. Nevertheless, improved running was always observed where pre-washing with dilute hydrochloric acid and water had been carried out.

Table I

Chromatography of pea phytin

Solvent mixture	Proportions	R_F values		
		Phytin	Pyrophosphate	Orthophosphate
*iso*Propanol–picric acid–water	40 ml. : 1 g. : 20 ml.	0·17	0·51	0·67
tert.-Butanol–picric acid–water	80 ml. : 3·5 g. : 25 ml.	0·12	0·41	0·73
tert.-Butanol–trichloroacetic acid–water	80 ml. : 4 g. : 20 ml.	0·10	0·22	0·59
tert.-Butanol–formic acid (98/100%)–water	60 ml. : 20 ml. : 30 ml.	0·17	0·29	0·48*

* Determined in pea extracts. In pure solution a value of 0·6–0·7 is obtained.

The procedure adopted was to apply about 1 μl. of the appropriate solution (usually 1% w/v with orthophosphate applied as the sodium salt) to a spot on a line drawn about 2 cm. from the bottom of the paper. Equilibration of the system was usually fixed at 3 hours and the chromatogram was run for 18 to 24 hours. Shorter periods of equilibration gave higher absolute R_F values, as did also longer periods of run. After air-drying the paper, the spots were located by means of a molybdic acid spray.[11]

The use of picric acid in solvent systems for sugar phosphates was suggested by Hanes & Isherwood[11] and proved quite suitable also for qualitative work with inositol phosphates. Trichloroacetic and formic acids were used in later work because of the absence of background colour in quantitative determinations by the densitometer;[12] *tert.*-butanol–formic acid–water was an especially suitable solvent mixture as all its constituents could be easily evaporated from the paper on drying.

(b) Chromatographic examination of commercial phytin

The sample used for this examination was taken from that used by Holt which had been kindly supplied by Dr. F. G. Peers of the Cereals Research Station, St. Albans, Herts. A preliminary comparison of pea phytin and this product revealed that there were slight differences in R_F values and the cause of these differences was shown in the results of a chromatogram run for seven days in a glass tank on a sheet of Whatman No. 1 paper 60 × 14 cm. using *iso*propanol–picric acid–water as solvent. In this time the solvent front had run about 55 cm. Details of the experiment are given in Table II. Approximately 3 μl. of a 1% w/v acetic acid solutions of pea phytin and of commercial phytin were applied to each spot, and where a mixture was applied in one spot the paper was dried between each addition so that the initial spot area was constant. The areas of the spots after development of the chromatogram were also measured and are designated ' stain areas ' in Table II.

Table II

Chromatography of pea phytin and commercial phytin

Sample	Spot A		Spot B	
	R_F	Stain area 10^{-2} sq. in.	R_F	Stain area 10^{-2} sq. in.
Pea phytin	0·21	16	—	—
Commercial phytin	0·20	8	0·25	10
Pea phytin and commercial phytin	0·22	35	0·29	10
,, ,, ,, ,, ,,	0·19	22	0·25	9
,, ,, ,, ,, ,,	0·19	35	0·25	23

From these results it appears clear that this sample of commercial phytin was a mixture of at least two compounds, probably very similar in chemical constitution, having R_F values of approximately 0·20 and 0·26 in this solvent mixture. The compound with the lower R_F constitutes pea phytin and appears pure. The augmentation of stain area in Spot A supports the view that this compound occurs both in pea and commercial phytin. Commercial phytin also contained orthophosphate.

(*c*) *Synthesis of phytic acid* (*Posternak's method*[6])

Twenty-eight g. of phosphoric acid which had previously been dried under vacuum at 100–110° for three hours were mixed, in a 200-ml. bolt-head flask fitted with a glass stopper, with 6 g. of anhydrous inositol and the mixture heated on an oil bath until it had completely dissolved. While the mixture was still hot 45 g. of phosphoric anhydride in fractions of about 10 g. at a time were added at intervals of several minutes, taking care to spread it throughout the mass by closing and shaking the flask. The temperature rose and the reaction was completed by holding the mixture in an oil bath at about 120° for 3 hours. The contents of the flask became a little brown and had a consistency like honey. On cooling, the mass became semi-solid.

Separation of mineral phosphates

By means of a nickel spatula the cooled reaction product was introduced in small portions into 500 ml. of 5N-sodium hydroxide until dissolution was complete. The flask and spatula were washed with 100 ml. water and the solution with the washings transferred to a weighed porcelain dish which was heated first on a hot plate to boiling for 5 minutes and then on a water-bath until the weight of the solution reached approximately 500 g. The solution was then placed in the cold-room (2–4°) for about 6 hours and the crystals formed filtered off on a Buchner funnel under vacuum. The yield in this experiment was 212 g. and was designated Precipitate I. This precipitate after washing with 75 ml. of cold water was dried and set aside. The filtrate which weighed 348 g. was evaporated on the water-bath until it reached a weight of 175 g. On keeping for 3 hours in the cold, further crystallization took place and the crystals formed were again filtered under vacuum. (Yield 76 g., designated Precipitate II.) After washing the precipitate with cold water, the volume of the mother liquor was 137 ml. To this was added an equal volume of 85% ethanol, when a white cloudy solution was produced. After keeping for 20 hours in the cold-room, a syrup was produced in the bottom of the flask, which, after decantation of the supernatant alcoholic soda, was filtered on a Buchner funnel and washed with 100 ml. of 50% ethanol. The syrupy residue obtained on the paper after many hours' filtration was dissolved in 20 ml. of distilled water and 20 ml. of 85% ethanol added; the mixture was again placed in the cold-room for 24 hours, when the alcohol was decanted from the syrup and the latter mixed with and dissolved in 500 ml. of distilled water to which 15 ml. of glacial acetic acid and 50 ml. of 20% calcium acetate solution had been added. A milk-white precipitate was produced, and the mixture was left in the cold overnight and then filtered through Whatman No. 540 paper on a Buchner funnel. After drying for 4 days in a vacuum desiccator, this calcium salt weighed 10·5 g. and was designated Fraction I. A further two fractions, II and III, were obtained by adding 50 ml. of 20% calcium acetate solution in the cold to the filtrate and keeping overnight in the cold-room. After filtering Fraction II, the filtrate was heated to boiling, a further 50 ml. of 20% calcium acetate added and the precipitate filtered hot. This was named

Fraction III. After vacuum drying, Fraction II yielded 4·2 g. and III 1·3 g. of material. All these salts were intensely white and closely resembled pea phytin, but no crystalline structure was visible in any of them under the microscope. The later investigations were made with Fraction I.

Examination of the calcium salt

Preliminary examination of this product by paper chromatography using *iso*propanol–picric acid–water as solvent mixture and Whatman No. 541 paper, showed the presence of three spots, the fastest moving being due to orthophosphate and the intermediate to pyrophosphate ($R_F = 0{\cdot}51$). The slowest moving, $R_F = 0{\cdot}15$, was thought to be the calcium salt of phytic acid. Attempts at fractionation of this mixture by adding a slight excess of 10% barium chloride solution to a 2% v/v hydrochloric acid solution of Fraction I and filtering the precipitate formed were unsuccessful, as was also precipitation by barium hydroxide.

Further chromatographic examination using *iso*propanol–picric acid–water indicated that this spot at $R_F = 0{\cdot}15$ might be composite and on the assumption that more valid comparisons could be made between inositol phosphates when combined with the same cation the preparation of an alkaline sodium phytate was attempted using the method of Harrison & Mellanby[13] with both Fraction I and a sample of commercial phytin kindly supplied by the Ciba Company. Apart from orthophosphate, the Ciba product gave one clear spot at $R_F = 0{\cdot}08$ using *tert.*-butanol–trichloroacetic acid–water as solvent mixture, while in another chromatogram the synthetic product gave three spots at $R_F = 0{\cdot}11$, 0·18 and 0·22. (The latter is almost certainly pyrophosphate.) The R_F of the Ciba product in this same chromatogram was 0·06, which is sufficiently divergent from the R_F of the slowest moving spot in Fraction I to create doubts as to the identity of the two.

Composition of Precipitate I

The heterogeneous nature of Fraction I was sufficiently interesting to invite an examination of other products formed in Posternak's synthesis. Accordingly Precipitate I was examined by the methods outlined above using *tert.*-butanol–picric acid–water mixtures. Four compounds certainly, and possibly five, were found to be present. The two fastest running were identified as ortho- and pyro-phosphate, R_F values 0·71 and 0·41 respectively. The slowest running gave an $R_F = 0{\cdot}11$. The spot appearing immediately below pyrophosphate was tentatively identified as inositol-2-monophosphate ($R_F = 0{\cdot}25$), because on treatment of a strong aqueous solution of Precipitate I with 10% barium chloride solution a heavy white precipitate was formed. This was filtered off, washed and dissolved in 2% v/v hydrochloric acid, when a little of the resulting solution was chromatographed on paper using *tert.*-butanol–picric acid–water as before, the spot appearing at R_F 0·25 was not obtained, indicating that the barium salt of the compound giving the spot was soluble. Both inositol monophosphate and diphosphate have soluble barium salts,[14a,b] but an authentic sample of inositol-2-monophosphate (barium salt) kindly supplied with other inositol phosphates by Professor A. Desjobert, gave a very similar R_F value and ran as one spot when chromatographed with Precipitate I in *iso*propanol–picric acid–water ($R_F = 0{\cdot}36 - 0{\cdot}37$). The possibility that inositol diphosphate may be present cannot however be ruled out, as an examination of a number of inositol phosphates on the paper chromatogram in other solvents showed great similarities in R_F values, which would make separations very difficult except by two-dimensional techniques. An interesting observation in the barium treatment of Precipitate I was that the R_F of the orthophosphate present rose appreciably after treatment, in one case from 0·61 to 0·70, in another from 0·74 to 0·80.

Discussion

The experimental results obtained are summarized in Table III, and certain facts are clearly established from them. The purest compound obtained in this work is undoubtedly pea phytin which has been obtained free from orthophosphate or any other organic phosphate. The wheat phytin used by Mattson and that of Holt, which was also presumably of cereal origin, appear to be contaminated with other inositol phosphates and also to contain orthophosphate. Posternak's

Table III

Chromatography of phytin samples

Solvent	Compound	R_F values			
		Pea phytin	Commercial phytin	Synthetic phytin	Precipitate I
*iso*Propanol–picric acid–water	Phytin	0·17	0·20	0·15	0·23
	Unknown	—	0·26	0·32	0·33
	Pyrophosphate	—	—	0·51	0·51
	Orthophosphate	—	0·73	0·71	0·75
tert.-Butanol–trichloroacetic acid–water	Phytin	0·10	0·08	0·11	0·12
	Unknown	—	?	0·18	?
	Pyrophosphate	—	—	0·22	0·25
	Orthophosphate	—	0·59	?	0·50
tert.-Butanol–picric acid–water	Phytin	0·12	—	—	0·11
	Unknown	—	—	—	0·16
	,,	—	—	—	0·25
	Pyrophosphate	—	—	—	0·41
	Orthophosphate	—	—	—	0·74

synthesis, while it gives one compound having an R_F value very similar to that of pea phytin, also gives rise at the same time to a number of other compounds, among which have been identified pyrophosphate, orthophosphate and possibly inositol mono- or diphosphate. The compound with the higher R_F in commercial phytin has not been identified, but it may well account for the 11 mg./g. of phosphorus unaccounted for in Holt's analysis of this product. As, moreover, it is presumably not precipitated as a ferric salt (phytin being the only compound to behave in this manner[15]), it would account for the possibility of preparing by Harrison & Mellanby's method[13] a chromatographically pure sodium phytinate from the Ciba product except for an admixture of a small quantity of orthophosphate. Courtois[14] has produced an interesting review on the phosphoric esters of inositol and mentions (p. 12) that many commercial preparations which are considered to be salts of phytic acid have a phosphorus/inositol ratio which more nearly approaches that of inositol tetraphosphate than phytic acid and speculates therefore on the existence of inositol tetraphosphate in plants. A mixture of phytin with the salt of a lower inositol phosphate (possibly triphosphate), inseparable except by chromatographic methods, would give a very similar phosphorus/inositol ratio. No lower inositol phosphates have, however, been found in peas.

While the full characterization and identification of a chemical compound by a chromatographic method alone is impossible, there can now be little doubt of the value of chromatography as an analytical tool in the critical examination of the purity of a product, especially when it is present in minute quantities. The verification of Mattson's theory demands that quantitative determinations should be made of phytin in peas and this present study has shown that a purer product for use as a standard may be obtained from the peas themselves rather than from other sources. The separation of phytin from orthophosphate can be effected with ease by the methods outlined above and, as both compounds are of interest in the problem of the hardness of peas, both can be simultaneously determined in a pea extract by measurement of stain areas[16] or by densitometer measurements on the paper chromatogram with more certainty and little less accuracy than the methods described previously.

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ALPHA-CAROTENE IN LEAVES OF THE CARROT PLANT

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About 13% of the carotene in leaves of pigmented carrots was in the α-form. α-Carotene was absent from the leaves of all types of 'white'-rooted carrots except second generation albinos, although the content of β-carotene was normal. α-Carotene was present in cotyledons of those carrots which had, or would later have, any carotene (α or β) in their roots even at a stage too early for the roots yet to be coloured.

Introduction

Total carotene comprises about 90% of the total carotenoids in the root of the carrot (Booth & Dark[1]). Of this total carotene, β-carotene comprises the major part, while the α-isomer makes up about 20%. (For references see Sadana & Ahmad[2] and Booth.[3]) In leaves of all plants β-carotene is universally present. Mackinney[4] found that α-carotene formed about 10% of the total carotene in the leaves of carrots. Strain[5] isolated α- and β-carotenes from leaves of a variety of white-rooted carrot: the α-isomer formed approximately 5% of the total carotene. Fujita & Ajiska[6] found that the α-isomer formed 15% of the carotene in the leaves of their carrots. In the present investigation, α-carotene was found in the leaves of certain kinds of carrots, but was absent from batches of the few species of other green leaves that were tested, including mint, parsley, nettle and tomato.

Experimental

Material

Carrots were grown on various sites near Cambridge in several years and cultivated normally. They were of three types, grouped according to the concentration of carotene in the roots, as follows:

(1) 'High carotene' carrots with exceptionally high root concentrations. Some of these

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are of German origin, as described by Dark & Booth,[7] and others are selections, not yet named, bred by Mr. S. O. S. Dark at the Horticultural Research Station, Cambridge.

(2) Commonly-grown or ' ordinary ' varieties with carotene contents near to 125 p.p.m. at maturity. They include Chantenay, Nantes, James's Scarlet Intermediate, Early Market and others.

(3) ' White ' carrots, with very low concentrations of carotenoids. Wild carrots, which come in this group, have only about 3·5 mg. of total carotenoids per kg. of root, and of this only about a quarter is carotene. In the varieties of fodder-type carrot that were tested (Dutch White, Wiltshire White and Carotte Blanche de la Meilleraye), the carotenoid concentrations of the roots were very low and no carotene was detectable. The group includes ' albinos '—the almost colourless-rooted freaks that occur occasionally in a plot of normal carrots. These have some of the characters of wild carrots; for instance, many of them ' bolt to seed ' in the first year. Their total carotenoid concentrations are comparable with those of wild carrots, but the variation is wider and a higher proportion of the pigment is carotene.

Sampling

The term batch is used to denote a quantity of carrot leaves taken from one plot at one time. Batches differed from one another either as to origin of seed, location (soil and climate) or year of growing. For each batch, leaves were gathered by snipping off a secondary axis with its leaflets from at least 10, and usually 25, growing plants. Only healthy green leaves were used and main axes were excluded. The leaves were assayed immediately after collecting them, and all proper precautions were taken to prevent loss of carotene between harvesting and extracting.

Extraction and identity of the carotene

The total carotene was extracted by grinding about 1 g. of fresh leaf snippings with quartz in a mixture of cold light petroleum, acetone and quinol, and purifying on alumina–Na_2SO_4 as described by the Analytical Methods Committee of the Society of Public Analysts and other Analytical Chemists[8, 9] and by Booth,[10, 11] followed by colorimetry. Carotene is expressed as parts per million (p.p.m.) of the fresh weight. This solution of total carotene in light petroleum was washed with water to remove acetone and passed through a chromatographic column of magnesium oxide. The percolation was hastened by applying pressure above the column.[12] The lightly-adsorbed lower yellow band was eluted with light petroleum and determined colorimetrically. The upper zones were eluted with 40% acetone in light petroleum and determined as a check on recovery. For simplicity, the easily-eluted lower pigment is called the α-carotene fraction although it was not proved to be entirely free from very small amounts of *cis*-isomers of β-carotene. The principal pigment of the upper zones was β-carotene.

The α-carotene fraction from a mixed batch of carrot leaves was compared with a similarly-obtained fraction from carrot roots. When the pigments from the two sources were mixed, they could not be resolved on a long column of magnesium oxide. Moreover, when the two fractions were compared in 40–60°-light petroleum solution on the Unicam quartz spectrophotometer, the light adsorption curves were nearly identical, with maxima for each at 444 and 473 mμ. These two results make it highly probable that these pigments from carrots and from their leaves were identical and that the leaf fraction was indeed α-carotene.

Results

Comparison of varieties

The α-carotene fraction was estimated in several batches of leaves of carrot plants and the results are shown in Table I. The concentration varied considerably from batch to batch. For instance, in the leaves of 20 batches of ordinary varieties the α-carotene content ranged from 1 to 35 p.p.m. The mean was 15 p.p.m. and the standard deviation of a single batch was 8·3, i.e., it was over half of the mean. There was no significant difference ($P = 0{\cdot}7$) between the average concentrations of α-carotene in ordinary varieties and in high-carotene varieties.

Table I

α-Carotene in carrot leaves

Type of carrot	Carotene in roots	Batches examined	α-Carotene in leaves, p.p.m.	
			Mean	S.E.*
High-carotene varieties	+++	10	16	1·58
Ordinary varieties	+	20	15	1·83
Cotyledons of ordinary seedlings	trace	4	9	
White, fodder types	0	5	0	
Wild carrots	0	5	0	
Albino sports	0	4	0	
Albino F_1	0	2	18	

* Of the mean.

Therefore the 30 batches (10 high carotene + 20 ordinary) were pooled and the concentrations of α-carotene compared with the concentrations of total carotene. The correlation coefficient was 0·24 ($P = 0{\cdot}18$). This small degree of proportionality between the concentrations of α-carotene and total carotene is not regarded as significant. The average content of total carotene in the leaves of these 30 batches was 117 p.p.m. Therefore the α-carotene comprised, on average, 13% of the total carotene.

Leaves of white carrots

No α-carotene could be detected in the leaves of fodder types or of wild carrots. These two types have different appearances, sizes and growth habits but they have this in common, that there is no more than a trace of any carotene in their roots.

In any stand of carrots there usually occurs an occasional almost colourless-rooted freak. No α-carotene was detected in the leaves of several batches of these ' albino ' carrots, although the β-carotene content was similar to that of ordinary carrot leaves. In order to study second-generation albinos, 20 of these ' sports ', collected from stands of several varieties of red carrots during the 1946 season, were replanted together and allowed to cross-pollinate. The seed was collected in 1947 and sown in 1948. In September 120 roots were examined. Most of these F_1 carrots resembled their parents: a large proportion ' bolted to seed ' in the first season; 95% had ' white ' roots, the other 5% being pale yellow; the roots were tough; and many were branched. In these particulars they also resembled wild carrots. The leaves of the ' white ' progeny (the 95%) had a total carotene content of 119 p.p.m., that is, they did not differ from their parents or from other varieties of carrot in this respect. α-Carotene comprised 15% of this total carotene: in this respect however the F_1 albinos differed from their parents which had no α-carotene. The F_1 plants were also allowed to grow on for seed production but, although flowering was prolific, seed failed to set, and the plants were presumed to be sterile.

Cotyledons

The roots of tiny seedlings of ordinary carrots at first resemble the white group of carrots in that they have very low concentrations of carotene. When the cotyledons of these ordinary carrots (they had no proper leaves at this stage) were extracted, the carotene values were: total 47·5 p.p.m., α-isomer 9·2 p.p.m. Thus α-carotene comprised 19% of the total. In this last respect seedlings resembled older carrots of the ordinary group.

Discussion

The principal pigment in carrot roots is β-carotene, but some α-carotene and smaller amounts of other carotenoids are also present. α-Carotene was always found in the leaves of any carrot having appreciable total carotene in its roots. Moreover, α-carotene was present in cotyledons from seedlings of varieties of red carrots which had as yet negligible concentration of carotene in their tiny roots but which would develop root pigment later. With one exception—the F_1 generation of albino carrots—white carrots having little or no carotene of any type in their roots

had no α-carotene in the leaves. Hence the absence of α-carotene from leaves of a carrot indicates that there is not, nor ever will be, appreciable concentrations of any of the carotenes in the roots.

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THE FATE OF γ-BENZENE HEXACHLORIDE IN NORMAL AND RESISTANT HOUSEFLIES. II*

By F. R. BRADBURY and H. STANDEN

Quantitative radiochemical studies have shown that houseflies can convert the four common isomers of benzene hexachloride into water-soluble metabolites which are excreted. α- and γ-isomers are most readily metabolized, δ- less and β- still less so. Both normal and resistant flies can metabolize benzene hexachloride although the resistant strain brings about the change more rapidly. Resistant flies also absorb less benzene hexachloride than normal ones, and the combined effect of reduced pick-up and increased decomposition rate is to reduce the γ-benzene hexachloride in a resistant fly to one-quarter of that in a normal one four hours after a 15-minute exposure to the vapour. It is concluded that both properties are contributory to the resistance of the flies to poisoning by γ-benzene hexachloride.

Introduction

In the previous paper,[1] quantitative studies of the pick-up and penetration of γ-benzene hexachloride by normal and resistant houseflies were reported. In that paper attention was drawn to the fact that part of the γ-benzene hexachloride picked up by the flies was converted to a water-soluble compound and that the amount of this water-soluble material produced during a 6-hour exposure to insecticide was greater in the resistant than in the normal flies.

The present paper records quantitative determinations of the amount of water-soluble material produced after 24 hours' exposure to the insecticidal vapour. Experiments have also been made with the non-toxic α- and δ-isomers in order to eliminate the complication of toxicity from the comparison of the behaviour of normal and resistant flies.

With the intention of throwing light on the significance of metabolism in resistance of insects to insecticides, the rate of conversion of γ-benzene hexachloride into its water-soluble products following a short exposure to γ-benzene hexachloride vapour has been studied by exposing flies for 15 minutes to a treated surface and then storing them away from the deposit for periods varying from 10 minutes to 24 hours before analysis.

The distribution of the insecticide and its metabolites between head, thorax and abdomen of the fly has been examined by analysis of the separated body parts.

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Method

The flies used in the experiments were normal Hawthorndale stock of *Musca domestica* and a strain having very high resistance to benzene hexachloride reared at Hawthorndale from an original strain from Uruguay. The relative toxicity of various insecticides to this strain have been determined by Busvine.[2] The flies were reared at Hawthorndale on a medium containing 0·5% γ-benzene hexachloride, but for the final breeding cycle prior to use of the flies in experiments the medium was benzene hexachloride-free. The insects were exposed to the vapour of the insecticide either by confining them in Petri-dishes over filter paper impregnated with γ-benzene hexachloride or by placing them in stoppered Erlenmeyer flasks containing a film of insecticide. The insecticide and its metabolites were extracted from the fly tissues as described in the previous communication[1] except that water extraction of both the flask and the insect tissues was used as a routine method for recovery of water-soluble radio-active products.

The radio-activity was normally determined by burning the sample to carbon dioxide in a furnace, except for water-soluble radio-active compounds where wet persulphate oxidation in the presence of silver nitrate was used to convert the compounds into carbon dioxide. In either case the gas was subsequently converted to barium carbonate for counting. During the work, a supply of highly active ^{14}C-labelled benzene became available and benzene hexachloride prepared from this by our colleagues Messrs. Hill, Johnson and Wimpenny had a specific activity of approximately 3 mc./g., which permitted the determination of quantities as little as 0·04 μg.

Experimental

Insect breeding

The flies were supplied by the Hawthorndale Laboratories as pupae which were hatched in cages and fed on lump sugar and diluted milk as described by Knipe & Frings.[3] Non-resistant flies were normal Hawthorndale stock and these were hatched from pupae in the same way. Flies 2–4 days old were used for quantitative experiments.

Exposure of flies to insecticide

Two exposure methods were used, the flask method and the Petri-dish method, both of which have been described in detail in Part I.[1]

Experiments in which the flies were exposed to benzene hexachloride for a short period followed by storage were carried out using the Petri-dish method, but in view of the short exposure time the carbon dioxide was administered sparingly to avoid prolonged anaesthetization. After 15 minutes' exposure the flies were re-anaesthetized, removed from the Petri-dish and stored in batches of 20 flies in 100-ml. stoppered conical flasks maintained at a temperature of 24–25°.

Dissection of treated flies

In experiments to determine the distribution of benzene hexachloride in the fly body, each fly, after exposure and storage, was cut into three parts: head, thorax and abdomen. The wings and legs were included with the thorax.

Recovery of radio-active material from insects

Carbon tetrachloride-soluble material and water-soluble material were removed from the outer tissues by shaking the flies with 5 ml. of carbon tetrachloride together with 5 ml. of water, using the cylindrical extraction funnel described previously.[1] Two washings were employed, the extracts then being combined and the two layers separated. In the case of the dissected flies this 'outside' extraction was omitted. The flies were then ground and extracted with carbon tetrachloride as described previously[1] except that 'Dicalite 4200', a coarse diatomaceous earth, was substituted for anhydrous sodium sulphate. The 'inside' carbon tetrachloride-soluble material was filtered off, the residue transferred to a 100-ml. conical flask and 30–40 ml. of hot water added. After keeping overnight the 'inside' water-soluble material was filtered off, the residue transferred to a Petri-dish and kept in air at room temperature until dry. The residual

benzene hexachloride and the excreted water-soluble material were extracted from the exposure flask by shaking with 5 ml. of carbon tetrachloride together with 5 ml. of water, this being carried out twice, followed by separation of the two layers. In those experiments in which the flies were stored after exposure, the excreted water-soluble material was extracted from the storage flasks by shaking twice with 5 ml. of water each time.

Determination of radio-activity of the samples

The method described by Calvin *et al.*[4] was used for solutions of radio-active material in organic solvents, the solvent being first removed by evaporation under controlled conditions as previously described.[1]

For aqueous solutions of radio-active material the wet combustion method described by Calvin *et al.*[4] was employed. The aqueous solution or an aliquot of it, and the appropriate amount of sugar to give sufficient carbon dioxide to form a pad of ' infinite ' thickness, was warmed with a solution of potassium persulphate and silver nitrate, the carbon dioxide so produced being subsequently boiled off and absorbed in caustic soda. The carbonate was precipitated as barium carbonate and mounted on a brass block for counting in a flow-type proportional counter. It was found that benzene hexachloride could be converted quantitatively to carbon dioxide by this technique, but the wet method had no special advantage over the furnace method when dealing with solutions in organic solvents.

Results

Metabolism of benzene hexachloride by flies during twenty-four hours' continuous exposure to the insecticide

Table I shows the radioactivity expressed as μg. of benzene hexachloride found ' outside ' and ' inside ' by carbon tetrachloride extraction, in the hot water extracts and on burning the residues. Experiments with α-, β-, γ- and δ-isomers in which normal and resistant flies were compared are shown.

Table I

Pick-up and metabolism of benzene hexachloride isomers by flies in 24 h. continuous exposure to the chemical

Fifty flies exposed to 200 μg. of benzene hexachloride in a 250-ml. stoppered conical flask for 24 h. at 24–25°. Results expressed in μg./fly

Isomer	Alpha			Beta		Gamma					Delta		
	N	R	R*	N	R	N	N*	R	R	R†	N	R	R*
Wt. of 50 flies	0·81	0·73	1·03	0·88	0·75	0·82	1·19	0·70	0·84	0·89	0·87	0·74	0·63
Picked up	3·46	3·36	3·56	1·57	2·24	3·69	4·03	3·64	3·43	3·84	3·83	3·75	2·81
' Outside ', CCl_4-soluble	0·17	0·08	0·09	0·72	1·59	0·53	0·34	0·23	0·16	0·18	0·19	0·39	0·35
' Inside ', CCl_4-soluble	0·44	0·15	0·08	0·65	0·46	0·98	1·16	0·34	0·29	0·39	2·25	1·60	1·40
Water-soluble	2·27	2·98	2·84	0·07	0·11	1·77	2·19	2·52	2·56	2·78	1·16	1·75	1·00
Residue	0·32	0·11	0·15	0·09	0·03	0·29	0·36	0·30	0·19	0·41	0·18	0·23	0·16
Total accounted for	3·20	3·32	3·16	1·53	2·19	3·57	4·05	3·39	3·20	3·76	3·78	3·97	2·91
Unaccounted for	0·26	0·04	0·40	0·04	0·05	0·12		0·25	0·23	0·08	0·05		

* = female flies only
N = normal
R = resistant

† = original Uruguay strain, not having been bred on a γ-benzene hexachloride medium

Metabolism of α-benzene hexachloride by flies

Table II records the results of analysing flies which have been exposed to a ^{14}C-labelled α-benzene hexachloride deposit for 15 minutes and then stored in an untreated flask. The loss of benzene hexachloride and the production of water-soluble radio-active compounds were measured at intervals. The results are expressed as μg. of benzene hexachloride and in each case are for 20 female flies.

Table II

Pick-up and metabolism of α-benzene hexachloride by flies in 24 h. after 15 min. exposure

Female flies exposed to benzene hexachloride on filter paper (11 μg./cm.²) for 15 min., then stored in batches of 20 in 100-ml. stoppered conical flasks at 24–25°. Results expressed in μg./20 flies

	Storage time (h.)							
	3/16	3/8	3/4	1½	3	6	12	24
α-Resistant flies (160 weighed 2·48 g.)								
' Outside ' (CCl_4)	0·39	0·29	0·08	0·04	0·03	0·03	0·01	0·01
' Inside ' (CCl_4)	0·32	0·33	0·39	0·29	0·22	0·13	0·07	0·01
Water-soluble	0·19	0·25	0·33	0·51	0·50	0·58	0·63	0·60
Residue	0·03	0·03	0·03	0·16	0·04	0·11	0·11	0·13
Total	0·93	0·90	0·83	1·00	0·79	0·85	0·82	0·75
Normal flies (160 weighed 2·98 g.)								
' Outside ' (CCl_4)	0·52	0·21	0·11	0·04	0·04	0·02	0·01	0·01
' Inside ' (CCl_4)	1·02	1·23	0·51	0·71	0·74	0·41	0·19	0·07
Water-soluble	0·11	0·33	0·24	0·23	0·42	0·52	0·81	0·70
Residue	0·04	0·02	0·11	0·19	0·17	0·17	0·21	0·13
Total	1·69	1·79	0·97	1·17	1·37	1·12	1·22	0·91

In Figs. 1 and 2 the quantities in Table II have been adjusted for variation between batches of flies by applying to the figures in each column the factor required to bring the total for that column to the mean value for the column totals. [The ordinates show the radioactivity expressed as BHC.]

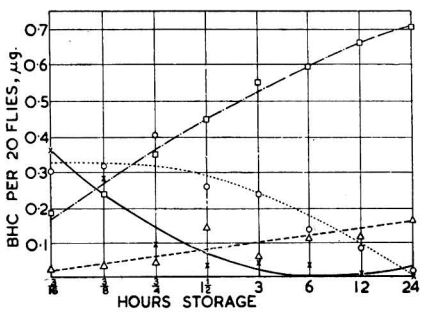

FIG. 1.—*Metabolism of α-BHC by resistant flies*

× ——— × Outside CCl_4-soluble
○ - - - - - - - - - ○ Inside CCl_4-soluble
□ ·—·—· □ Metabolite
△ — — — △ Residue

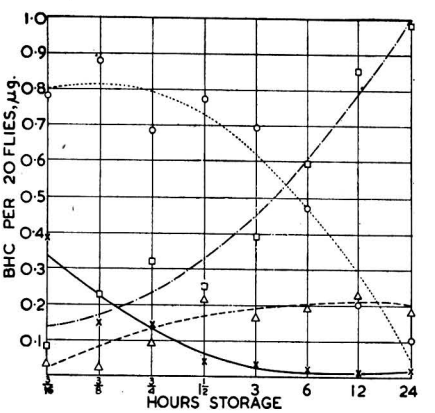

FIG. 2.—*Metabolism of α-BHC by normal flies*

× ——— × Outside CCl_4-soluble
○ - - - - - - - - - ○ Inside CCl_4-soluble
□ ·—·—· □ Metabolite
△ — — — △ Residue

Distribution of γ-benzene hexachloride and metabolites in the fly body

The results of experiments with γ-benzene hexachloride on the distribution of insecticide and metabolites between head, thorax and abdomen are set out in Table III. The results are also represented graphically in Figs. 3–5 after adjustment as above. [The ordinates give the radio-activity expressed as BHC.]

Discussion

The experiments with the four benzene hexachloride isomers, in which flies were exposed continuously to the chemicals for 24 hours, permit a comparison of the maximum value for pick-up, penetration and metabolism of the four chemicals by normal and resistant flies. The figures

Table III

Distribution of γ-benzene hexachloride and metabolites in the fly body

Female flies exposed to γ-benzene hexachloride on filter paper (11 μg./cm.²), then stored in batches of 20 in 100-ml. stoppered conical flasks at 24–25°. Determinations carried out on head, thorax and abdomen separately. Results expressed as μg./20 flies

Extract	Source	Storage time ½ h.		1 h.		4 h.		6 h.		24 h.	
Resistant flies (100 weighed 1·62 g.)											
	Head	0·05		0·07		0·02		0·03		0·01	
CCl_4-soluble	Thorax	0·79	1·24	0·38	0·69	0·10	0·28	0·09	0·25	0·05	0·15
	Abdomen	0·40		0·24		0·16		0·13		0·09	
	Head	0·09		0·06		0·03		0·03		0·05	
Water-soluble	Thorax	0·18	0·60	0·26	1·21	0·11	0·55	0·20	0·88	0·36	0·92
	Abdomen	0·33		0·89		0·41		0·65		0·51	
	Head	0		0·01		0·02		0		0·01	
Residue	Thorax	0	0·05	0·01	0·09	0·01	0·05	0	0·06	0·04	0·09
	Abdomen	0·05		0·07		0·02		0·06		0·04	
Storage flask (water-soluble)		0·22		0·44		1·87		1·34		1·00	
Total		2·11		2·43		2·75		2·53		2·16	
Normal flies (100 weighed 1·63 g.)											
	Head	0·16		0·19		0·08		0·05		0·02	
CCl_4-soluble	Thorax	1·06	1·86	1·06	2·09	0·60	1·19	0·46	0·80	0·15	0·33
	Abdomen	0·64		0·84		0·51		0·29		0·16	
	Head	0·03		0·08		0·08		0·08		0·10	
Water-soluble	Thorax	0·13	0·41	0·17	0·64	0·24	1·16	0·34	1·33	0·50	1·60
	Abdomen	0·24		0·39		0·85		0·90		1·00	
	Head	0·01		0		0·01		0·02		0·02	
Residue	Thorax	0·03	0·06	0·06	0·10	0·08	0·17	0·07	0·18	0·08	0·22
	Abdomen	0·02		0·04		0·08		0·09		0·12	
Storage flask (water-soluble)		0·19		0·07		0·17		0·21		0·42	
Total		2·52		2·91		2·69		2·52		2·57	
Mortality %		0		15		70		100		100	

in Table I are maximum in the sense that throughout the 24-hour period the flies are enclosed in a saturated vapour of the chemical, and chemical removed by penetration and metabolism can be made good by further absorption.

The amount picked-up per fly is similar for α-, γ- and δ-isomers being between 3 and 4 μg. In the case of the β-isomer, however, the figures are lower by 1½ to 2 μg., probably on account of the very low vapour pressure of this isomer. The ' inside ' carbon tetrachloride-soluble matter, which for the γ-isomer had previously been shown to be unchanged γ-benzene hexachloride,[1] is greatest for the δ-isomer (2·25 and 1·60 μg./fly). The figures for ' inside ' carbon tetrachloride-soluble material also show that the normal fly contains more than the resistant, the ratio normal to resistant varying from under 2 (δ and β) to 3 or 4 (α and γ).

The amount of water-soluble material produced from the chemicals, on the other hand, is greatest for the α- and γ- (2 to 3 μg./fly) and least for δ- and β-isomers (1·75 μg. or less/fly). Taking together the figures for water-soluble and for carbon tetrachloride-soluble matter, it appears that the α- and γ-isomers are most readily converted in the fly to water-soluble metabolites, the δ- and β- much less readily. The small extent to which the δ-isomer is converted is striking, since the quantity of this isomer picked-up and therefore available for the conversion is as great as for γ- and α-isomers. The slow breakdown of the δ-isomer in flies has also been observed by Oppenoorth.[5]

The bottom row of figures in Table I, the benzene hexachloride unaccounted for by analysis, shows that the sum of the ' inside ', ' outside ', ' water-soluble ' and ' residue ' figures approximates reasonably well to the total absorbed. It follows that only a very small proportion, if any, of the dose is unaccounted for under the foregoing headings and that there is no substantial conversion of the chemical to gaseous or volatile constituents which would be lost in our analyses.

The occurrence of an appreciable ' residue ' may be of importance. This portion of the absorbed radio-activity is not extractable either by carbon tetrachloride or water and must be

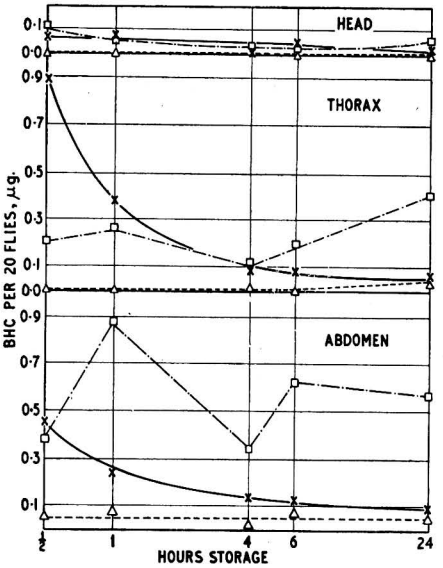

FIG. 3.—*Distribution of γ-BHC and metabolites in resistant flies*

× ——— × CCl_4-soluble
□ ·—·—· □ Metabolite
△ — — — △ Residue

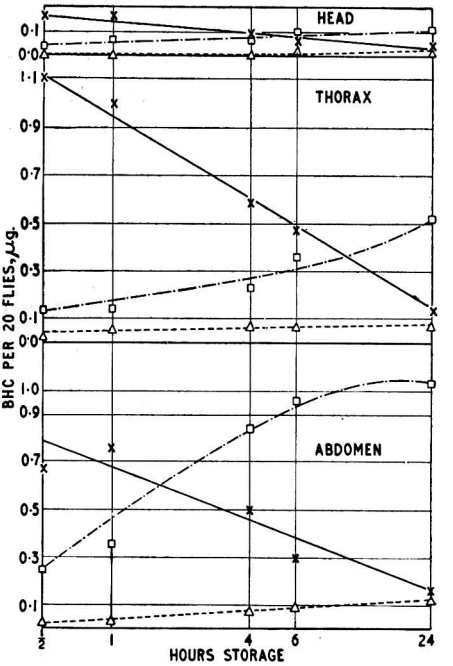

FIG. 4.—*Distribution of γ-BHC and metabolite in normal flies*

× ——— × CCl_4-soluble
□ ·—·—· □ Metabolite
△ — — — △ Residue

firmly bound up by adsorption or chemical combination in the insect tissue. The radio-activity is recovered by combustion of the fly body, after the extraction processes, when it appears in the carbon dioxide absorbed in the train from the furnace.

To get a picture of the pattern of events following the absorption of a small dose of chemical, flies were exposed to deposits on filter paper for 15 minutes and then removed to an untreated flask. Analyses were made at intervals during the ensuing 24 hours. Table II records results for normal and resistant flies exposed to α-benzene hexachloride.

The variations among the totals recorded in Table II must be due, at least in part, to sampling errors involved in selecting groups of 20 flies from the total batch of 160 initially treated with benzene hexachloride. To eliminate this the figures have been adjusted as described under 'Results' before being plotted in Figs. 1 and 2.

The first point to be made in connexion with these storage tests is that the totals of radio-active matter are greater for normal than for resistant flies. This is partly due, no doubt, to the greater weight of the normal flies, but the ratio of the mean absorption totals (1·5) is greater than the ratio of the weights (1·2). It therefore appears that resistant flies absorb less benzene hexachloride than normal ones.

The second point to be drawn from the storage test results is that in the course of 24 hours almost all the absorbed dose of chemical has been converted to water-soluble material or un-extractable residue, both by normal and resistant flies. If, however, we follow the shape of the respective curves for normal and resistant flies, it is evident that the resistant flies metabolize benzene hexachloride much more rapidly in the early stages after exposure. This is even more striking if the figures are plotted against an arithmetical rather than a log time scale. In the course of 3 hours' storage, the benzene hexachloride 'inside' resistant flies is reduced to little more than a quarter of that in normal flies.

The third point of interest in Figs. 1 and 2 is the course of events revealed by the shape of the

'outside', 'inside' and 'water-soluble' curves. The 'water-soluble' material increases steadily during storage indicating steady conversion of benzene hexachloride to metabolite. The 'outside' (benzene hexachloride) curve decreases sharply at first, whilst the 'inside' (benzene hexachloride) curve shows only a slight fall. Two explanations are possible: (i) that metabolism takes place preferentially in 'outside' tissues and only later is the 'inside' benzene hexachloride converted; (ii) that benzene hexachloride removed from 'inside' by the metabolic process is constantly made good from 'outside' until supplies from this source are exhausted. The second proposition appears more attractive although Sternberg & Kearns[6] claim that DDT is metabolized extensively in the insect cuticle.

The experiments summarized in Table II were carried out with α-isomer to avoid complications due to toxic effects of the insecticide when γ-isomer is used. However, examination of the sub-totals in Table III show a very similar picture for γ- to that described for the α-isomer. In Table III the percentage of mortalities amongst the normal flies are recorded. It is noteworthy that the process of conversion of insecticide to metabolite proceeds smoothly despite the mounting death rate. It appears therefore that the biochemical detoxication process is continued unaffected by the physiological changes regarded as criteria of death (absence of all movement when observed in bright light). Tahori & Hoskins[7] have made a similar observation on the metabolism of DDT by flies.

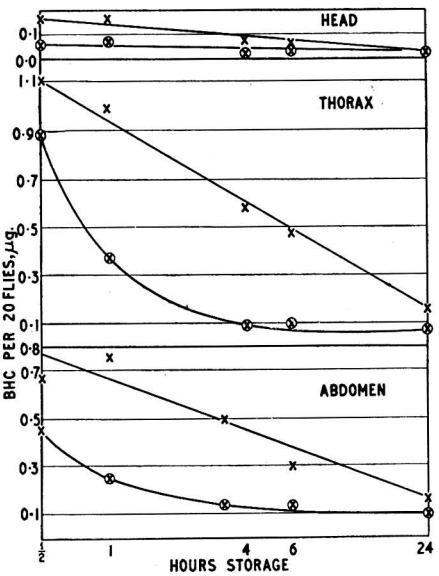

FIG. 5.—*Comparison of distribution of γ-BHC in resistant and normal flies*

⊗——⊗ Resistant, CCl_4-soluble
×——× Normal, CCl_4-soluble

The distribution experiments were done by dissecting the fly body after treatment and analysing the parts. The γ-isomer was used in these experiments and the results for normal and resistant flies are set out in Table III. The figures, after adjustment for batch-to-batch variation, have been plotted in Figs. 3 and 4. The picture is similar to that obtained for the whole fly body. The normal flies absorb a little more insecticide than the resistant ones: this is largely converted to water-soluble compounds by the flies but most rapidly by resistant ones in the early stages after treatment. The sub-division into head, thorax and abdomen, shows the metabolite predominantly in the abdomen, which is to be expected in a detoxication process leading to excreted products. Reference to Table III shows that the resistant flies excrete a large proportion of the water-soluble material, which is then found in the storage flask washings. Normal flies do not do this to the same extent, presumably on account of the toxic action of the insecticide; the metabolite accumulates in the abdomen instead of being discharged.

Leroux & Morrison[8] claim that there is slower distribution of DDT, especially to the head, in resistant flies. To simplify the comparison, the figures of benzene hexachloride content of the body parts of normal and resistant flies have been brought together in Fig. 5. The relative weights of head, thorax and abdomen were approximately 1 : 5 : 4 and the distribution of insecticide in normal flies immediately after treatment was similar, 1 : 7 : 4. Resistant flies showed the proportions 1 : 16 : 8 for the distribution of insecticide between head, thorax and abdomen and Leroux's contention would appear to receive some support from this. However, the rate of removal of insecticide by metabolism is much greater in the thorax and abdomen than in the head, and after 4 hours' storage the ratios are: normal 1 : 8 : 6; resistant 1 : 5 : 8, and the picture of distribution favouring other parts than the head in resistant flies is obliterated.

In a review of the subject Chadwick[9] concludes that the mechanism of resistance of flies to DDT, whilst still uncertain, probably includes decreased rate of penetration, increased rate of detoxication, and different distribution within the insect body. The experiments reported here

suggest that two, at any rate, of these factors, reduced penetration rate and increased detoxication rate, contribute also to γ-benzene hexachloride resistance in flies.

Work on this subject is being continued.

Acknowledgments

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VOLATILE COMPOUNDS PRODUCED BY APPLES. I.—Aldehydes and Ketones

By D. F. MEIGH

Methods were developed for analysing the volatile aldehydes and ketones present in the air surrounding gas-stored apples. These methods were used to compare the compounds produced by three varieties of apple, one susceptible and the other two resistant to superficial scald. The comparison did not reveal any fundamental differences between them. Of the aldehydes and ketones produced by the three varieties, acetone predominated.

Introduction

Some varieties of apple, when kept in cold storage, are subject to a physiological disorder known as superficial scald. This affects the skin of the fruit only and its severity varies from season to season. The opinion has been widely held that the disorder is caused by one or more of the volatile substances which are produced by the apples themselves and which accumulate in the fruit and in the air around them when they are stored in a confined space.

In previous studies of these substances which apples produce, their extraction has been achieved in one of three ways, (*a*) by distillation from the apple tissue or its juice, (*b*) by adsorption on to activated carbon from the air of a refrigerated apple store, followed by extraction of the carbon, and (*c*) by passing air over apples at ordinary temperature and collecting the products in reagent solutions or cold traps.

Power & Chesnut,[1] and Thomas[2] examined steam distillates of apple tissue and showed acetaldehyde to be present, Power & Chesnut by the Rimini test and the preparation of a silver salt after oxidation, Thomas by the preparation of a *p*-nitrophenylhydrazone and a dimedone derivative. White[3] examined an apple juice concentrate obtained by a flash-evaporation process intended for commercial use. He found (in decreasing order of amounts) acetaldehyde, hexanal, hex-2-enal and acetone by preparing the 2 : 4-dinitrophenylhydrazones. These he identified by elementary analysis and mixed melting point determination.

The active carbon method was used by Henze, Baker & Quackenbush,[4] who identified acetone, propanal and acetaldehyde by the preparation of dinitrophenylhydrazones. These were co-chromatographed with authentic compounds on columns of two different adsorbents, their ultra-violet spectral absorption curves were compared, and the acetone derivative was identified by melting point. Numerous smaller fractions were separated, seven of which appeared by their absorption curves to be a closely related series of dienal or trienal carbonyl compounds, while one other might have been hex-2-enal.

Power & Chesnut,[1] by passing air over whole apples and absorbing the products in bisulphite, identified acetaldehyde, using the Rimini test. Huelin,[5] using the same method of collection, was able to identify acetaldehyde, acetone and propanal by chromatographing the dinitrophenylhydrazones on paper. The identity of the acetone derivative was confirmed by a spectral absorption curve and that of the aldehydes by conversion to hydroxamic acids, which were separated by paper chromatography.

Of work that has been published, only the analysis of active-carbon adsorbates from a cold store has a direct bearing on the behaviour of apples at cold storage temperatures. Since there is at least one way in which apples behave quite differently at normal and low temperatures, namely in their reaction to ethylene stimulation,[6] it is clear that more information is needed about their behaviour in cold storage. Fidler has shown that under normal English gas-storage conditions, in which the carbon dioxide produced by the fruit is allowed to accumulate to a controlled extent in the store, the cooking apple Edward VII produces volatile substances (excluding carbon dioxide and ethylene) at a rate of less than 10 mg. carbon/ton of fruit/day.[7]

The aim of this work was to collect and analyse volatile substances from the air of a store of apples held in conditions approximating to those of a good commercial gas store, using methods appropriate to the small concentrations of material available. The air was drawn through a series of cold traps and the substances thus collected were converted to derivatives which were separated by chromatography on paper or columns. This technique was used to compare the substances given off by two varieties of apple, the one susceptible to scald, the other resistant; the comparison was at first qualitative but it was later possible to provide an approximate quantitative study. The analysis of volatile alcohols and esters will be described in a later paper.

Methods

Fruit storage equipment

The fruit was stored in gas-tight cabinets made of welded mild steel sheet supported on frameworks of angle iron. They were either ' ton ' cabinets, holding about 2000 lb. of fruit in 54 galvanized steel boxes with slatted bottoms, or ' half-ton ' cabinets holding about 900 lb. of fruit in 20 aluminium boxes perforated on sides and bottom. The design of the ' ton ' cabinets has been described by Fidler;[7] that of the ' half-ton ' cabinets follows the same principles. In these, an axial flow fan (9 in. diam. Ventaxia), mounted in a false ceiling, circulated air down through a duct formed at the back of the cabinet by an internal false wall. The air rose through a perforated false floor, up through the boxes of fruit, packed tightly in the cabinet, and so returned to the fan. The cabinets were provided with steel doors for loading purposes, bolted to the door frame and sealed with rubber gaskets. The general arrangement was such that the rate of air flow was considerably higher than is usual for a commercial store.

The cabinets stood in a refrigerated chamber with a central cooling tower. Each cabinet was provided with a U-tube manometer, and a thermometer (calibrated in 0·1° F divisions) fitted so that the bulb was suspended in the space on the delivery side of the fan. From high outside the building a tube led fresh air into the chamber, where branch tubes led a supply of air into each

cabinet on the delivery side of the fan. The rate at which air entered was controlled by the rate of withdrawal of air through sampling tubes on the sides of each cabinet. 'Ton' cabinets were provided with three glass sampling tubes, two of which were fitted with ground-glass ball joints (Quickfit & Quartz Ltd., MS 9/18), so arranged that a volatile-collecting train provided with the corresponding cup joint could be attached to them. 'Half-ton' cabinets were provided with two sampling tubes, one of which was fitted for collection of volatile substances. From each sampling point a tube led to a manometer and an adjustable manostat of the bubbler type filled with butyl phthalate. From these, the air was led through needle valves for coarse adjustment of air flow and so to a vacuum pump.

Experimental material

The variety Laxton Superb was chosen as the scald-resistant apple and Edward VII as the scald-susceptible one. Cox's Orange Pippin, a scald-resistant variety, was also used to provide further qualitative data. The apples were picked at the normal date and sorted to remove unsound fruit. The Edward VII apples were stored in two ways, (*a*) unwrapped and (*b*) wrapped in oiled tissue paper, the normal commercial treatment to prevent scald. The intention was to compare the volatile production of the fruits under the two conditions, but this was partially frustrated in 1952 because the crop in the Coxheath orchard was found to be insufficient and had to be supplemented. The source of the fruit, with times of picking, storing and unloading, are given in Table I.

After unloading the fruit at the end of the storage period, ten boxes from each cabinet were immediately examined for signs of rotting and superficial scald. Another ten boxes from each cabinet were kept at room temperature for three weeks to allow incipient injuries to develop and were then examined. Fidler has given a detailed description of the appearance of superficial scald on the Edward VII apple.[7]

Table I

Fruit used for analytical work

Storage season	Apple variety and treatment	Location of orchard	Date of picking	Date of loading and sealing	Date of unloading
1952–3	Edward VII unwrapped	Coxheath	14.X.52	15.X.52	6.V.53
	Edward VII wrapped	Sutton Valence	15.X.52	16.X.52	6.V.53
	Laxton Superb	Coxheath	3.X.52	4.X.52	23.IV.53
1953–4	Edward VII unwrapped	Sutton Valence	26.X.53	27.X.53	22.IV.54
	Edward VII wrapped	,, ,,	26.X.53	27.X.53	22.IV.54
	Laxton Superb	,, ,,	11.X.53	12.X.53	22.IV.54
1954–5	Cox's Orange Pippin	,, ,,	11.X.54	12.X.54	11.II.55

Storage conditions

The cabinets were loaded with weighed boxes of fruit, the doors were sealed and cooling begun. During the early stages of storage, the cabinet temperatures were noted daily and the temperature of the refrigerated chamber adjusted to bring them within the range of 38 to 39° F. Gas samples from each cabinet were analysed daily, using a Katharometer, throughout the storage period. When the carbon dioxide concentration had risen to 8%, ventilation was begun and the rate of suction was adjusted, when necessary, so that the carbon dioxide concentration remained between 8 and 9% in each cabinet. The aeration required was obtained in such a way that the flow rate in the trains collecting the volatile matter did not exceed 8 l./h., any required excess being drawn through the spare tube provided.

Collection of volatile substances

Once a fortnight a collecting train was attached to each cabinet so that air from the store could be passed through it for a period of about 6 days. Thus about 1000 l. of air were scrubbed during a period. During the early part of the 1952–3 season a period of 11 days was used. Fig. 1 shows the final form of the design used for the train collecting the volatile matter. The air

entered a wide U-tube (A) through an FS 9/18 cup joint; the tube was immersed in ice–salt mixture (at about — 10°) and here the bulk of the water vapour was removed from the air. It then passed through two sintered-glass gas-washing bottles (B, C) which contained a pure solvent to wash the air free of volatile substances. Each of these was immersed in solid carbon dioxide–light petroleum mixture (at about — 80°). The first bottle was provided with a wide entry tube where residual water vapour froze out. A suitable box made of hard-board contained the vacuum flasks, enclosed in plywood boxes, and a thick cork lid provided thermal insulation.

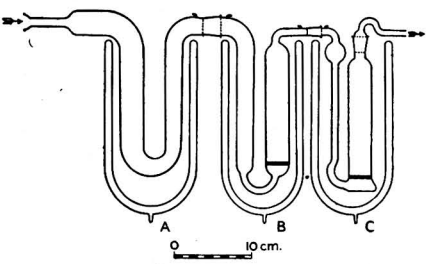

FIG. 1.—*Apparatus for collecting volatile substances from the air of apple stores*

A. Wide U-tube cooled by ice–salt mixture
B. Sintered-glass gas-washing bottle with wide entry, cooled by solid carbon dioxide–petroleum ether mixture
C. Sintered-glass gas-washing bottle cooled as B

Before assembly, the glass apparatus was washed with chromic acid mixture in the usual way and dried in an oven. A thin band of Apiezon-M or -N grease was applied to the wider end of each ground cone joint in such a way that the vapour in the train could not come in contact with it when the joints were assembled. Specially pure light petroleum (b.p. 60–80°) was poured into the sintered-glass gas-washing bottles (50 ml. each) and the apparatus assembled. A sample (100 ml.) of the light petroleum used for each batch of analyses was stored at — 20° and treated along with the solutions of volatile matter as a reagent blank.

Later, it was found that hexane (special for spectroscopy, B.D.H. Ltd.) was a sufficiently pure solvent to use. In the early stages, however, light petroleum (b.p. 60–80°, A.R., free of aromatic hydrocarbons) was purified with sodium–potassium alloy and then used after redistillation.[8]

During the period of collection of the volatile matter, the cooling mixtures were replenished every 48 hours. At the end of the period, the large U-tube was disconnected and lifted from the vacuum flask, the joint cleaned of grease with cotton wool moistened with carbon tetrachloride, its ends closed with bungs and the ice allowed to melt at room temperature. The first sintered-glass bottle was then lifted out, the joints cleaned, the light petroleum drained through the sintered-glass filter in such a way that the ice crystals remained behind, and transferred to a clean stoppered bottle. The ends of the gas-washing bottle were then closed with bungs and the ice allowed to melt. The melted ice from both pieces of apparatus was collected in a separating funnel and each rinsed with light petroleum from the second sintered-glass bottle. These washings were used to extract the melted ice after saturating it with sodium chloride (A.R.). All the light petroleum was then collected in the stoppered bottle and stored at — 20° to await analysis.

Conversion of the aldehydes and ketones to 2 : 4-dinitrophenylhydrazones

The solution for analysis was allowed to warm to room temperature and transferred to a 150-ml. glass-stoppered flask, and to it were added 5 ml. of a freshly prepared 0·1% solution of 2 : 4-dinitrophenylhydrazine (A.R.) in 2N-hydrochloric acid which had been extracted twice with an equal volume of carbon tetrachloride (A.R.) to remove interfering impurities.[9] The flask was stoppered and shaken at medium speed on an automatic flask shaker for 18 hours. The mixture was allowed to separate in a separating funnel, and the light petroleum layer extracted with

2N-hydrochloric acid (2 × 5 ml.), and then evaporated to dryness at room temperature under vacuum. The residue was transferred to a 10-ml. flask using a few ml. of carbon tetrachloride (A.R.) and once more evaporated to dryness before transferring it to a paper chromatogram with a few drops of the same solvent.

Chromatography of 2 : 4-dinitrophenylhydrazones

The derivatives were separated on paper, using methanol–heptane as solvent (methanol was A.R. quality anhydrous, and the heptane a fraction from petroleum). The method, which has been described elsewhere,[10] was somewhat modified as follows. A piece of Whatman No. 1 paper (53 × 15 cm.) was prepared in the usual way and the solution of derivatives for separation applied in equal quantities to five adjacent spots on the starting line. Suitable marker spots were also applied. After overnight equilibration with the methanol-rich phase in both side troughs, the spots were developed with the heptane-rich phase for about 4 hours to obtain maximum separation. The paper was then pressed against a sheet of Rhodamine-treated paper[10] and examined under a long-wave ultra-violet lamp with Wood's glass filter (Hanovia Ltd.). The spots appeared as dark shadows on a fluorescent background. For permanent records contact prints exposed in ultra-violet light were suitable. One of these, in Fig. 2, shows the separation of some artificial mixtures of the derivatives of normal saturated aliphatic aldehydes and methyl ketones, together with a number of miscellaneous compounds. It will be seen that in a homologous series the compounds spread out in order of their molecular weights. Ketones run faster than the corresponding aldehydes and saturated faster than unsaturated compounds. The way in which the furfural derivative streaked is typical of a number of simple aromatic compounds.

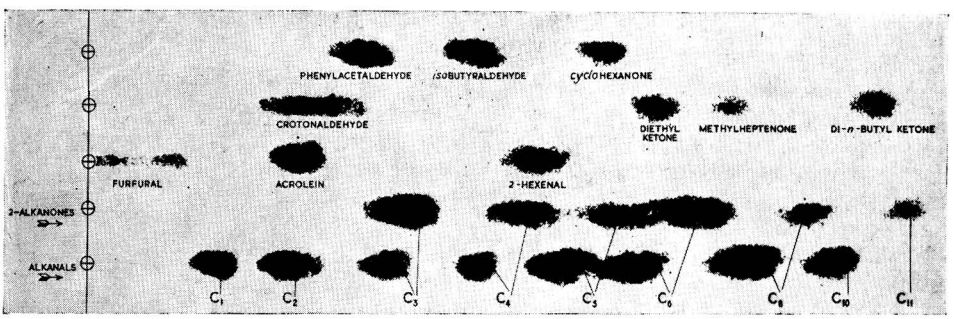

FIG. 2.—*Separation of 2 : 4-dinitrophenylhydrazones by paper chromatography using methanol–heptane : contact print of chromatogram in ultra-violet light*

The areas of paper containing the separated bands of derivatives were cut out, trimmed to a point at one end and the spots eluted with purified methanol,[10a] using Dent's method.[11] After evaporation of the methanolic solution at room temperature, the derivatives were dried in a vacuum desiccator and then dissolved in *cyclo*hexane (special for spectroscopy, B.D.H. Ltd.) and transferred to a volumetric flask of suitable size. In this way much of the extraneous matter eluted from the paper was discarded. The solutions were then examined with an ultra-violet spectrophotometer (Unicam SP 500).

Spectroscopic examination of 2 : 4-dinitrophenylhydrazones

Dinitrophenylhydrazones generally show three absorption bands in the ultra-violet region, of which that of longest wavelength is normally the most intense, and the one best used for analytical work. Within a group of related compounds its wavelength and molecular extinction coefficient are fairly constant. Values for solutions of these compounds in ethanol or chloroform have been collected by Gillam & Stern.[12] For our work it was more convenient to use *cyclo*hexane. The spectral absorption curves of a number of dinitrophenylhydrazones in *cyclo*hexane solution were plotted and the following generalizations made: Saturated, unsaturated and aryl-substituted aliphatic aldehydes (excluding $\alpha\beta$-unsaturated compounds) have a $\lambda_{max.}$ of about

340 mμ, while $\alpha\beta$-unsaturated aldehydes have a $\lambda_{max.}$ of about 360 mμ. Saturated, unsaturated and aryl-substituted ketones (excluding $\alpha\beta$-unsaturated compounds) have a λ_{max} of about 345 mμ, while $\alpha\beta$-unsaturated ketones have a $\lambda_{max.}$ of about 370 mμ. In aromatic or polyene conjugated aldehydes and ketones the bands become broader and shift to higher wavelengths. Solutions in ethanol or chloroform have been classified in a similar manner. Table II lists a number of carbonyl compounds with the approximate $\lambda_{max.}$ of solutions of their derivatives in *cyclo*hexane.

Table II

Longest-wavelength absorption bands of 2 : 4-*dinitrophenylhydrazones dissolved in* cyclo*hexane*

Aldehydes	λ mμ	Ketones	λ mμ
Formaldehyde	327	Acetone	344
Acetaldehyde	337	Ethyl methyl ketone	345
n-Butanal	340	Methyl *iso*propyl ketone	346
*iso*Butanal	339	Methyl *n*-propyl ketone	347
Hexanal	342	Diethyl ketone	347
Decanal	340	Allyl methyl ketone	346
Phenylpropanal	339	2-Methylhept-2-en-6-one	347
Crotonaldehyde	358	Benzyl methyl ketone	345
Hex-2-enal	358	Mesityl oxide	367
Cinnamaldehyde	377	β-Ionone	372

If the amount of derivative available for investigation was insufficient for recrystallization, then it was purified by chromatography on paper, using Whatman No. 120 if the amounts were large. A blank sheet of paper was developed at the same time and the area containing the spot, together with the corresponding area from the control sheet, were eluted and dissolved in *cyclo*hexane as described above. The optical density of the solution of derivative was then measured, using the control as zero reference solution. This procedure gave spectral absorption curves which could be compared with those of authentic compounds. The comparison was less satisfactory at very low concentrations and results with less than 10 μg. of derivative were not reliable.

Fig. 3 shows a graph, with optical density on a logarithmic scale, of the superimposed absorption curves of four derivatives. These are examples of the four aliphatic groups that can be distinguished in this way and they would run at similar rates in methanol–heptane.

Quantitative estimation of dinitrophenylhydrazones using paper chromatography

Measured volumes of standard solutions of authentic dinitrophenylhydrazones were developed on paper and the derivatives recovered and estimated at the appropriate $\lambda_{max.}$ for the compound. In these preliminary experiments the amounts recovered varied between about 85 and 95%. Various attempts to curb these fluctuations were unsuccessful. Slight variations in the R_F values obtained were also unexplained and it was concluded eventually that adsorption by the cellulose of the paper was responsible. This might well be favoured by the use of a non-aqueous solvent system.

The early separations of derivatives from the volatile matter from apples showed a division into four fractions, corresponding to C_2-, C_4- and C_6-aldehydes and a C_3-ketone. Acetaldehyde, acetone, *n*-butanal and hexanal were therefore chosen as representative compounds for calibrating a quantitative method. These were fractionally distilled immediately before use and from them were prepared standard solutions of the individual compounds in the specially purified light petroleum, in dilutions corresponding to those encountered in the apple analysis. Using the methods described above, and estimating the aldehydes at 440 mμ and the ketone at 445 mμ, about 70% recoveries were obtained, based on the amount of aldehyde or ketone used. From the results of duplicate analyses of each compound, calibration curves were drawn for the ranges 1 to 10 $\times$ 10^{-8}, 1 to 10 $\times$ 10^{-7} and 1 to 10 $\times$ 10^{-6} g.-mol. (concentrations of 1 $\times$ 10^{-7} to 10 $\times$ 10^{-5} g.-mol./l.). The curve for the range that was found most useful is given in Fig. 4.

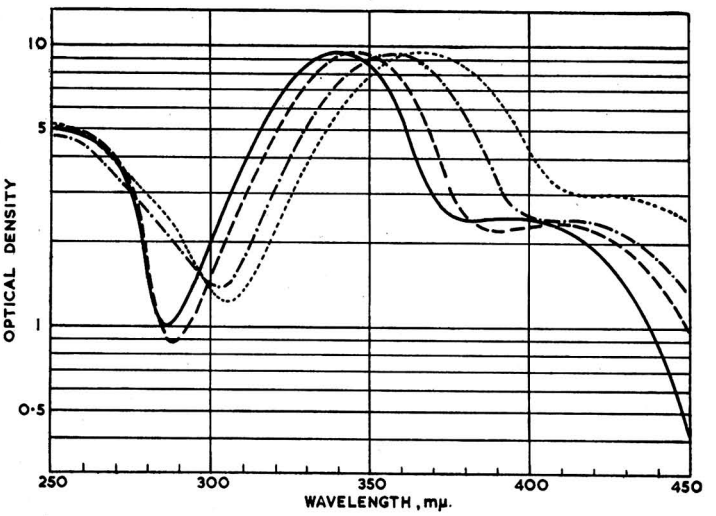

FIG. 3.—*Superimposed spectral absorption curves of solutions of 2 : 4-dinitrophenylhydrazones in* cyclo*hexane*

n-valeraldehyde
methyl ethyl ketone
hex-2-enal
mesityl oxide

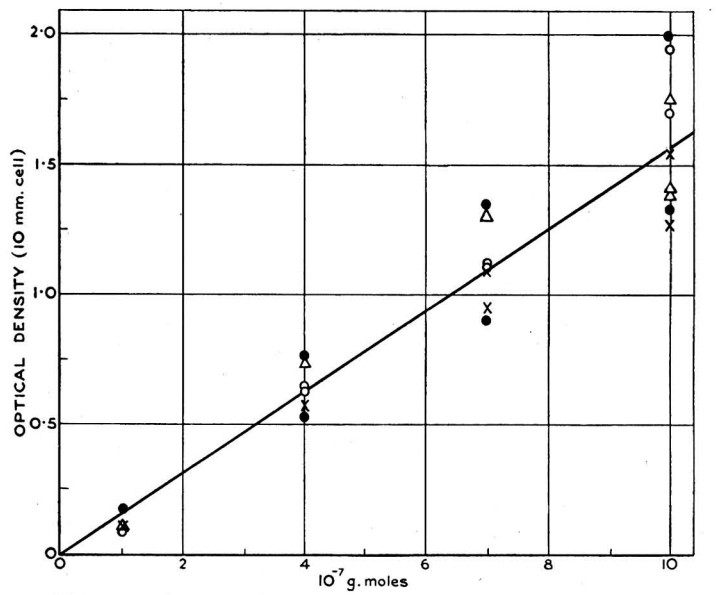

FIG. 4.—*Calibration curve for colorimetric determination of 2 : 4-dinitrophenylhydrazones after separation by paper chromatography : solutions in 10 ml. of* cyclo*hexane*

acetaldehyde △
acetone ●
n-butanal ×
hexanal ○

The standard deviation from the calibration curve value, calculated for eight analyses each of 0·07, 0·1, 0·4, 0·7, 1·0 and 4·0 μmoles of carbonyl compound were 33, 34, 17, 15, 18 and 17%, respectively. Below 0·07 μmoles, the reproducibility deteriorated rapidly.

Recovery of volatile substances from an air stream

The efficiency of a dry-ice trap system of the type used in these experiments has not been directly determined before, although Thompson & Huelin[13] found that when two spiral gas absorbers, cooled with solid carbon dioxide, were connected in series and used to trap volatile esters evolved by apples, the first trap accounted for 95% of the esters trapped in the pair of them. An estimate of the efficiency of our system was obtained by vaporizing, during a six-day period, a known amount of acetone into an air stream which was entering a train for collecting volatile matter at a rate of 8 l./h., the whole apparatus being housed in a constant-temperature room at 2°. To obtain conditions comparable with those encountered in the apple investigation, it was necessary to dissolve the acetone in specially purified light petroleum and inject the solution into the air stream from a fine capillary-tipped pipette actuated by a modified ' Agla ' micrometer syringe containing mercury and driven by a suitably geared synchronous motor (1 rev./day). This apparatus has been used in experiments on the physiological effect of volatile substances on stored apples.[14] Using the same apparatus, identical quantities of acetone solution were pipetted into specially purified light petroleum (100 ml.) for a control analysis.

In a similar experiment, acetaldehyde was used. This compound was both the most volatile and most reactive carbonyl compound encountered in gas stores. The amounts collected in the apple experiments were smaller than might have been expected from the behaviour of the fruit at ordinary temperatures. The behaviour of small concentrations of acetaldehyde in damp air at about 3° does not seem to have been investigated and it is possible that the amounts actually produced by the fruit would be depleted, partly through oxidation and partly through heterogeneous reactions on the surfaces with which the vapour can come in contact.

The results of these recovery experiments are given in Table III. The agreement between estimations is of the order to be expected. Acetone was recovered in good yield, but about half the acetaldehyde was lost.

Table III

Recovery of air-borne volatile compounds in light petroleum at about — 80°

	Amount added μmoles	Concentration in air μmoles/1000 l.	Amount recovered μmoles	Recovery %
Acetone	1·64	2·05	1·45	89
	0·62	0·78	0·68	110
	7·88	9·85	6·40	81
Acetaldehyde	0·17	0·21	0·08	44
	0·09	0·11	0·08	76
	0·27	0·34	0·08	31

Qualitative analysis of dinitrophenylhydrazones in bulk

After each quantitative estimation the solutions used were set aside in appropriate groups, so that bulk samples of derivatives of apple volatiles gradually accumulated. At the close of the quantitative experiments the bulked samples were first fractionated on columns of bentonite, following White's method[15] with slight modifications. For these derivatives, bentonite separations are complementary to those obtained by paper chromatography with methanol–heptane; bentonite has a greater affinity for ketones than the corresponding isomeric aldehydes, and it can be used to separate some isomeric branched and unbranched compounds. Thus the acetone and propanal derivatives could be separated easily on bentonite, and what appeared on a paper chromatogram to be acetone could be shown to contain minor amounts of propanal.

The bentonite was activated by heating at 70–80° in a vacuum oven for 2 hours at approx. 15 mm. pressure. Three sizes of column were used—13 × 200 mm. for 10–20-mg. quantities, 10 × 200 mm. for 5–10-mg. quantities, and 4 × 200 mm. columns for quantities of derivative less than 5 mg. The solvents used for development were light petroleum (b.p. 60–80°), ether and methanol in a graded series of mixtures. On elution from the column, the bands were collected;

if a large quantity of material was available it was repeatedly recrystallized from an appropriate solvent and its identity investigated by melting point and mixed melting point determinations; smaller bands were purified by chromatography on paper. This eliminated a number of compounds which were eluted from the bentonite itself and revealed themselves on the paper by their fluorescence in ultra-violet light. An attempt to purify some bentonite by passing methanol through it in a column, followed by reactivation, was not successful, as the product had a much reduced separating ability. The smaller bands were eventually rechromatographed on paper with suitable authentic compounds to establish their identity and to provide solutions for the determination of spectral absorption curves.

Results

The compounds which were identified in the bulk samples of apple derivatives are listed in Table IV with a rough estimate of the amount of derivative present in the sample analysed. These figures are an indication of the relative amounts of the corresponding carbonyl compound produced by the variety of apple, but no quantitative comparison can be made between varieties since the samples were of different sizes. Melting points and mixed melting points are given where these could be determined. Otherwise the identifications were made on the basis of chromatographic behaviour on paper and column, and on comparison of spectral absorption curves, except where 10 μg. or less of derivative are recorded, when reliable absorption curves could not be obtained.

Table IV

Aldehydes and ketones produced by gas-stored apples: analysis of 2 : 4-dinitrophenylhydrazones in bulk

Apple variety	Edward VII			Laxton Superb			Cox's Orange Pippin		
Compound	Estimated wt., mg.	M.p.	Mixed m.p.	Estimated wt., mg.	M.p.	Mixed m.p.	Estimated wt., mg.	M.p.	Mixed m.p.
Acetaldehyde	0·063	—	—	2·0	160°	161°	0·005	—	—
Propanal	0·025	—	—	0·2	—	—	—	—	—
Acetone	9·0	124°	125°	13·0	125°	126°	5·6	125·5	126·5
*iso*Butanal	—	—	—	0·047	—	—	0·005	—	—
n-Butanal	1·3	—	—	2·4	110°	115°	0·005	—	—
Ethyl methyl ketone	0·1	—	—	0·13	—	—	0·07	—	—
*iso*Valeraldehyde*	—	—	—	0·033	—	—	—	—	—
Methyl *n*-propyl ketone*	—	—	—	0·05	—	—	0·01	—	—

* Tentative identification

In the quantitative experiments the recorded data consisted of the total volume of gas withdrawn from each cabinet during any period, the volume of gas from which volatile compounds were extracted over a known collecting period, the estimated amounts of collected compounds (each corrected for the appropriate reagent blank) and the weight of apples that produced them. From these figures were calculated the concentration of the volatile compounds in the atmosphere of each store and the rate at which they were produced by the fruit. In Tables V–VII are shown the concentration figures. It can be deduced from the qualitative results that the four fractions into which the dinitrophenylhydrazones were separated consisted of C_2—acetaldehyde, C_3—acetone with minor amounts of propanal, C_4—*n*-butanal with minor amounts of *iso*-butanal and ethyl methyl ketone, C_5 and C_6—probably mostly *iso*valeraldehyde and methyl *n*-propyl ketone. In Figs. 5–8 are shown the corresponding rates of production (μmoles/1000 kg./day) in the form of graphs, relating the rate of production (logarithmic scale) and the storage period in days. The value for each storage period, whose duration is shown in the concentration tables, is represented by a point lying at the middle of the period. Neglecting the errors involved in collecting the compounds, the region above about 0·15 μmoles/1000 kg./day is subject to analytical errors of about $\pm 20\%$ rising to errors of about $\pm 35\%$ in the region below 0·05 μmoles/1000 kg./day. Values in the more erratic region below 0·1 μmoles/1000 kg./day have not been plotted. The two curves for acetaldehyde production which have been omitted from Fig. 8 would lie in the region occupied by the C_4 curves. As will be seen from Table VII,

after an initial rise, the values fell off. Although a reliable estimate of the total errors inherent in the analytical process would be difficult to obtain, a general impression of the degree of reproducibility of the process is given by the data shown.

Table V

Concentration of volatile compounds in stores of Laxton Superb apples

1952–3					1953–4				
Collecting period days from loading	Fractions, μmoles/1000 l. of air				Collecting period days from loading	Fractions, μmoles/1000 l. of air			
	C_2	C_3	C_4	C_5 and C_6		C_2	C_3	C_4	C_5 and C_6
13–24	0·05	1·69	1·25	0·15	10–16	0·07	4·28	2·78	0·05
26–37	0·16	2·77	1·31	0·17	23–29	—	0·69	0·08	0·02
40–51	0·41	1·58	1·02	0·12	37–43	0·08	—	0·01	—
54–65	1·44	1·72	0·75	0·12	51–57	0·10	0·37	—	0·02
68–79	0·59	0·65	0·47	0·09	65–71	0·20	0·36	0·05	—
80–88	0·86	1·73	0·25	0·07	86–92	0·17	0·46	0·04	—
96–102	1·10	2·41	0·42	0·15	100–106	0·24	0·35	0·06	—
110–116	1·91	2·80	0·17	0·08	114–120	0·27	0·43	0·04	0·00
124–130	1·38	2·00	0·17	0·07	128–134	—	0·38	0·01	0·01
138–144	1·20	1·81	0·21	0·07	142–148	—	0·38	0·01	0·00
152–158	1·20	2·04	0·21	0·06	156–162	—	0·37	0·01	0·00
166–172	1·97	2·02	0·14	0·06	170–176	—	0·26	0·04	0·01
187–193	0·98	2·02	0·13	0·05					

Table VI

Concentration of aldehydes and ketones in stores of Edward VII apples, 1952–3

Collecting period days from loading	Unwrapped				Wrapped*			
	Fractions, μmoles/1000 l. of air				Fractions, μmoles/1000 l. of air			
	C_2	C_3	C_4	C_5 and C_6	C_2	C_3	C_4	C_5 and C_6
5–16	—	0·21	0·01	—	0·02	0·81	0·06	0·04
17–28	—	0·37	—	—	0·00	1·83	0·05	0·04
29–40	0·02	0·48	0·02	—	0·01	2·05	0·04	—
43–54	0·02	0·48	0·01	—	0·04	6·15	0·04	—
57–68	0·03	0·72	0·01	—	0·00	3·95	0·07	0·01
69–77	0·06	0·30	0·01	—	0·04	5·40	0·04	0·01
85–91	0·06	0·88	0·03	—	0·03	3·12	0·03	0·03
99–105	0·07	0·91	0·02	0·01	0·07	3·60	0·05	0·04
113–119	0·11	0·99	0·02	—	0·02	2·14	0·02	0·01
127–133	0·11	0·75	0·02	0·02	0·04	2·07	0·02	0·03
141–147	0·09	0·75	0·01	0·03	0·01	1·67	0·03	0·03
155–161	0·16	0·35	0·00	0·03	0·03	1·94	0·01	0·01
176–182	0·18	0·72	0·02	0·01	0·05	1·26	0·02	0·01
190–196	0·39	0·91	0·02	0·01	0·04	1·20	0·02	0·02

* All collecting periods one day behind those of the unwrapped apples

Table VII

Concentration of aldehydes and ketones in stores of Edward VII apples, 1953–4

Collecting period days from loading	Unwrapped				Wrapped			
	Fractions, μmoles/1000 l. of air				Fractions, μmoles/1000 l. of air			
	C_2	C_3	C_4	C_5 and C_6	C_2	C_3	C_4	C_5 and C_6
9–15	0·08	3·10	0·08	0·03	0·02	2·34	0·17	0·05
23–29	0·06	0·72	—	0·03	0·07	1·63	0·15	—
37–43	0·21	1·58	0·12	0·03	0·11	3·84	—	—
51–57	0·18	0·55	0·13	0·06	0·14	1·36	0·09	—
72–78	—	1·47	0·04	—	0·08	1·34	0·05	—
86–92	0·07	1·12	0·06	—	0·23	1·08	0·09	—
100–106	0·06	0·76	0·06	—	0·09	0·96	0·02	0·02
114–120	—	0·60	0·04	0·02	—	0·94	0·02	0·02
128–134	—	0·29	0·01	0·00	—	0·88	0·04	0·02
142–148	—	0·38	0·01	0·01	—	0·70	0·03	0·01

Discussion

Qualitative analysis

Acetaldehyde, acetone, propanal, hexanal and hex-2-enal have been identified in previous work on apples, but the analytical samples were obtained in a variety of ways—from the flesh

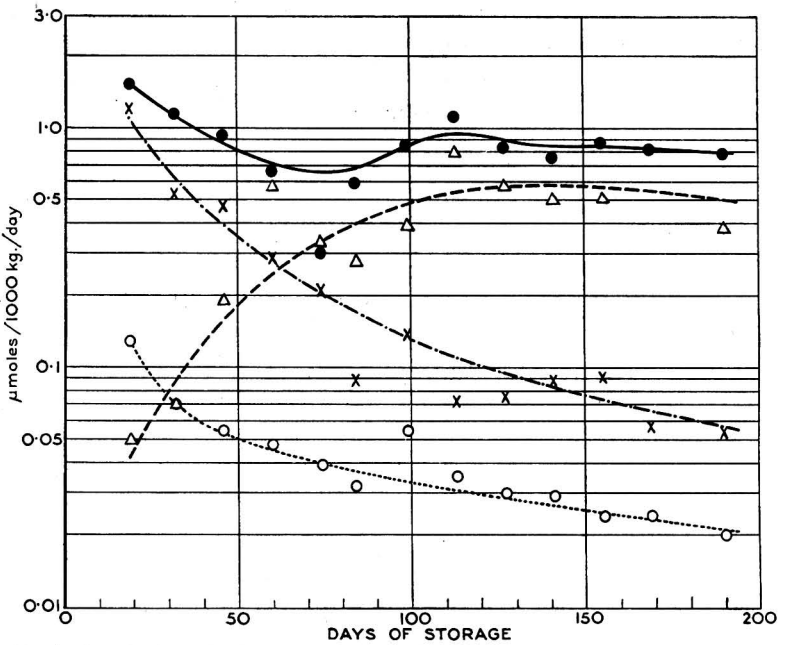

FIG. 5.—*Rates of production of aldehydes and ketones by Laxton Superb apples in gas storage* 1952–53

C_2 compounds
C_3 compounds
C_4 compounds
C_5 and C_6 compounds

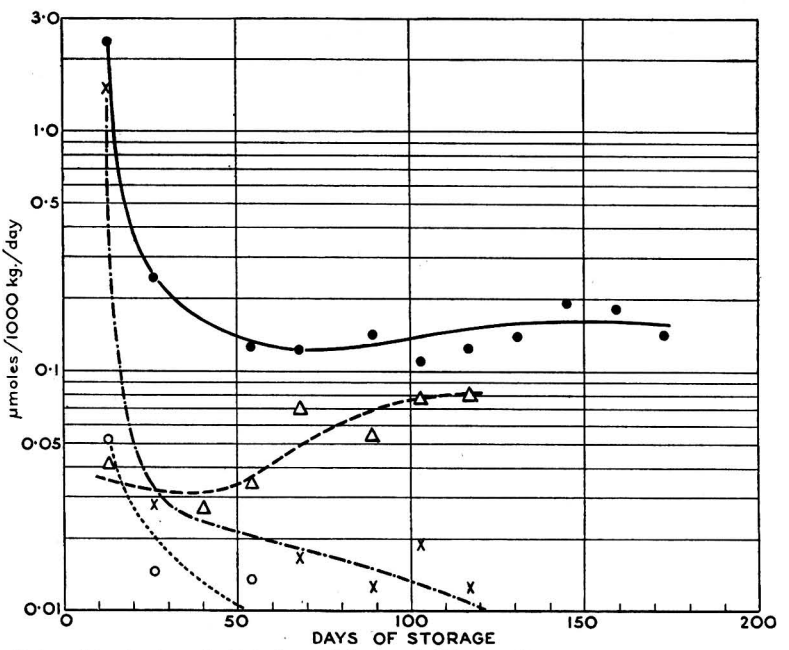

FIG. 6.—*Rates of production of aldehydes and ketones by Laxton Superb apples in gas storage* 1953–54

C_2 compounds
C_3 compounds
C_4 compounds
C_5 and C_6 compounds

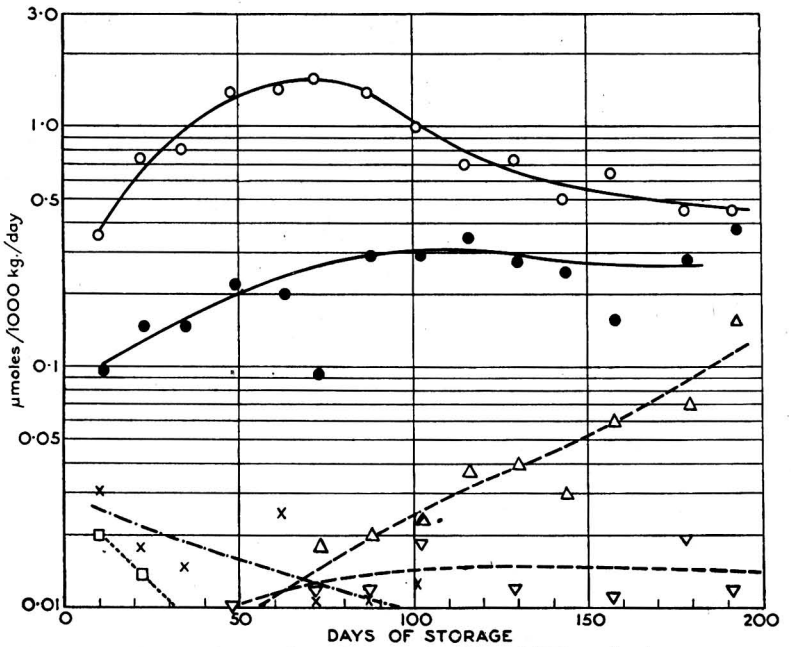

FIG. 7.—*Rates of production of aldehydes and ketones by Edward VII apples in gas storage* 1952–53

C_2 compounds from unwrapped apples
C_2 ,, ,, wrapped apples
C_3 compounds from unwrapped apples
C_3 ,, ,, wrapped apples
C_4 compounds from wrapped apples
C_5 and C_6 compounds from wrapped apples

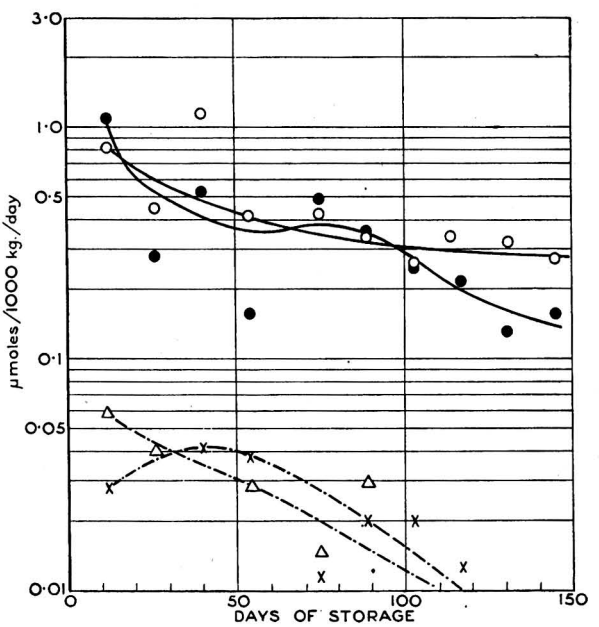

FIG. 8.—*Rates of production of aldehydes and ketones by Edward VII apples in gas storage* 1953–54

C_2 compounds from unwrapped apples
C_2 ,, ,, wrapped apples
C_4 compounds from unwrapped apples
C_4 ,, ,, wrapped apples

of the fruit, from air drawn once over a small sample of fruit, or, as in cold storage, from a large volume of air constantly recirculating over the fruit. Comparisons between the various results are therefore difficult ; there is no simple way of relating the composition of the flesh to that of the evolved compounds ; the effects of temperature on the rate of production of these compounds and the reactions which they may spontaneously undergo in the vapour phase and on available solid surfaces in the store are imperfectly understood.

There seems to have been general agreement that acetaldehyde is the most abundantly produced carbonyl compound at ordinary temperatures. At cold storage temperatures, however, Henze *et al.*[4] found mostly acetone, and in our work acetone was the most abundant compound, even if it is assumed that only half the acetaldehyde was collected. Their starting material was extracted from activated carbon filters and it is possible that under such conditions condensation reactions would occur to convert acetaldehyde to compounds of higher molecular weight. Polyene compounds, of which they found indications, might be formed in this way.

White[15] has identified hexanal and hex-2-enal. These compounds are less volatile than the others and might be evolved from the fruit only in small quantities. Hex-2-enal would in any case not be found when trapping methods involving the use of bisulphite were used, since it would not be regenerated from its bisulphite compound. Again, hex-2-enal might be an artefact, as Nye & Spoehr[16] have shown to be the case in their experiments on leaves ; the amounts recovered when oxygen was excluded or enzymic action inhibited during the distillation were negligible.

In our work acetone, acetaldehyde, *n*-butanal, propanal, ethyl methyl ketone, *iso*butanal, methyl *n*-propyl ketone and *iso*valeraldehyde were identified—this representing roughly their order of abundance in the apple varieties used. All, except perhaps *iso*butanal and ethyl methyl ketone, have been found in plants before.[17] It is in the nature of the column and paper chromatographic methods of separation used here that the compounds of lower molecular weight can be more definitely characterized than the higher. Thus it is difficult to conceive of other compounds, which with a spectral absorption curve like that of acetaldehyde dinitrophenylhydrazone, would behave in the same way in a chromatographic separation. With a C_5- or C_6-compound however the number of other possibilities becomes too great to estimate. Where, as in this case, other evidence is lacking, the identification of *iso*valeraldehyde and methyl *n*-propyl ketone can only be considered tentative. From the work of Ross[18] on the infra-red spectra of dinitrophenylhydrazones it seems probable that in work of this sort, where microquantities must be used, infra-red spectra would provide further confirmation of identity.

If we compare the results obtained with Edward VII apples, which are susceptible to scald, and Laxton Superb and Cox's Orange Pippin apples which are resistant, no clear differences appear. There seems more reason to distinguish between Laxton Superb and Cox's Orange Pippin, since, if the relative amounts are compared, the Cox variety appears to produce ketones at the expense of aldehydes.

Quantitative analysis

Fidler[7] has shown that during the first ten days of gas storage the rate at which apples respire follows a series of changes ; it drops rapidly during about the first three days and then rises rapidly to a peak at about the seventh day before beginning a slower decline to a more or less constant level. This transitory stimulation and decline obliterate any indications that there might be of a climacteric.

In our analysis the necessary amounts of volatile substances could only be obtained by collecting over a period of days, which made it impossible to follow clearly the early behaviour of the fruit, in which rapid changes of activity might occur ; the same may be said of the estimates that have previously been made of production of ethylene and total non-ethylenic volatile matter.[7] However, the rate at which volatile substances are produced must inevitably drop rapidly as the apples cool from the higher outside temperature to that of the store. Whether a subsequent rise and fall ensues remains uncertain and the analytical results obtained from the first collecting period must be markedly affected by its precise relation to the time when storage began.

The results from the two crops of Laxton Superb apples, which came from different orchards and in different years, resembled each other in certain ways. In each, after an initial period of about 60 days in which there were rapid changes, the rates of production tended to settle in the order $C_3 > C_2 > C_4 > C_5$ and C_6 compounds (Figs. 5 and 6). The acetaldehyde curves can be distinguished from the others by their continued tendency to rise, and if corrected for an assumed loss in collection, would meet the acetone curves in the latter half of the storage period. The actual rates of production differed greatly in the two samples, being some five times as great in the first as in the second. Similar comparisons may be made between the concentrations in the air of the two stores (Table V).

The various compounds produced by Edward VII apples show a similar order of abundance to those of the Laxton Superb. In the first year the wrapped and unwrapped fruit came from different orchards and the wrapped were most productive of volatile substances, with the notable exception that acetaldehyde production remained low (Table VI, Fig. 7). In the second year both were from the same orchard and the rates of production did not differ significantly (Table VII, Fig. 8). The results for acetaldehyde, after an increase early in the season, became unusually erratic; this was attributed to overlong storage of the volatile solutions before analysis. For fruit grown in Sutton Valence, a comparison between the two years shows that for a considerable period acetone production was three or four times as great in the first season as in the second.

Table VIII

Condition of apples removed from gas storage

Season	Apple variety and treatment	Loss in weight %	% number of apples affected		
			Rotting	Scald 1st examination	Scald 2nd examination
1952–3	Edward VII unwrapped	2·0	19·8	31·8	30·4
	Edward VII wrapped	1·6	1·34*	0·73*	8·7*
	Laxton Superb	1·75	8	—	—
1953–4	Edward VII unwrapped	1·8	27·9	75·7	85·7
	Edward VII wrapped	1·6	2·8	0	0·9
	Laxton Superb	2·87	55·8	—	—
1954–5	Cox's Orange Pippin	1·88	35·5	—	—

* The figures for fruit from the same orchard, unwrapped, were 2·6, 16·6, 41·8

A study of the figures for incidence of scald in the various samples (Table VIII) does not show any correlation between a high rate of carbonyl compound production and a heavy incidence of scald. Protection of the fruit from scald by wrapping does not appear to have had any significant effect; nor, if the wraps are ignored, can the scald figures for unwrapped fruit be correlated. If there is a connexion between them, then it must be a subtle and indirect one. Clearly the concentration of these compounds in gas stores can vary widely; whether orchard or weather conditions, the time of picking or some other factor determines the level would require considerable data to determine. If for example there were a precise means of predicting a standard picking date for an apple, then one variable at least could be controlled.

In the same table are given figures for apples affected by rotting. From the results for Edward VII apples it will be seen that in both seasons the extent of rotting was correlated with the incidence of scald and/or the wrapping of the apples. Where, in the 1953–4 season, the behaviour of the wrapped and unwrapped apples can be directly compared, it can be seen that the heavier rotting of the unwrapped apples did not cause a significant increase in the production of carbonyl compounds.

It seems reasonable to suppose, as Huelin has suggested,[5] that, in the apple, acetaldehyde probably arises by decarboxylation of pyruvic acid, or as an intermediate in the interconversion of ethanol and acetic acid, while acetone, often a by-product of lipid metabolism, arises by the decarboxylation of acetoacetic acid. Our results are consistent with this as they suggest that the rate of production of acetaldehyde varies independently of the rate of production of the C_3-, C_4-, C_5- and C_6-compounds. The unbranched aldehydes and methyl ketones might be formed

as by-products of the fatty acid cycle,[19] while the branched chain compounds *iso*butanal and *iso*valeraldehyde could perhaps be by-products of terpene synthesis or breakdown. Bonner, Parker & Montermoso[20] have suggested the following route for the synthesis of isoprene units by the rubber plant: acetoacetate + acetyl-co A $\rightleftharpoons$ β-hydroxy-β-methylglutaryl-co A $\rightleftharpoons$ β-methylcrotonyl-co A (co A = co-enzyme A) and *iso*valeraldehyde in particular could conceivably be formed in a similar way.

Conjectures such as these are, however, of little value until more accurate and less laborious analytical methods are available for the study of apple volatile metabolism in adequate detail. Whether the rate of production of these compounds would provide a sensitive index of the development of the apple, through its climacteric rise to senescence, or whether the compounds have no real metabolic significance in the fruit are problems which await investigation.

Summary

1. A method is described for the collection of volatile compounds from the air above apples kept in cold store at 38–39° F.

2. Carbonyl compounds present in the volatile matter were converted into 2 : 4-dinitrophenylhydrazones, which were separated by paper chromatography and identified by ultra-violet spectroscopy. Bulk samples of these derivatives were separated on bentonite columns and some were identified by melting point.

3. Good recovery of acetone, but only 50% of acetaldehyde, was obtained from air streams under conditions similar to those of the apple experiments.

4. The main constituent of the volatile matter from apples was acetone, with smaller amounts of acetaldehyde, *n*-butanal, propanal, ethyl methyl ketone and *iso*butanal, with (probably) *iso*valeraldehyde and methyl propyl ketone in traces.

5. The rate of evolution of the various carbonyl compounds during storage of apples has been determined. The results from Edward VII and Laxton Superb varieties were similar.

6. There was no correlation between high rate of carbonyl evolution and heavy incidence of scald, and protection of the fruit from scald by wrapping had no significant effect on the production of volatile compounds. Rotting of the fruit did not increase significantly the evolution of carbonyl compounds.

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FLAVOUR OF DEHYDRATED POTATOES MADE FROM MATERIAL TREATED WITH TETRACHLORONITROBENZENE*

By E. G. B. GOODING, C. G. TUCKER and J. M. HARRIES

Tests by two independent taste panels showed that potatoes, treated with TCNB* to reduce sprouting during storage, yielded a dehydrated product with a definite ' earthy ' taint, which increased with the period of storage of the raw potatoes. Heavy leaching during processing slightly reduced the intensity of the taint. When the dehydrated product had been stored for a year or more under temperate conditions the taint was still apparent.

Introduction

The season for commercial dehydration of potatoes lies between the end of the carrot season and the beginning of the cabbage season, viz., roughly from December to May or June. Long storage of the raw tubers is inevitable, but even at the normal temperatures of storage in the clamp, sprouting of the tubers may become a serious problem before the end of the season, while during cold spells when clamp temperatures in this country may fall well below 40° F, the sugar content of the potatoes may rise to levels which adversely affect the quality of the dehydrated product. There is evidence suggesting that if potatoes could be held at higher temperatures than normal (e.g., not below 45° F) for the period of storage between lifting and processing a better dehydrated product would be achieved,[1] but this has so far not been feasible on account of the acceleration of sprouting brought about by such conditions.

The advent of sprout depressants brings long storage or storage at temperatures higher than usual within the bounds of practicability, and in a recent experiment at the Experimental Factory of the Ministry of Agriculture, Fisheries and Food at Aberdeen, technical grade tetrachloronitrobenzene (TCNB) was tried in this connexion. The effect of TCNB on the flavour of stored potatoes has received adverse comment,[2] and one of the objects of the Aberdeen experiment was to ascertain whether the flavour associated with this material would be transmitted to and retained in the dehydrated product.

* In the tests described in this paper, the technical grade material was used. Work is at present in progress in which a more highly purified TCNB preparation is being tested.

Experimental

Storage

Twenty-four tons of King Edward potatoes grown at Tarland, Aberdeenshire, were lifted in October and stacked 5–6 feet high in a stone building. The walls were lined internally with straw bales and the potatoes were covered with loose straw. The tubers were dusted during stacking with a commercial sprout depressant understood to contain 3% by weight of 2 : 3 : 5 : 6-tetrachloro-1-nitrobenzene in an inert carrier ; this preparation was applied at the rate of 10 lb. per ton of potatoes. The control material was 130 tons of King Edward potatoes from the same field, clamped on the farm, with no sprout depressant.

Sampling and dehydration

Three-ton lots of the control tubers were taken from the clamp after storage for 10, 14, 18 and 26 weeks (January, February, March and May), and dehydrated. Similar quantities of the treated material were taken from store after 11, 15, 19 and 27 weeks' storage. Dehydration was by the usual technique practised in Britain.[3] Scalding in water was employed, and in this connexion it should be noted that batches produced early in a run may be expected to have suffered heavier leaching of solutes during scalding than those processed later in a run. The product was in the form of strips, $\frac{3}{16} \times \frac{5}{16}$ in. in cross-section.

At the end of the season (May 1954), three cans of the dehydrated product were taken from an early batch (heavily leached) and two from a late batch (lightly leached) of each run ; one can was retained for taste panel examination at Aberdeen, the other cans were sent for examination to the laboratories at headquarters in London.

Twelve months later (May 1955) further cans were taken from control and treated materials which had been retained at room temperature, and the contents of the cans were submitted to a taste panel at Aberdeen.

Culinary tests

Three separate sets of taste panel tests were carried out, two at Aberdeen and one in London. The methods used differed in all three tests and they are described separately below ; in all of them the panel consisted of members of the staff of the Scientific Adviser's Division of the former Ministry of Food. In all tests 20-g. samples of the dehydrated potato strips ($\frac{3}{16} \times \frac{5}{16}$ in. cross-section) were reconstituted by the addition of 190 ml. of hot water + 10 ml. of 10% sodium chloride solution (or greater quantities in proportion), allowing to soak for 2 hours, and then cooking for 15 minutes.

Taste panels in Aberdeen, May 1954

Eight taste panels were held, four on first-batch material and four on last-batch. On each occasion five samples were presented, composed of a pair and a triad, i.e., there were either two treated and three untreated samples, or two untreated and three treated samples, all of the same month's production. Tasters were told that the five samples, coded at random, could be divided into two groups and were asked to do so on the basis of their flavour, denoting in addition any samples which they thought had a natural flavour by the letter *a*, and any samples which they thought possessed an off-flavour with the letter *b*. This is a simple extension of the two-out-of-five-test described elsewhere.[4] The tasters sat in separate cubicles and tasting was carried out under red lights to eliminate any possible differences in colour between the samples.

Taste panels in London, May 1954

Eight sessions were held, four samples being presented at each session. Tasters were asked to allot a score for flavour only according to a system previously used for fresh potatoes.[2] A scale from 0 to −5 was intended to indicate the intensity of any off-flavour present and any score other than 0 was to be accompanied by an adjective which the taster thought best described the off-flavour. Positive marks up to +2 were also allowed to indicate any natural flavours they thought were present.

In any one session two treated samples were directly compared with two untreated samples of the same batch and month of production. It was also arranged that, separately for first and last batches, direct comparisons were obtained between the following times of storage in clamp:

January with May
February with March
January with March, and
February with May

In this way each of the above comparisons was obtained twice, once for first-batch material and once for last-batch. It was not possible also to compare first-batch material with similar last-batch material directly in the same session. There were thus 32 samples in all, each of the 16 treatment combinations being tasted twice.

Taste panels in Aberdeen, May 1955

The material used in this test had been stored, after dehydration, for 12 to 16 months at room temperature (40–70° F). No untreated samples were available from the February and March production batches, so that it was not possible to adopt an orthogonal tasting plan. Six sessions were held and at least one untreated sample was tasted at each session. Each sample was tasted four times and direct comparisons between different times of storage in clamp were standardized as far as possible. In the results, there were twice as many readings for treated samples as for untreated samples.

Tasters were asked to record off-flavour only, indicating the intensity on a scale from 0 to −5, and to use an adjective to describe the nature of the off-flavour, but the adjectives were drawn from a limited list [see Table III(*c*)] which had been found by experience to cover most of the off-flavours found in dehydrated potatoes.

Results

Aberdeen panel, May 1954

The results of this set of tests are shown in Table I. It will be seen from column 3 how many of each type of sample were presented, and from column 5 the number of correct groupings. The results for March (first batch) and for March and May (last batch) are significant in that the odds against this number of correct groupings, had there been no real difference between the two sets of samples, are 19 : 1 or greater. Shown in columns 6 to 9 are the number of assignments of the letters *a* and *b* to treated and untreated samples, made by all tasters whether correct in their groupings or not. On only one occasion did a taster who separated the five samples

Table I

Flavour of dehydrated potatoes made from stock treated with TCNB (Aberdeen panel 1954)
Five samples presented at each tasting

Batch	Month taken from clamp	Number of control samples	Number of tasters present	Number of correct groupings	Number of assignments (total)			
					Control		Treated	
					a	*b*	*a*	*b*
First	Jan.	2	4	1	5	3	7	5
	Feb.	2	5	0	4	6	8	7
	March	3	4	3	8	4	2	6
	May	2	4	0	6	2	5	7
Last	Jan.	2	4	0	3	5	7	5
	Feb.	2	4	0	6	2	5	7
	March	3	5	3	12	3	1	9
	May	3	4	2	10	2	0	8

Tasters were asked to assign letter *a* to samples with normal flavour, *b* to those possessing a taint.

correctly into their two groups allot the letter *a* to treated samples and *b* to untreated samples (March, first batch). It will be seen from the Table that, when a significant number of differentiations were made, the assignments of the letters were predominantly *a* to untreated and *b* to treated samples.

There is a presumption from these results (though this evidence is not on its own sufficient) that there was no detectable difference between treated and untreated potatoes either of first or last batches, taken from the clamp in January and February, but that for material taken from the clamps in March and for last-batch material taken from the clamp in May there was an appreciable difference between treated and untreated potatoes, the latter predominantly meriting the description ' no off-flavour ' and the former predominantly meriting an assignment of some degree of off-flavour. There was also a suggestion that first-batch material produced from potatoes stored in clamp until May, though not so clearly differentiated as similar last-batch material, also showed some degree of off-flavour in the treated samples.

London panel, May 1954

The results of this set of tests are shown in Table II(*a*), II(*b*) and II(*c*). Table II(*a*) shows the average marks given to each type of material and is based on all the tasters present (usually 8). Table II(*b*) shows an analysis of variance of the results of the four tasters who were present at all sessions. Table II(*c*) shows the frequency with which certain adjectives were used to describe off-flavours, again based on all tasters present.

Since two samples of each treatment combination were tasted, the residual error in the analysis of variance [Table II(*b*)] is based on the reproducibility of the results by the tasters. As there were no significant interactions, they have all been pooled with the residual to give a final estimate of error. All the main factors gave significant results, the most significant being that due to treatment, and it will be seen from Table II(*a*) that the TCNB-treated material

Table II(*a*)

Flavour of dehydrated potatoes made from stock treated with TCNB (London panel 1954)

Month taken from the clamp	Average scores			
	Treated		Untreated	
	First batch	Last batch	First batch	Last batch
January	− 0·25	− 1·00	− 0·32	− 0·19
February	− 0·50	− 1·25	− 0·30	− 1·29
March	− 1·29	− 1·81	− 0·64	− 0·96
May	− 1·30	− 1·46	− 0·50	− 0·29

Table II(*b*)

Analysis of variance

Source of variance		Sums of squares	Degrees of freedom	Mean square	Significance
Between treatments	T	13·781	1	13·781	***
,, batches	B	3·781	1	3·781	*
,, months	M	11·602	3	3·867	*
,, tasters	O	14·946	3	4·982	**
Interactions	T × B	0·634	1	0·634	
	T × M	5·297	3	1·766	
	T × O	5·359	3	1·786	
	B × M	3·518	3	1·173	
	B × O	3·984	3	1·328	
	M × O	6·882	9	0·765	
	T × B × M	0·692	3	0·231	1·006
	T × B × O	4·570	3	1·523	
	T × M × O	12·313	9	1·368	
	B × M × O	5·217	9	0·579	
	T × B × M × O	0·729	9	0·081	
Residual		70·500	64	1·102	
Total		163·805	127		

Table II(*c*)

Frequency of adjectives describing off-flavour

	Treated	Untreated
Stale (storage)	11	6
Musty	7	0
Mouldy	7	1
Sulphite	20	14
Sulphurous	6	2
Moth balls	2	0
Chemical disinfectant	5	6
Metallic	2	1
Earthy	26	8
Rancid	3	0
Smoky	4	13
Burnt	3	10
Bitter	1	1
Cardboard	3	3
Bland	2	2
All others	5	8
Total	107	75

Levels of significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

showed an off-flavour which was, in general, more intense in late batches than in first batches, and which increased significantly with time of storage in clamp. Table II(*c*) shows that the words 'earthy' and 'sulphite' were used most frequently to describe the treated material, although the choice of adjectives was entirely free and the range was considerable. It is noteworthy that these flavours have in common the leaving of a persistent after-taste in the mouth.

Aberdeen panel, May 1955

The results of this set of tests are shown in Table III. Table III(*a*) shows the average marks given and Table III(*b*) an analysis of variance of the scores given to the treated samples only. (This was because of the lack of balance between treated and untreated samples.) Table III(*c*) shows the frequency with which the available adjectives were used to describe the above sets of samples.

It is clear from these results that potatoes treated with TCNB retained a strong taint during the 16 months of temperate storage and that the increase in taint with longer storage in clamp was still shown, an effect which the analysis of variance [Table III(*b*)] shows to be significant.

Table III(*a*)

Flavour of dehydrated potatoes made from stock treated with TCNB (Aberdeen panel 1955*) : dehydrated product stored for* 12–16 *months after manufacture*

Month taken from clamp	Average scores: Treated	Untreated control
January	− 0·75	− 0·50
February	− 1·25	—
March	− 2·05	—
May	− 2·05	− 0·70

Table III(*b*)

Analysis of variance (treated samples only)

Source of variance	Sums of squares	Degrees of freedom	Mean square	Significance
Between months	22·76	3	7·58	***
Between tasters	28·45	4	7·11	***
Interaction	10·35	12	0·86	—
Residual	69·69	60	1·16	—
Total	131·25	79	—	—

Table III(c)

Frequency of adjectives describing off-flavour

	Treated	Untreated
Stale (storage)	11	1
Sulphite	0	0
Earthy	23	2
Cardboard	2	0
Sour	0	0
Alkaline	0	6
Scorched	8	2
Any other off-flavour	0	0
Total	44	11

Note: Twice as many treated as untreated samples were tasted.

Discussion

The results of the tests indicate very clearly that treatment of raw potatoes with technical grade TCNB at the rate normally recommended (10 lb. of the 3% dust per ton of potatoes) has led to a taint in the dehydrated product, and that the taint was strongest in the material which had suffered least leaching during processing. There was a striking increase in strength of taint from a minimum in the material taken from store in January (after 10 weeks' storage) to a maximum in that taken from store in May (after 26 weeks' storage). It was thought after the 1954 tests that the probable explanation was a loss of taint during storage of the dehydrated potato (those dehydrated in May were tasted almost immediately after production, while those dehydrated in January had been stored for 4 months), but in the 1955 tests the same effect was found when the potatoes had been stored for 12–16 months, thus ruling out this hypothesis. In an earlier paper[2] there was some indication that taint might be decreasing during storage in the clamp, but, since those tests were made on fresh potato, it was, of course, impossible to make direct comparisons between material stored for different lengths of time.

No controlled consumer tests were arranged but one batch of TCNB-treated material (taken from store in March) was served as mash in a canteen which had been receiving untreated dehydrated potatoes for several weeks. The reception was markedly hostile.

Conclusion

Tests conducted by panels in Aberdeen and London showed that King Edward potatoes, treated with technical grade TCNB, yielded dehydrated products with a persistent taint, which increased with the period of storage of the raw potatoes. Heavy leaching during processing slightly reduced the extent of the taint.

Acknowledgments

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HEAT AND WATER TRANSFER DURING THE DEHYDRATION OF HERRING FILLETS

By M. M. DEL CAMPO and C. L. CUTTING*

The rate of drying of herring fillets in an over-draught dryer was very sensitive to temperature, but practically unaffected by normal ranges of wet-bulb depression and air velocity. Fatty herrings took longer to dry than lean ones and considerable fat oxidation occurred during the process. Variation of thickness within the range usually encountered was of some importance.

The storage life of fillets, which depends very much on taste, was limited chiefly by (1) the oxidation of the fat, which could be prevented by packing in an inert gas, and (2) the development of moulds, which occurred unless the moisture content was below about 16%. It was found from a determination of equilibrium moisture contents at a number of relative humidities that this corresponded to about 70–75% R.H. It is considered that if suitably packaged, the best products could be kept in condition acceptable to some native races in tropical regions for several months under the prevailing climatic conditions.

Introduction

In connexion with a more economic exploitation of our herring resources than the present widespread manufacture of fish meal and oil, an attempt has been made to preserve herring fillets by a dehydration process and to obtain a palatable product that can easily be reconstituted, and that would provide a means of meeting the protein deficiency so common in undeveloped countries. Primitive methods of drying and smoking are commonly used in many parts of the world (e.g., the preparation of ' bonga ' in West Africa), and it was thought that it might be economically possible by large-scale mechanical methods to produce a smoked dried herring for export from this country.

Preliminary experiments by one of us[1] had shown that although whole herrings could be hot-smoked and dried to produce a commodity similar to ' bonga ', the drying process, which took several days compared with a few hours for cooked mince, was likely to be too lengthy for commercial purposes when the fresh fish contained more than about 5% of fat. Since microbiological activity continues in dehydrated fish during storage down to moisture contents of about 10%,[2] thorough drying was essential, but the removal of the last 20 or 30% of the moisture took up about half the total drying time. The drying time had also been reduced by filleting the fish, thus reducing thickness, and by storing at a moisture content of between 25 and 30% in closed containers in an atmosphere of carbon dioxide.

This paper presents the results of experiments on the effect of such variables as temperature, relative humidity, air velocity, size and fat content of the fillets, and the arrangement of the fillets on the rate of drying of herring fillets in an ' over-draught ' truck and tray dryer. The effect of smoke was not investigated but this would not be likely to affect the rate of drying. No systematic attempt was made to assess the palatability or storage properties of the product and no detailed consideration was given to problems of packaging the product or to economic aspects of the drying process.

Experimental

Double fillets were cut from a group of herring of average size and weight by hand or machine, one from each fish with the dorsal fin in the middle. These were placed on wire-mesh trays in an experimental dryer, similar to that previously described by Ede & Hales,[3] and subjected to an even temperature (ranging from 80° to 180° F), humidity (20° to 40° wet-bulb depression) and air speed (1·5 to 21 feet per second).

Length, maximum width and maximum thickness of each fillet were recorded and averages obtained for each batch which usually consisted of 36 fillets, selected to be as uniform as possible. The values in different experiments ranged from 16·75 to 19·25 cm. (6·6 to 7·6 in.) in length,

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3·45 to 3·75 cm. (1·36 to 1·4 in.) in width and 0·8 to 1·05 cm. (0·32 to 0·4 in.) in thickness, and the weights from 28·2 to 39·5 g. Although differences of this order, particularly in thickness, have an appreciable effect on the rate of drying, it is considered that the variation over the size of batch employed would not greatly affect the consistency or comparability of the average results.

The batch of fish was weighed at regular intervals, and drying rates obtained from two successive values of water content and the time elapsed. Water contents were expressed as the ratio of the weight of water to solids plus oil.

Final water contents were determined by drying samples to constant weight in an oven at 218° F (103° C) and occasionally by distillation with toluene.[4]

In most cases the final water contents did not agree exactly with that calculated from the original water contents and the corresponding losses of weight. This was chiefly because of the difficulty of sampling material of variable composition, especially as regards fat content which varied from fillet to fillet. Occasionally, too, oil was lost during the drying process, particularly in the case of the fattier fish and the higher temperatures.

Fat contents were determined by Soxhlet extraction with ether. Peroxide values of the oil extracted by light petroleum were determined from time to time as an index of the oxidative rancidity by Banks' modification[5] of the method of Lea.[6]

Results and discussion

Figs. 1–3 show the effects of dry-bulb temperature, wet-bulb depression (W.B.D.) and air speed on the rate of drying of fish of medium to low fat content (about 5–10%). Only temperature has any significant effect.

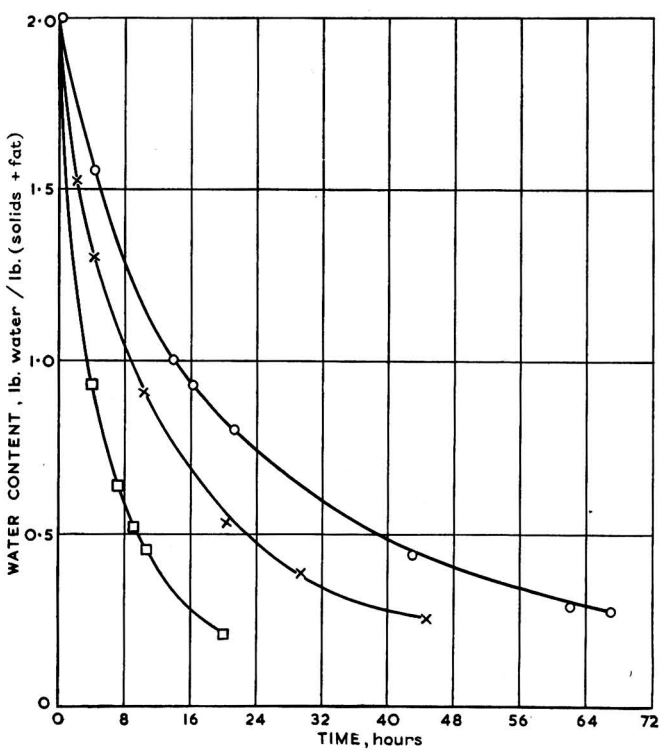

FIG. 1.—*Effect of temperature on the drying of herring fillets*

○ Dry bulb 80° F; air speed 12 ft./sec. W.B.D. 20° F
× Dry bulb 100° F; air speed 12 ft./sec. W.B.D. 20° F
□ Dry bulb 140° F; air speed 12 ft./sec. W.B.D. 20° F

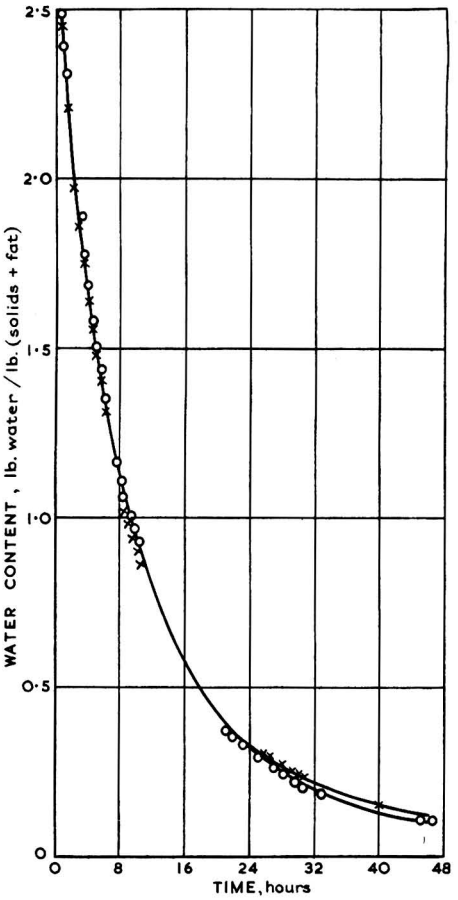

FIG. 2.—*Effect of wet bulb depression (W.B.D.) on the drying of herring fillets*

O Dry bulb 120° F; air speed 12 ft./sec. W.B.D. 40° F; R.H. 15%

× Dry bulb 120° F; air speed 12 ft./sec. W.B.D. 30° F; R.H. 30%

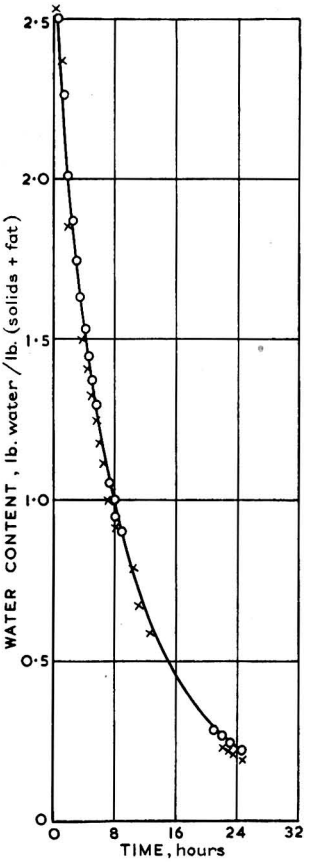

FIG. 3.—*Effect of air speed on the drying of herring fillets*

O Dry bulb 120° F; air speed 6 ft./sec. W.B.D. 40° F; R.H. 15%

× Dry bulb 120° F; air speed 18 ft./sec. W.B.D. 40° F; R.H. 15%

Effect of temperature

An increase of temperature at a constant wet-bulb depression increased the rate of drying, but at 160° F, and even more so at 180° F, the thinner portions of the fillet such as the tail end were scorched, and the flesh tended to crumble when handled so that packaging would be difficult, if not impossible. At 180° F, too, the fillets were very dark and adhered to the trays and the oil of fatty fish was badly oxidized. At a maximum permissible temperature of 140° F the total drying time from, say, 3·0 to 0·2 lb. of water per lb. of solids would not be below about 20 hours. However, at 140° F and 20° F wet-bulb depression the time in the dryer could be reduced by about 40% if the fish were taken out at a water content of about 0·4 lb. per lb. of solids and drying completed under external atmospheric conditions.

Jason[7] has shown with ' white ' fish that the water content during drying falls off exponentially with time. Neither the present experiments, nor some published data for the drying of whole, raw capelin[8] (a fish containing only a few % of fat) displayed this relationship, even when allowance was made for the equilibrium water content corresponding with the humidity of the drying air.

Fig. 4 shows that fairly straight-line relationships, apart from a warming-up period at the beginning, were obtained by plotting the logarithm of the water content against the logarithm of the rate of drying.

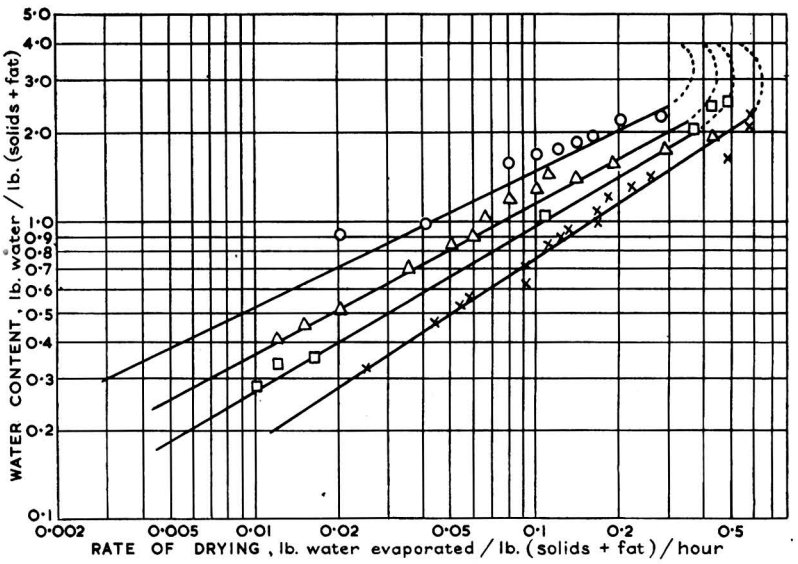

FIG. 4.—*Effect of temperature on the rate of drying of herring fillets*

○ Dry bulb 80° F
△ Dry bulb 100° F
□ Dry bulb 120° F
× Dry bulb 140° F
W.B.D. 20° F Air speed 12 ft./sec.

Although the absolute increase in rate of drying with temperature is greater at the beginning of the process, the greatest proportional effect is at the end (see Table I). At 0·3 lb. water per lb. of (solids + fat) the rate of drying at 140° F is 7·5 times that at 80° F for a wet-bulb depression of 40° F. The average rate of drying to a moisture content of 0·3 lb. per lb. solids is increased by about 80% for a 20° F rise in temperature.

Table I

Drying rates of herring fillets [in lb. water per lb. (solids + fat) per hour] at various air temperatures and water contents

Water content (lb./lb. solids + fat)	Temperature of air (° F)				Drying rate at 140° F / Drying rate at 80° F
	80	100	120	140	
2·0	0·17	0·27	0·33	0·44	2·6
1·0	0·038	0·070	0·096	0·145	3·8
0·5	0·0086	0·018	0·028	0·048	5·6
0·3	0·0028	0·0066	0·015	0·021	7·5
Total time of drying to 0·3 (hours)	65	38	21	15	4·3

Effect of relative humidity

For a water surface the rate of evaporation is proportional to the wet-bulb depression (W.B.D.). Ede & Hales[3] showed that for stripped and diced blanched vegetables initial drying rates were in fact directly proportional to W.B.D., and although the effect decreased as drying proceeded, even at water contents of 0·2 it was still noticeable. In the present experiments,

W.B.D. seemed to have little or no effect over the bulk of the drying range from 2·0 to 0·8 water content. At the very beginning of the process there was a slight effect, probably corresponding to the ' constant rate period '. At the end of the process, the total amount of free water was greater at higher W.B.D. because of the water relationships (see later).

Surface temperature

Neglecting conduction through the trays and radiation from the surroundings, all the latent heat required for the evaporation of water during drying is obtained by direct heat exchange between the air and the surface of the fillets.

The rate of heat transfer is given by

$$\frac{dQ}{dt} = hA\Delta\theta$$

where h is the heat-transfer coefficient, A = surface area, and $\Delta\theta$ is temperature difference between air and fish,

whence

$$\frac{dQ}{\lambda dt} = \frac{dw}{dt} = \frac{hA\Delta\theta}{\lambda}$$

where λ is the latent heat of water and $\frac{dw}{dt}$ is the rate of evaporation.

The rate of drying under constant conditions should therefore be proportional only to the temperature difference between air and the surface of the fish. Table II gives some typical values which show this approximately, in most cases, within the limits of the accuracy of measurement.

Table II

Relation between temperature differential and rate of drying

Dry-bulb temperature (° F)	120	120	120	120	120	120	120	120
Surface temperature (° F)	102·5	112	115	117·5	118	117	117·5	117·5
Temperature differential (° F)	17·5	8	5	2·5	2	3	2·5	2·5
Rate of drying (lb. water/h./lb. solid)	0·80	0·50	0·32	0·24	0·22	0·20	0·17	0·16
Temperature differential/rate of drying	22	16	16	10	9	15	15	16

Effect of air speed

Fig. 3 shows that air speed (within the range 6–21 ft./sec.) has little or no influence on the rate of drying below water contents of 2·0 lb. water per lb. solids. In another experiment even 1·5 ft./sec. seemed sufficient, showing that the rate at which moisture diffuses from the interior of the fish to the surface is the controlling factor.

Effect of size

In Fig. 5 the results are given for two different sizes of fillets weighing on average 28·7 g. (1·01 oz.) and 35·7 g. (1·26 oz.) respectively, corresponding to surface/weight ratios (taking weight as a sufficiently close index of volume) of approximately 2·83 and 3·03, i.e., a 7% difference. There was a constant ratio of the rates of drying in the two cases, the smaller fillets drying approximately 25% faster. Although fillets were selected for uniformity, those with the same weight or thickness could have considerably different surface/volume ratios and consequently dry at different rates. Variation in fat content also markedly affected rate of drying. Comparison of the results with the theoretical treatment relating to uni-directional flow of water in a flat slab[9] was not found helpful. Fig. 5 also shows that whole round fish took a good deal longer to dry than fillets under the same conditions.

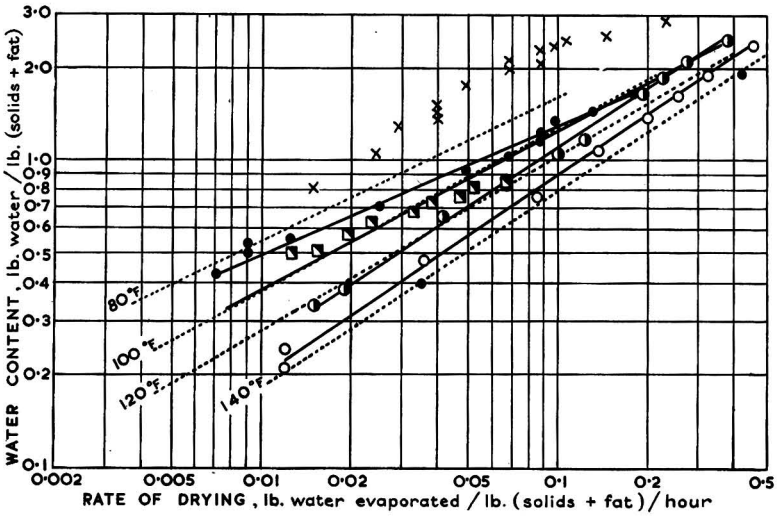

FIG. 5.—*Effect of size, fattiness, etc. on the rate of drying of herring fillets*

Temperature 120° F W.B.D. 40° F
× Round fish; air speed 12 ft./sec.
● Small fatty fillets; air speed 12 ft./sec.
◑ Large lean fillets; air speed 12 ft./sec.
○ Small lean fillets; air speed 12 ft./sec.
◥ Salted fillets; air speed 6 ft./sec.

Effect of fat content

Fig. 5 further shows that with fillets of similar average size dried under the same conditions, the drying rate is very much retarded at high fat contents in addition to the difficulties already mentioned. The (broken) reference lines in Fig. 5 reproduce for comparison the data already given in Fig. 4.

Effect of means of support

Fillets lying on small wire mesh trays (9 × 9 in.) with their tails upstream dried somewhat more quickly than fillets of the same size and composition suspended by hooks on tenters. However, drying was appreciably slower on large trays (3 ft. by 1 ft. 8 in.). This effect was tentatively attributed to geometrical configuration.

The rate of drying was not affected by (i) the design of the trays, whether made of sheet metal or wire mesh or (ii) the placing of the fillets, whether skin side up or down so that the effects of radiation and conduction were negligible in comparison with that of convection. The rate of drying was very much slower in the case of salted fish which were of course already partly dehydrated by the osmotic action of the salt before drying, and dried more slowly even than whole fish under comparable conditions (see Fig. 5). Fillets of cod and haddock, which contain only about 0·3% of fat,[10] dried only very slightly more quickly than lean herring and mackerel fillets. Drying was accelerated by infra-red heat, supplied by a 1-kw filament lamp, so that it was about as fast as at an air temperature 40° F higher without radiation.

Summary of results on rate of drying

Table III gives the constants A and B in the empirical equations derived from log-log plots as in Fig. 4 for different conditions in the form.

$$W = A\left(\frac{dw}{dt}\right)^B$$

where W is water content in lb. of water per lb. of solids (including oil) and $\frac{dw}{dt}$ is the rate of drying in lb. of water evaporated per lb. of (solids + fat) per hour.

Table III

Drying equation constants for herring fillets

Temperature (° F)	W.B.D. (° F)	Air speed (ft./sec.)	A	B	Fat content (%)	Comments
80	20	12	4·6	0·46	—	
100	,,	,,	4·0	0·52	—	
120	,,	,,	3·7	0·57	5·7	
140	,,	,,	3·3	0·63	7·6	
120	40–45	6–21	5·2	0·77	6·0–7·4	
120	40–45	6–12	4·6	0·77	3·8	Small trays (9″ × 9″)
,,	,,	,,	4·6	0·63	—	Large trays (3′ × 1′ 8″)
,,	,,	,,	4·6	0·69	—	Fillets suspended
,,	45	12	3·8	0·83	3·0	7″ long
,,	,,	,,	4·6	0·82	3·3	11·4″ long
,,	,,	,,	3·3	0·53	13·3	9·8″ long

The higher the value of A the slower is drying at a given water content, and the lower the value of B the slower is the drying especially at the end of the process.

Water relations of dried herring fillets

As already stated, the water relations of the product determine the rate of drying towards the end of the process. Dried fillets of lean herrings were placed in desiccators over solutions maintaining constant relative humidities in a room at a constant temperature of about 80° F and weighed at intervals until they had come to equilibrium, usually after about 10 days, when they were transferred to another humidity. In this way the water relations were obtained for the product as shown in Table IV, which gives the average results obtained from determinations on about two dozen individual fillets.

Table IV

Equilibrium water contents of dried herring fillets at different relative humidities

R.H. %	Water content (lb. per lb. of solids + fat)
44	0·064
52	0·100
75	0·160
82	0·180
75	0·140
44	0·082

The water contents were obtained by drying the specimen in an oven at 102° C (216° F) where it required about 48 hours to reach constant weight. The hysteresis effect in drying as compared with moistening the specimens is very noticeable. No significant difference in equilibrium moisture content was found with dried herrings which contained 3·25% of oil in the wet fillet as compared with those containing 6·9%. The figures are of about the same order as those obtained for dehydrated minced cooked herring.[11]

Storage properties and palatability

The effect of atmospheric humidity on equilibrium moisture content is important in connexion with the storage properties of the product. Above a level of about 10% (i.e., 0·11 lb. water per lb. of solids + fat), moulds begin to develop slowly whilst above about 16% (0·19 lb. water per lb. of solids + fat) mould growth is quite rapid.[2] Although none of the products

obtained could be regarded as palatable to British tastes, there is reason to believe that they would be as acceptable, in parts of Africa, for example, as the native products. However, the relative humidity in some tropical areas is in the region of 90% which would obviously make storage in passable condition a difficult problem.

Storage experiments on the product were carried out under conditions approximating to what would be expected in the tropics, i.e., at 80° F and 90° F corresponding approximately to ' average ' and ' average worst ' conditions respectively, and relative humidities of 80 and 90%. At the latter R.H. moulds developed within a few days and at 80% R.H. samples stored with water contents of above 25% showed similar symptoms within a few weeks. In one experiment, mould growth occurred at both 80° and 90° F when the fillets contained 18% of moisture, whilst no visible mould growth was found at 14% of moisture.

Samples dried at the higher temperatures, particularly 140° F and above, were rather disagreeable on account of brittleness, toughness and darkening even before storage, and had a flavour of oxidized oil in the case of the fattier fish. All these characteristics developed to a greater degree under the relatively severe storage conditions to which they were exposed in these experiments. Thus the product darkened in colour with storage, to a greater extent at the higher temperature. Marked rancidity to the palate developed when the fillets were stored in air in sealed cans, but not when the air was replaced with nitrogen, although another flavour appeared, described by tasters as ' meat extract ' (possibly associated with changes in the protein or extractives), which was not noticeable in the air-pack, perhaps because it was masked by rancidity of the fat. Fillets wrapped in paper behaved similarly to samples stored in cans, although mould growth was not so marked, perhaps because access of the dry air of the store resulted in continued loss of moisture and prevented the conditions of high equilibrium humidity that are built up in a sealed container.

The maximum tolerable storage life of the product in edible condition even at 10% moisture content was about 5 months at 80° F and somewhat less at 90° F. However, samples of native cured fish obtained from West Africa, South-east Asia, etc., have appeared to be in even less palatable condition than the worst of those rejected in this experiment on almost all counts except mouldiness. It is also true that the experimental samples were merely dried, and not smoked, and it is likely that the added flavour and preservative action of the smoke imparted in a suitable combined hot smoking and drying process might have rendered most of the products less unappetizing to all tastes.

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THE CRYSTALLIZATION OF COCOA BUTTER AND ALTERNATIVE FATS. II.*—Palm Kernel Stearins and their Mixtures with Cocoa Butter and Butter Fat

By E. H. STEINER

The results are presented of calorimetric and cooling curve measurements on eight palm kernel stearins. Consideration is given to phase composition and crystallization of the stearins alone and in admixture with cocoa butter and butter fat in binary and ternary mixtures. The practical implications of the results are discussed in regard to the use of the stearins as alternatives to part of the cocoa butter in chocolate.

Introduction

In a previous paper[1] was described a calorimeter for the measurement of the thermal characteristics of fats and the determination of their solid and liquid phases, and a number of results were given for cocoa butter. The present paper is concerned with the thermal analysis of some cocoa butter alternatives of the palm kernel stearin type. These are obtained from palm kernel oil, or from mixtures of palm kernel and coconut oils, by pressing out part of the lower-melting glycerides.[2] The purpose of the investigation has been to examine the crystallizing properties of these alternatives in comparison with those of cocoa butter with a view to assessing their suitability for use in chocolate products.

While the calorimetric measurements provide a guide to the extent of crystallization and also, therefore, to the hardness of the stabilized fat they provide no indication of supercooling or of rate of crystallization. In view of the importance of these factors in regard to tempering requirements, cooling curve data are also included.

The investigation covers the crystallizing properties of the palm kernel stearins both by themselves and in admixture with cocoa butter. Also, for assessing their behaviour in milk chocolate products, the effects of butter fat are examined in both binary and ternary mixtures with cocoa butter and the alternative fats.

Experimental

Materials

Eight typical commercial fats of the palm kernel stearin type have been studied, covering a range in chemical composition and physical properties. Analytical data for the usual constants are presented in Table I, the samples being arranged in order of their complete melting points. Melting points were determined by the method of mercury flotation,[3] and the other constants in the usual manner.

Table I

Analytical characteristics of palm kernel stearins

Sample	Refractive index at 40° (Zeiss)	Iodine value	Saponification	Reichert value	Polenske value	Kirschner value	Melting point	
							Incipient (° c)	Complete (° c)
A	37·7	13·4	247·5	4·4	9·7	0·8	27·6	31·3
B	37·4	12·4	246·0	5·0	9·3	1·0	29·9	32·5
C	36·2	8·2	249·4	3·0	8·0	0·3	30·8	32·8
D	36·6	8·0	246·0	3·0	7·7	0·3	31·6	33·2
E	36·1	4·6	249·7	2·4	8·5	0·3	32·0	33·8
F	36·0	7·4	249·7	3·4	8·9	0·6	32·5	33·8
G	36·7	2·8	237·0	1·8	6·1	0·4	34·1	36·2
H	36·0	3·3	244·6	5·6	9·7	1·0	35·1	38·0

For the preparation of mixtures with cocoa butter, sample B of the series examined in Part I was used throughout. Where butter fat was incorporated, this was prepared from unsalted butter by separating out at 60° and decanting through a Whatman No. 4 filter paper. The butter fat was left to solidify for a week at room temperature and stored at 0°. Mixtures of fats

* Part I: *J. Sci. Fd Agric.*, 1955, **6**, 777

were prepared at 40° and cooled at room temperature with stirring until a pasty consistency was reached. To ensure maximum crystallization in the mixtures they were solidified at 0° overnight then held at 25° for 3 days to accelerate stabilization and finally stored at room temperature (17–20°) for two or more weeks until required.

Methods

Calorimetric measurements.—Thermal analyses were carried out with the calorimeter in the manner described previously[1] except that a more rapid method of heating was adopted. The heating was maintained continually (except for certain intervals) at a constant rate of about 1 watt, temperature measurements being taken at 5-minute intervals. The differential thermocouple setting was controlled in accordance with the expected average between each pair of temperature measurements. As this procedure allowed no time for the contents of the calorimeter to reach thermal equilibrium, the amount by which the measured temperature lagged behind the equilibrium temperature was determined from time to time (by cutting off the current for 5 minutes and measuring temperature at the end of that time) and a graph was plotted relating temperature error to observed temperature. This error varied from −1° C when the fat was solid to practically zero during melting, returning to −0·8° C when the fat was liquid. By adopting this technique it is possible to carry out runs in approximately half the time of the standard procedure although the accuracy is less, particularly in regard to specific heats. Where data on the existence of maxima in the specific heat–temperature curve are required, the standard procedure must be followed. This was done in the case of three of the stearin samples (Fig. 1).

All measurements on the individual fats were commenced at −70° to obtain data on the completely solidified fat. In the case of the mixtures, the extrapolated curve of heat content of the solid fat was calculated in proportion from the components, the experimental run being carried out only from 0° upward. From some preliminary trials it was found necessary to make a small correction to the calculated heat content of the solid 50/50 mixtures of cocoa butter and stearin because of a lowering of the latent heat of fusion by approximately 1·5 cal./g. A reduction in latent heat of 2 cal./g. below the calculated value was found, similarly, for a 70/30 mixture of cocoa butter with butter fat (Table II). A relatively low value for the latent heat in these mixtures may be expected from the increased diversity of the molecules, leading to a looser packing and, consequently, smaller heat of crystallization.[4]

In computing the percentages of liquid phase, therefore, the extrapolated (calculated) values of relative heat content were reduced numerically by 1·5 cal./g. for 50/50 cocoa butter–stearin mixtures and by 2 cal./g. for a 70/30 cocoa butter–butter mixture. A proportional correction was made in the case of mixtures of other composition. A reduction of 3 cal./g. was made for ternary 40/40/20 mixtures of cocoa butter, stearin and butter fat.

Cooling curve.—For obtaining cooling curves a technique was employed based on that of Jensen,[5] but adapted to a smaller sample. A 6 × 1 in. boiling tube was inserted into a cork carrying a thermometer reading to 0·02° C and a loop stirrer capable of up and down movement. The assembly was supported in a wide-mouthed bottle which was immersed up to the neck in a water-bath maintained for convenience at 17°. Ten ml. of fat heated to 50–55° were placed in the tube and allowed to cool to 40°. Cooling was continued with slow stirring to 33°, when the tube was inserted into the air jacket. From 31°, without stirring, temperatures were plotted every minute. When solid fat first became visible, stirring was recommenced with 3 strokes at ½-minute intervals until 10 minutes beyond the maximum temperature.

Approximate quantitative information on the extent of crystallization which occurs during the rise from minimum to maximum may be obtained from a knowledge of the heat losses and thermal capacity of the apparatus.

Let Q = heat content of apparatus at time t and temperature θ
x = proportion of crystallized fat at time t and temperature θ
s = specific heat of contents at time t and temperature θ
m = mass of contents
W = thermal capacity of apparatus
L = apparent latent heat of fusion of solid fat at temperature θ.

The rate of loss of heat from the apparatus during cooling is given (Newton's law of cooling) by $-\frac{dQ}{dt} = k(\theta - \theta_0)$. The effective air temperature θ_0 in the apparatus was determined from the experimental data (see below).

If crystallization occurs,

$$\frac{dQ}{dt} = -k(\theta - \theta_0) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

also

$$\frac{dQ}{d\theta} = W + ms - mL\frac{dx}{d\theta} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Strictly speaking, L is variable, but with sufficient accuracy its value may be fixed as the latent heat at mid-fusion. According to calorimetric measurements, the apparent latent heat of fusion (vertical intercept between the extrapolated solid and liquid lines) did not vary by more than 3% from room temperature up to melting.

Dividing (1) by (2) gives

$$(W + ms)\frac{d\theta}{dt} = -k(\theta - \theta_0) + \frac{mL\,dx}{dt} \quad .. \quad .. \quad .. \quad (3)$$

Values of W, k and θ_0 are determined by plotting cooling curves from an initial temperature θ_1 on two non-crystallizing liquids of known specific heats. Putting $\frac{L\,dx}{dt}$ equal to zero, equation (3) can be directly integrated giving

$$\log_e(\theta - \theta_0) = -\frac{kt}{W + ms} + \log_e(\theta_1 - \theta_0) \quad .. \quad .. \quad .. \quad (4)$$

From measurements on water and oil, it was found that straight lines were obtained on plotting $\log_e(\theta - \theta_0)$ against t by arbitrarily taking $\theta_0 = 18{\cdot}0$. From the slopes of the two lines, k and W were calculated as 0·86 and 7·8, respectively.

Equation (3) may be applied to the rise in the cooling curve of fats, after inserting values of the constants. If the increase in the proportion of crystalline phase producing the temperature rise is denoted by x_m, integration of (3) gives ($s = 0{\cdot}45$, $m = 9$, approx.)

$$9Lx_m = 12(\theta_{max.} - \theta_{min.}) + 0{\cdot}86\int_{t_{min.}}^{t_{max.}} (\theta - 18)dt \quad .. \quad .. \quad .. \quad (5)$$

The integral can be evaluated graphically from the cooling curve and L is known from calorimetric data, hence x_m is determined.

Results

The thermal characteristics of the eight samples of stearins are given in Table II, together with data for the samples of butter fat and cocoa butter used in the preparation of the mixtures.

Curves relating apparent specific heat and temperature for the three samples A, E and G are shown in Fig. 1. No regular or characteristic maxima appear before the main melting temperature as with cocoa butter. The equations connecting specific heat, s, and temperature, θ, of the solid and liquid fats were as follows:

	Solid	Liquid
Sample A	$s = 0{\cdot}39 + 0{\cdot}0014\theta$	$s = 0{\cdot}50$
B	$s = 0{\cdot}39 + 0{\cdot}0016\theta$	$s = 0{\cdot}50$
C	$s = 0{\cdot}39 + 0{\cdot}0017\theta$	$s = 0{\cdot}49$
D	$s = 0{\cdot}38 + 0{\cdot}0014\theta$	$s = 0{\cdot}54$
E	$s = 0{\cdot}38 + 0{\cdot}0015\theta$	$s = 0{\cdot}50$
F	$s = 0{\cdot}39 + 0{\cdot}0015\theta$	$s = 0{\cdot}52$
G	$s = 0{\cdot}39 + 0{\cdot}0017\theta$	$s = 0{\cdot}50$
H	$s = 0{\cdot}39 + 0{\cdot}0016\theta$	$s = 0{\cdot}51$

Extrapolation of the solid and liquid portions of the heat content–temperature curves was

carried out in accordance with these equations after integration, the constants being determined from the data of Table II at − 60°. Calculation of the percentage of liquid phase was made in the usual way and the results are plotted in Fig. 2 from 10° upward.

Table II

Thermal characteristics of palm kernel stearins, butter fat and cocoa butter

Sample	Latent heat at mid-fusion (cal./g.)	Temp. of mid-fusion (° c)	Temp. of 100% liquid phase (° c)	Relative heat content* (cal./g.) at −60°	0°	20°	25°	30°
Stearin A	30·0	22·1	31·7	−74·6	−50·1	−32·1	−25·3	−12·2
B	31·8	23·5	33·0	−75·5	−52·6	−33·8	−27·0	−13·0
C	33·9	28·5	33·4	−76·7	−55·4	−41·0	−34·8	−22·1
D	34·3	28·7	32·8	−77·4	−56·6	−42·3	−37·2	−23·8
E	34·7	31·0	34·8	−76·9	−56·3	−44·1	−39·2	−29·9
F	33·1	28·8	33·2	−76·8	−55·5	−41·6	−36·1	−22·5
G	34·7	28·8	36·7	−77·7	−57·0	−43·2	−36·0	−24·8
H	33·0	25·7	38·0	−76·6	−55·5	−38·7	−30·8	−18·8
Cocoa butter	39·1	31·1	34·4	−80·8	− 59·1	−47·3	−43·0	−33·6
Butter fat	24·0	12·1	37·4	−71·5	−41·4	−21·4	−16·3	−12·2
Cocoa butter + butter fat (70/30)	32·5	25·2	31·0	−76·5	−52·0	−34·8	−28·6	−11·7

* Heat content at 50° assumed to be zero

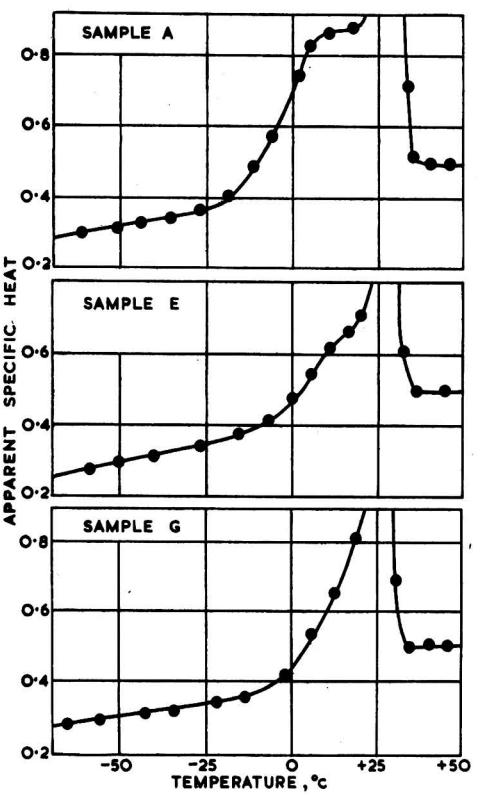

FIG. 1.—*Specific heat and temperature of palm kernel stearins*

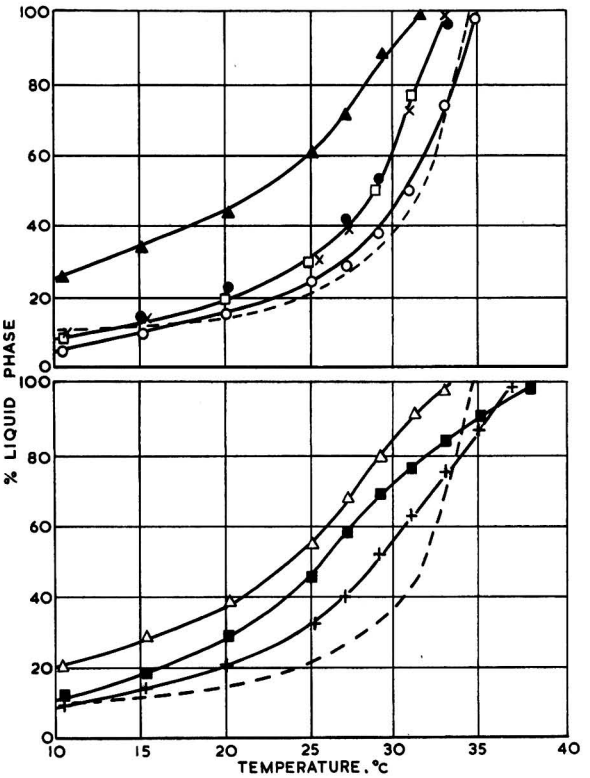

FIG. 2.—*Percentage of liquid phase and temperature of palm kernel stearins*

In Fig. 3 are given the curves of liquid phase and temperature for the 50% mixtures of cocoa butter and stearin. The typical manner in which the percentage of liquid phase changes with composition of mixture is illustrated in Fig. 4 for two samples E and G.

Cooling curve data both for the fats themselves and their 50% mixtures are shown in Table III.

Table III

Cooling curve data of palm kernel stearins and cocoa butter

Sample of stearin	Unmixed fat				50% mixture of stearin and cocoa butter			
	Minimum (° c)	Maximum (° c)	Time of rise, min.	Crystallization %	Minimum (° c)	Maximum (° c)	Time of rise, min.	Crystallization %
A	22·7	27·1	8	38	18·9	22·1	38	44
B	23·2	26·5	6	28	19·7	22·4	8	26
C	25·1	29·5	8	42	20·5	23·3	11	23
D	25·7	29·8	5	32	21·5	23·6	7	16
E	26·5	31·4	6	37	21·2	24·6	8	23
F	25·4	30·0	8	42	20·4	23·3	7	20
G	25·2	30·5	7	40	21·6	24·6	6	23
H	28·5	30·4	4	22	22·9	24·5	5	14
Cocoa butter	21·2	28·4	11	46				

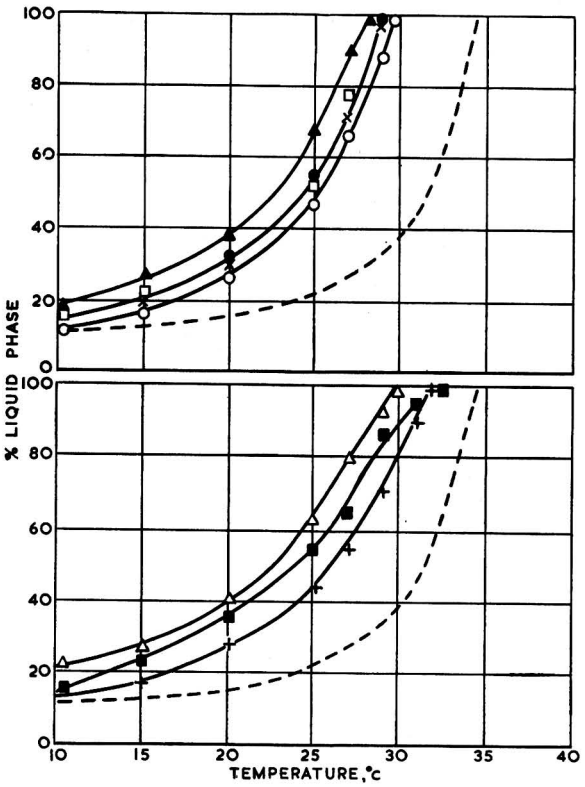

FIG. 3.—*Percentage of liquid phase and temperature of mixtures of palm kernel stearin with cocoa butter* (50/50)

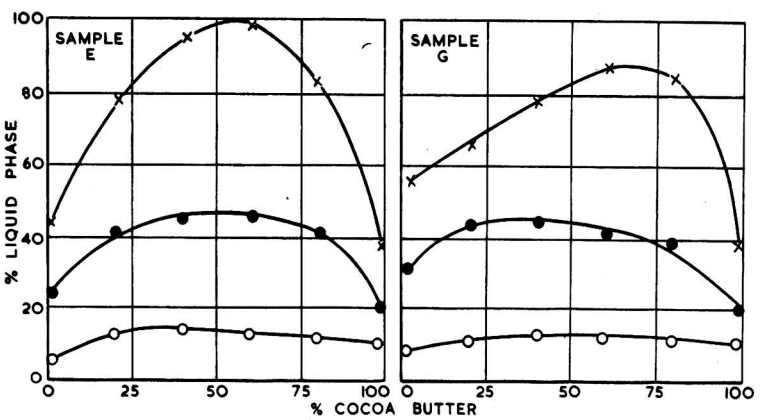

FIG. 4.—*Effect of composition of stearin–cocoa butter mixtures on percentage of liquid phase*

× at 30° ● at 25° ○ at 10°

The change with percentage composition in both cooling curve maxima and minima may be seen in Fig. 5 for samples E and G, together with the change in the temperature of 100% liquid phase.

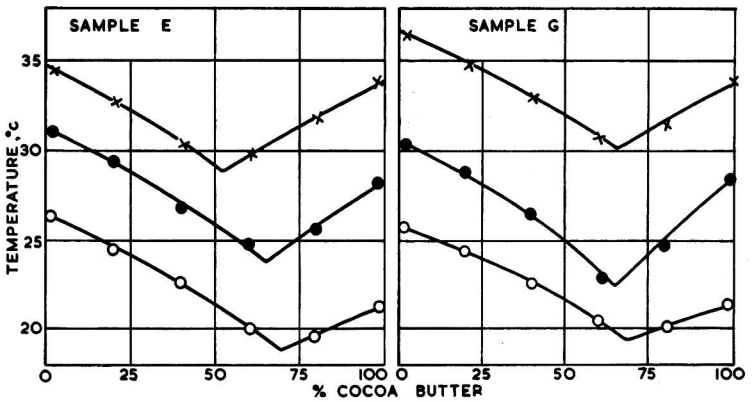

FIG. 5.—*Effect of composition of stearin–cocoa butter mixtures on melting temperature and cooling curve data*
× = Temperature for 100% liquid phase
● = Cooling curve maximum ○ = Cooling curve minimum

Data for the heat content at 0° and 20°, and temperature at which there is 100% liquid phase are given in Table IV for mixtures of the stearins with cocoa butter and butter fat. In most cases the thermal characteristics were not determined below 0°.

Table IV

Thermal characteristics of palm kernel stearin mixtures

Sample of stearin	Temp. of 100% liquid phase (°C)			Relative heat content* (cal./g.) at 0°			Relative heat content* (cal./g.) at 20°		
	SC	SB	SCB	SC	SB	SCB	SC	SB	SCB
A	28·5	29·0	28·5	−53·3	−47·7	−50·6	−35·0	−28·6	−30·2
B	29·9	32·6	30·5	−54·0	−49·3	−51·4	−35·2	−29·0	−31·0
C	28·8	31·2	31·0	−54·8	−50·8	−51·6	−39·2	−35·1	−34·2
D	29·6	31·4	29·5	−55·6	−52·2	−52·5	−39·9	−36·5	−34·0
E	29·5	32·4	30·1	−56·0	−52·2	−52·5	−41·4	−37·7	−35·0
F	28·4	31·0	29·0	−54·0	−51·3	−51·1	−38·3	−34·8	−32·5
G	31·6	35·8	33·3	−55·8	−53·1	−52·6	−40·5	−37·7	−35·0
H	32·2	38·2	33·8	−55·3	−52·6	−52·6	−37·2	−33·2	−33·4

* Heat content at 50° assumed to be zero
SC = stearin + cocoa butter (50/50)
SB = stearin + butter fat (80/20)
SCB = stearin + cocoa butter + butter fat (40/40/20)

Fig. 6 indicates the specific heat–temperature relationship for the sample of butter fat by itself and in admixture with cocoa butter (30/70). Fig. 7 shows the phase composition of cocoa butter and butter fat mixtures in varying proportions and Fig. 8 gives the percentage of liquid phase in mixtures (20/80) of butter fat with the stearins. Finally in Fig. 9 is shown the liquid phase composition in ternary mixtures (20/40/40) of butter fat, stearin and cocoa butter. Cooling curve data for both the binary and ternary mixtures with butter fat are provided in Table V.

Discussion

Thermal characteristics

The values obtained for the latent heat at mid-fusion of the stearins (Table II) were in all cases lower than those for cocoa butter,[1] although palm kernel oil itself contains a considerably higher proportion of tri-saturated glycerides. This result, which is in apparent contradiction to

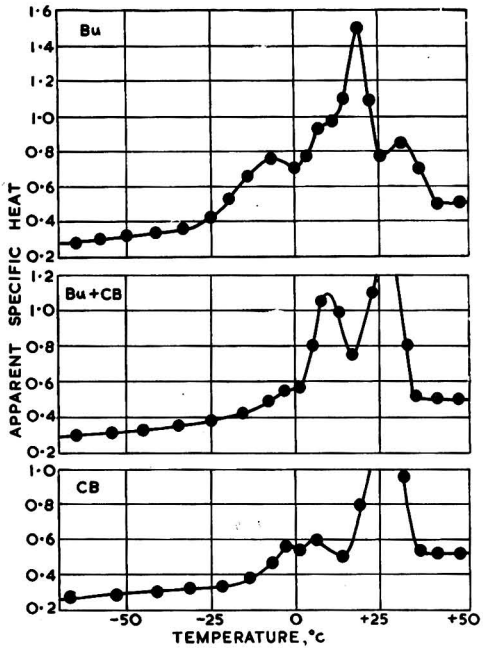

FIG. 6.—*Specific heat and temperature relation for butter fat and cocoa butter*

	Solid	Liquid
Bu = Butter fat	$s = 0.445 + 0.0019\theta$	$s = 0.51$
CB = Cocoa butter	$s = 0.37 + 0.0013\theta$	$s = 0.51$
Bu + CB = Butter fat + cocoa butter (30/70)	$s = 0.40 + 0.0015\theta$	$s = 0.50$

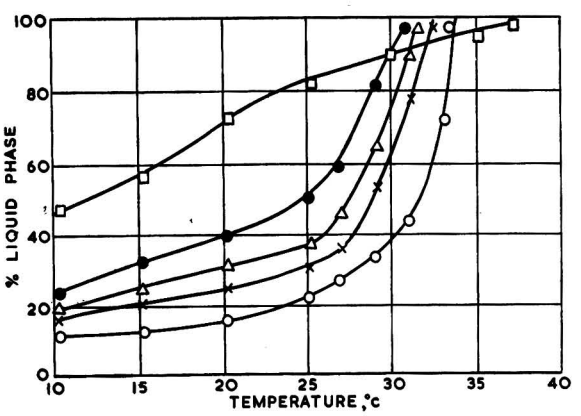

FIG. 7.—*Effect of butter fat on percentage of liquid phase in cocoa butter*

□ = Butter fat
○ = Cocoa butter
● = Cocoa butter + 30% butter fat
△ = Cocoa butter + 20% butter fat
× = Cocoa butter + 10% butter fat

the increase in latent heat which occurs with saturation,[6] is presumably a reflection of the relatively large number of component fatty acids in the glycerides of palm kernel oil,[7] leading to a less compact molecular arrangement in the crystal. It is of interest that among the samples of stearins themselves a significant negative correlation existed between latent heat and iodine value (correlation coefficient -0.79).

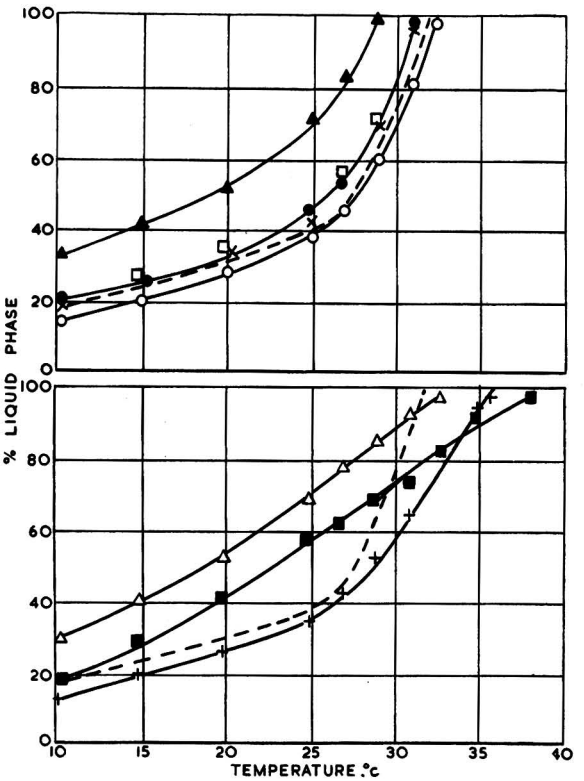

FIG. 8.—*Percentage of liquid phase and temperature of mixtures of palm kernel stearin with butter fat* (80/20)

▲ Sample A ○ Sample E
△ Sample B □ Sample F
● Sample C + Sample G
× Sample D ■ Sample H
------- Cocoa butter + butter fat (80/20)

Temperatures at which there was 100% liquid phase were, in several instances, slightly higher than the visually determined melting points. This result may well be expected owing to the difficulty of observing small amounts of crystal in the presence of a large amount of liquid phase.

Phase composition

From Fig. 2 it is evident that there is an appreciable divergence between the samples in respect of the amount of liquid phase at any temperature. Samples A and B which had the highest iodine values exhibited the largest proportion of liquid phase. Samples C, D and F showed substantially similar proportions of liquid phase and were also alike in their analytical characteristics. In general, however, neither the chemical analysis nor the melting point provided a guide to the thermal behaviour of these fats. Compared with cocoa butter itself the stearin curves tended to rise less sharply. In most cases the proportion of liquid phase was similar to that in cocoa butter below 15°, but was appreciably greater than that in cocoa butter at higher temperatures.

Cocoa butter–stearin mixtures

The percentages of liquid phase in the 50% mixtures with cocoa butter were considerably greater, particularly in the higher temperature range, than those for either cocoa butter or the stearin alone. The effect was least for those samples possessing the largest amount of liquid phase initially, sample A actually showing a decrease in the liquid phase of its 50% mixture with

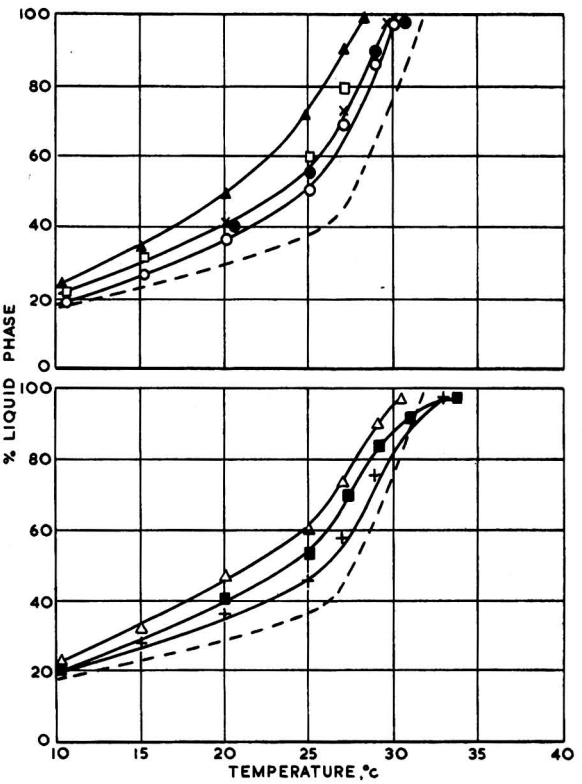

FIG. 9.—*Percentage of liquid phase and temperature of mixtures of palm kernel stearin, cocoa butter and butter fat (40/40/20)*

▲ Sample A ○ Sample E
△ Sample B □ Sample F
● Sample C + Sample G
× Sample D ■ Sample H
------- Cocoa butter + butter fat (80/20

Table V

Cooling curve data of butter fat, palm kernel stearin and cocoa butter mixtures

Sample of stearin	Butter fat–stearin (20/80)				Butter fat–stearin–cocoa butter (20/40/40)			
	Minimum (° c)	Maximum (° c)	Time of rise, min.	Crystallization %	Minimum (° c)	Maximum (° c)	Time of rise, min.	Crystallization %
A	21·8	25·2	7	30	19·0	21·6	13	22
B	23·8	26·1	5	23	20·0	21·6	7	14
C	23·8	27·7	4	29	19·1	21·3	7	14
D	26·2	27·9	4	20	20·2	22·0	4	12
E	24·7	29·3	4	33	20·2	22·3	5	14
F	24·5	27·9	5	31	20·1	22·7	6	18
G	24·7	29·6	5	35	20·8	22·9	6	18
H	27·2	29·4	5	27	22·7	24·3	4	14
Cocoa butter +10% butter fat	19·8	25·1	12	42				
Cocoa butter +20% butter fat	19·0	24·5	8	32				
Cocoa butter +30% butter fat	18·7	23·3	7	28				

cocoa butter at temperatures below 20°. In mixtures with cocoa butter, therefore, there was less diversity in the behaviour of these stearins than when they were used alone.

The typical manner in which the amount of liquid phase in either cocoa butter or palm kernel stearin was increased by the presence of the other is illustrated for two of the stearins in Fig. 4. The occurrence of a maximum in the percentage of liquid phase–percentage composition curve corresponds to the incidence of minima in the curves relating percentage composition with melting temperatures and with cooling curve maxima and minima (Fig. 5). The existence of a minimum in the melting-point curve and maximum in the liquid-phase curve presumably arises from the differences in the glyceride components of the fats. Whereas cocoa butter contains almost entirely oleic, palmitic and stearic glycerides, fats of the palm kernel stearin type comprise largely oleic, lauric and myristic glycerides.[7]

Mixtures with butter fat

An addition of 20% butter fat to a palm kernel stearin or to cocoa butter increased the percentage of liquid phase over the range 10 to 25° by between 10 and 15% (of the total fat) approximately. At higher temperatures the increase tended to be greater and the temperature for the presence of 100% liquid phase was usually lowered by 2° to 3°.

At any given temperature the percentage of liquid phase in the ternary mixtures was usually slightly higher than that in any of the corresponding binary mixtures, although compared with the stearin–cocoa butter mixtures there was no marked difference above 25° (Figs. 3, 8 and 9).

The increase in liquid phase on mixing cocoa butter and a palm kernel stearin in the presence of butter fat was least for those fats showing the greatest amount of liquid phase themselves, as observed in the case of the binary stearin–cocoa butter mixtures. Although the highest values for the percentage of liquid phase occurred in the ternary mixtures, these differed less from cocoa butter containing 20% butter fat than did the binary mixtures of fat and cocoa butter from cocoa butter itself.

With regard to melting points, the ternary mixtures showed temperatures at which there was 100% liquid phase which were usually lower, by varying small amounts, than those of the corresponding stearin–butter fat mixtures (Table IV). In most cases, however, they were slightly higher than those of the 50% stearin–cocoa butter mixtures and it is clear that the melting temperatures of these mixtures bear little relationship to their plastic behaviour at other temperatures.

Rate of crystallization

According to the cooling curve data of Table III, crystallization of the palm kernel stearins commenced at a higher temperature, and with less supercooling, than with cocoa butter. The onset of crystallization was slightly retarded by butter fat and considerably so by admixture with cocoa butter.

The comparatively low rise from minimum to maximum temperatures in the cooling curves of all mixtures with cocoa butter indicates a slower crystallization rate for these mixtures than for cocoa butter. This was confirmed by the figures for the percentage of crystallization during the rise, although a strict comparison of the fats on this basis is not valid since the percentage of crystallization (and also the temperature rise) is dependent partly on the minimum temperature reached. It is of interest that the percentage crystallization of the individual stearins approached the total possible percentage at the maximum temperature, whereas in mixtures with cocoa butter the amount of crystallization occurring was, in most cases, less than half the total possible (Figs. 2 and 3). Thus, for example, sample C showed 42% crystallization at a maximum temperature of 29·5° and 23% crystallization in admixture with cocoa butter at a temperature of 23·3° (Table III). Reference to Figs. 2 and 3 indicates that the total amounts of crystallized phase produced in the stable fats at these temperatures were 45% and 56% respectively. The above calculation does not include any crystallization which may have occurred before the minimum temperature, but in all cases this was found to be small (less than 5%).

The apparent anomaly of sample A in showing a high percentage of crystallization both alone and in its 50% mixture with cocoa butter (Table III) was due to the abnormally long

time taken in reaching the maximum. This was caused by a flattening of the cooling curve near the maximum, which was apparent also in the mixtures of this sample containing butter fat. In general the crystallization rate of the palm kernel stearins was retarded by butter fat although the effect was less than that produced by cocoa butter.

Conclusions

Regarding the percentage of liquid phase as a measure of the plastic properties of the stearins, at room temperature or below, most of these alternative fats exhibited a similar hardness to cocoa butter, although on warming they showed a greater degree of softening.

The results obtained on the mixtures of stearin and cocoa butter, in a proportion which would occur if the fats were used as alternatives to added cocoa butter in plain chocolate, showed that such products would be very soft and liable to show finger marks readily. It is improbable that such mixtures could be used satisfactorily in chocolate manufacture unless the amount either of alternative fat or of cocoa butter was kept to a low proportion (less than 10% in the total fat). At room temperature, the 50% mixtures were similar in plastic behaviour to cocoa butter containing about 20% milk fat, but on warming they became considerably softer relative to this.

From the points of view of hardness at room temperature and of softening on warming, a milk chocolate containing equal amounts of palm kernel stearin and cocoa butter would at best resemble a chocolate made with cocoa butter and a high proportion of milk fat (about 30% of the total fat). As the proportion of milk fat in the product is reduced, the brittleness should increase, but there will not be much improvement in the tendency to soften unless the amount of either cocoa butter or of palm kernel stearin is kept to a very low proportion. In general, however, if these fats are used as alternatives for added cocoa butter less difference in the properties of the chocolate may be expected in a milk product than in a plain one.

Data on the commencement and rate of crystallization obtained from the cooling curve measurements, considered in relation to cocoa butter, provide information on tempering requirements in the manufacture of chocolate containing the alternative fats. So far as products containing palm kernel stearin alone are concerned, they should be tempered at a higher temperature and for a shorter time than an equivalent cocoa butter product. If these fats are used with cocoa butter, the conditions should be altered in the directions of longer time and lower temperature than is usual for cocoa butter alone. If milk fat is also present, the tempering temperature should be lowered still further but, relative to an equivalent cocoa butter product, the changes in tempering required by the replacement of cocoa butter by a particular stearin are not likely to be influenced by the milk fat content.

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THE IDENTIFICATION AND ESTIMATION OF SOIL INOSITOL PHOSPHATES

By G. ANDERSON

A technique is described for the identification and estimation of inositol phosphates by paper partition chromatography, in either one or two dimensions. Development with methanol/aqueous ammonia resolved inositol mono-, di-, tri-, tetra- and hexa-phosphates into four spots, the tri- and tetra-phosphate moving together. Acid solvents, such as acetone/acetic acid, moved all these compounds in a compact group.

The method has been used to estimate the inositol phosphates in soils. The inositol phosphates were extracted with sodium hydroxide and, after the removal of many organic and inorganic concomitants, were precipitated as the barium salts, converted to the free acids or sodium salts, and examined chromatographically. In the soils so far examined, inositol hexaphosphate accounted for about one-third of the soil organic phosphate; the tetra-/tri-phosphate was present in very small quantity, but neither the di- nor the mono-phosphate was detected.

Introduction

The occurrence of phytin has been reported in contrasting soil types from widely separated areas. The work of Wrenshall & Dyer,[1] Bower,[2] Pedersen[3] and others showed that a substance having the properties of phytin could be extracted from soil with alkali, after interfering metal ions, such as calcium, had been removed by acid leaching; the substance was then precipitated as the ferric salt in acid medium, or as the calcium salt in alkaline medium, after a hypobromite oxidation to destroy the bulk of the organic matter.

Products obtained by Yoshida[4] and Bower[2] also indicated that lower phosphate esters of inositol were present. Smith & Clark[5, 6] developed a chromatographic technique for the separation of inositol phosphates on an ion-exchange column, but on applying it to soil products they obtained no clear evidence that lower phosphate esters did occur in the soil. Their method was rather tedious, however, and the identity of all the compounds eluted from the column was not certain even when authentic inositol phosphates were tested.

In the present work, a separation of inositol phosphates has been achieved by a paper chromatographic technique, already outlined (G. Anderson[7]), and full details are given of the application of the method to analysis of the 'phytate' component of soils.

Experimental

I. Chromatographic studies of inositol phosphates

Test materials

An extremely pure sample of phytin (the calcium–magnesium salt of inositol hexaphosphoric acid) was kindly supplied by Messrs. Ciba, Ltd. Sodium phytate was prepared by dissolving phytin in the minimum amount of dilute hydrochloric acid, precipitating the ferric salt with 5% ferric chloride solution, and treating the washed precipitate with excess dilute sodium hydroxide. Ferric hydroxide was removed by filtration, and the filtrate treated with several volumes of alcohol. Sodium phytate separated as a colourless oil which, on triturating with alcohol, finally solidified to a semi-crystalline mass. This was centrifuged and dried, further purification being unnecessary.

Preparation of sodium phytate from wheat bran was effected thus: 300 g. of wheat bran were suspended in 3 l. of water, and 1 l. of concentrated hydrochloric acid added. The mixture was heated for five hours in a boiling water-bath, filtered and made slightly alkaline with aqueous ammonia. The bulky white precipitate so formed was filtered, washed and redissolved in hydrochloric acid, and a slight gum-like residue discarded. Ferric chloride solution was added to precipitate the ferric salt, which in turn was converted to the sodium salt as already described.

Hydrolysis of sodium phytate.—(*a*) Two g. of sodium phytate were dissolved in 20 ml. of water, and acetic acid added until the pH was 4·0. The solution was boiled gently under reflux for 20 hours, during which samples were withdrawn at intervals for chromatographic examination and determinations of inorganic and total phosphate.

(*b*) Two g. of sodium phytate were dissolved in 100 ml. of water, the pH adjusted to 6 with N-HNO_3 and 1 g. of wheat bran and 1 ml. of toluene added. The mixture was incubated at 38° for a week and samples were withdrawn periodically, passed through a cation-exchange resin and reduced to small volume under reduced pressure.

Other inositol phosphates.—Inositol tetraphosphate and monophosphate were prepared by the method of Posternak,[8] and the former additionally purified by dissolving the barium salt in dilute hydrochloric acid, precipitating the barium by addition of dilute sulphuric acid, and adding several volumes of ethanol in which the free inositol phosphoric acids are soluble. Precipitated impurities were centrifuged and the supernatant liquor treated with concentrated aqueous ammonia, separating the ammonium salt of the tetraphosphate as an oil which was, in turn, centrifuged, washed with alcohol and dried. It was redissolved in water, barium hydroxide solution added, and the precipitated barium inositol tetraphosphate was filtered. Finally this was dissolved in dilute hydrochloric acid, reprecipitated with aqueous ammonia, filtered, washed with water and dried. In addition, a mixture of the tri- and di-phosphates was prepared, but attempts to separate them by fractional precipitation of the barium salts in aqueous alcoholic hydrochloric acid were only partially successful. Authentic samples of these four substances were also obtained from private sources.

The formation of inositol pentaphosphate during hydrolysis of phytin was not taken into consideration as it does not seem to have been proved. R. J. Anderson[9] claimed to have isolated such a compound, but Posternak[10] showed that it could be resolved into a mixture of the hexa- and tetra-phosphates. No authentic sample could be obtained for chromatographic study in the present work, and no spot was obtained from phytin hydrolysates to indicate its presence. However, the possibility that it moved with one of the other compounds cannot be ruled out.

Solvents and R_F values

Tests carried out with a range of solvents showed that acid solvents did not differentiate between the various inositol phosphates, and mixtures of these compounds moved in a compact spot. An alkaline solvent (70 vol. of methanol/30 vol. of 0·5N-aqueous ammonia), on the other hand, resolved them into four spots (Table I).

Table I

R_F values of inositol phosphates

Solvent	Compound(s)	R_F
Methanol, 70 vol./0·5N-aqueous ammonia, 30 vol.	Inositol hexaphosphate	0·06
	Inositol tetra- and tri-phosphate	0·17
	Inositol diphosphate	0·32
	Inositol monophosphate	0·48
Acetone, 50 vol./30% acetic acid, 50 vol.	Inositol phosphate group	0·44

The same R_F values were obtained with mixtures prepared by enzyme hydrolysis of the hexaphosphate, but orthophosphate masked the presence of inositol monophosphate, at R_F 0·48. The presence of the latter was confirmed either by elution and estimation of orthophosphate and total phosphate, the difference being a measure of the monophosphate, or alternatively by two-dimensional chromatography. At 13% hydrolysis, only the hexa- and tetra-triphosphate were visible; at 26% hydrolysis, traces of the diphosphate had appeared, while at 46% only a trace of the hexaphosphate remained; at 64% hydrolysis the monophosphate could be detected in very small amount. At no point was the diphosphate or the monophosphate present in great quantity. With acid hydrolysis, the hexaphosphate persisted to a much later stage, but there was no other notable difference.

Purification of papers

Before use, the papers, Whatman No. 1, were thoroughly washed with 2N-HCl, water, and a 0·5% aqueous solution of ethylenediaminetetra-acetic acid (Versene), to which a slight excess of aqueous ammonia had been added. They were dried, and developed upwards in open tanks

with very dilute aqueous ammonia, removing basic impurities to the top of the papers in thin strips, which were then cut off. After drying, the papers were ready for use.

Development

(*a*) *One-dimensional chromatograms.*—The inositol phosphates were applied as the free acids, or the alkali salts, in bands about 2 cm. long, the amount of any one ester not exceeding the equivalent of 25 μg. of P. The chromatograms were developed upwards, at about 23° until the solvent (ammoniacal methanol) front had moved 33 cm. After drying, the compounds were detected either by the ferric chloride–sulphosalicylic acid spray of Wade & Morgan,[11] or the molybdate–perchloric acid spray of Hanes & Isherwood.[12] The latter was found to be more sensitive, but after partial air-drying it was necessary to heat the papers between glass plates in an oven at 90° for 30 min. in order to detect trace amounts of the esters, a treatment which made the papers very fragile. Exposure of the papers to daylight also helped in the detection of trace amounts; with prolonged exposure, ammonium sulphate also gave a blue spot with R_F of about 0·65, but it could readily be distinguished from that given by phosphates by exposure to ammonia fumes, when a vivid blue-green colour was produced.

Separations on a larger scale were carried out on Whatman-E17 paper, and also on a cellulose column, with the same solvent, but, though effective, they did not give greater resolution.

(*b*) *Two-dimensional chromatograms.*—Mixtures containing other phosphate esters in addition to inositol phosphates may be conveniently examined by a simple two-dimensional technique. In this case the solution of esters is applied in a small spot, multiple applications being as a rule necessary to provide sufficient phosphate. Upward development with an acid solvent (acetone, 50 vol./30% acetic acid, 50 vol.) moved the inositol phosphates in a compact spot. Subsequent upward development at right angles with methanol–aqueous ammonia resolved them in a straight line (Fig. 1).

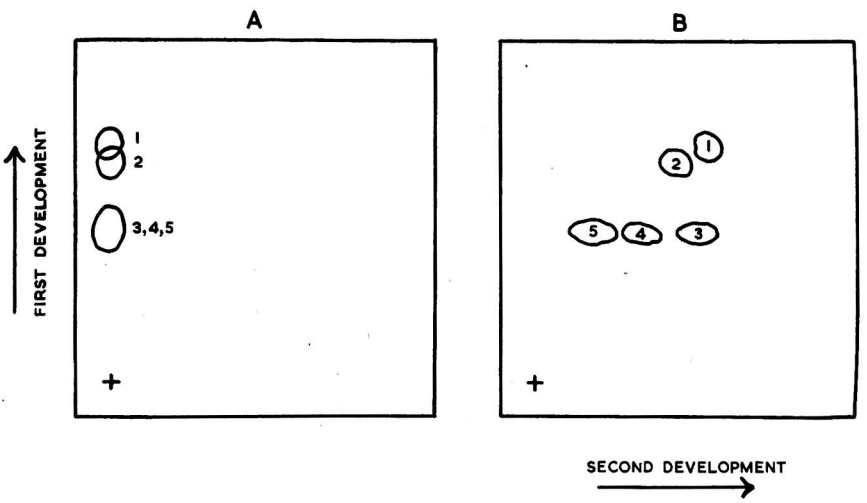

FIG. 1.—*Two-dimensional chromatogram of a mixture of phosphates containing inositol phosphates*
A. Chromatogram developed upwards with acetone, 50 vol./30% acetic acid, 50 vol.
B. Chromatogram subsequently developed upwards, at right angles, with methanol, 70 vol./0·5N-aqueous ammonia, 30 vol.
The compounds are: 1. β-glycerophosphate, 2. orthophosphate, 3. inositol monophosphate, 4. inositol diphosphate, 5. inositol triphosphate

Alternatively, the alkaline solvent can be used first, a procedure which has advantages with certain soil extracts (see below).

II. Analysis of soil inositol phosphates

The extraction and precipitation of the soil 'phytate', prior to chromatographic analysis, were similar in principle to the methods of other workers, but the rather severe hypobromite

oxidation was avoided. The soil, after acid leaching, was extracted with hot aqueous sodium hydroxide under conditions designed to minimize hydrolysis of inositol phosphates, and yet dissolve them quantitatively. Treatment of authentic inositol phosphates in the same way caused no measurable hydrolysis, a fact which is in accord with the findings of Fleury[13] and more recently of Desjobert & Fleurent.[14] The latter have shown that the hydrolysis of inositol hexaphosphate proceeds relatively rapidly under strongly acid conditions, falls to a minimum at a pH of 0 to 1, rises to a maximum about 4 and then falls to zero in weakly alkaline medium. All inositol phosphates behave in the same general fashion, but the pH above which they are stable depends upon the number of phosphate groups present, ranging from 7·5 for the monophosphate up to more than 11 for the hexaphosphate.

After extraction, four precipitations were carried out, and at no point was the solution allowed to stand for any length of time under acid conditions. The first of these precipitations, achieved by acidification to a low pH, removed a great part of the organic material without co-precipitating inositol phosphate. Neutralization with aqueous ammonia then yielded a bulky solid, mostly inorganic in nature, but containing a small amount of organic phosphate. Tests failed to detect any inositol phosphate in this precipitate. Treatment with barium acetate, and finally with alcohol, gave two more fractions which it will be convenient to name the ' barium ' and ' alcohol ' precipitates, respectively, and which contained the soil inositol phosphates. The last two precipitations might possibly have been combined by addition of barium acetate and alcohol in succession before centrifuging. It was considered, however, that if several inositol phosphates were present in markedly different proportions, with the hexaphosphate predominating, a partial separation would be advantageous at this stage. It seemed likely that barium acetate alone would precipitate most of the higher phosphate esters, but that lower esters, such as the diphosphate, might remain in solution in such dilute medium. Treatment with alcohol, however, would precipitate even the more soluble barium inositol monophosphate, and chromatographic examination could then be carried out without the complication of a grossly overloaded hexaphosphate spot.

Soil fractionation

Air-dried soil (500 g.), passing a 2-mm. sieve, was leached with cold 0·2N-hydrochloric acid until the filtrate gave no precipitate with ammonium oxalate solution. It was then washed with water, and extracted with 2 l. of N-sodium hydroxide at 60° for 4 hours with occasional shaking. After filtering, 1200 ml. of the extract were treated with 70 ml. of glacial acetic acid, with cooling, and further acidified to pH 0·5 by addition of hydrochloric acid. The precipitate of humic material was centrifuged and washed with a small quantity of water. The combined supernatant and washings were made alkaline with aqueous ammonia and the bulky precipitate removed by centrifuging, washed with water (twice), then ethanol and dried. To the supernatant liquid and aqueous washings was added excess of a 10% solution of barium acetate and the liquid set aside overnight, before the barium precipitate was centrifuged, washed with water and alcohol, and dried in a vacuum-oven. This precipitate contained the bulk of the soil inositol phosphates. Since the volume of the filtrate was large, however, and the barium salt of inositol monophosphate is soluble even under alkaline conditions, the volume was decreased under reduced pressure to about 1000 ml. and two volumes of 95% ethanol added. After keeping overnight, the precipitate was centrifuged, washed with alcohol and dried. This precipitate contained barium orthophosphate and small amounts of inositol phosphate. The final aqueous–alcoholic liquid contained only very small amounts of phosphate.

Analytical procedure

Phosphate analysis.—Total soil organic phosphate was determined by the hot hydrochloric acid–cold sodium hydroxide extraction method, and the ignition method, described by Saunders & Williams.[15] The values by these widely differing methods are in good agreement (Table II).

Inorganic phosphate in extracts was determined colorimetrically (Truog & Meyer ;[16] Williams & Stewart[17]). Tests were carried out to ensure that inositol phosphate, and other common phosphate esters, did not interfere with this colorimetric estimation.

To determine the total phosphate in extracts, a suitable aliquot portion was ignited with 0·5 ml. of a 12% solution of crystalline magnesium nitrate in a silica basin, taken up with 15 ml. of N-hydrochloric acid and evaporated to dryness on the water-bath. The residue was dissolved in 10 ml. of hot 0·1N-hydrochloric acid, transferred to a 100-ml. graduated flask and diluted to about 70 ml. To adjust the pH, two drops of an aqueous 0·25% solution of *p*-nitrophenol were added, then N-aqueous ammonia until the liquid became yellow, and the colour was discharged with the minimum amount of N-sulphuric acid. Phosphate was estimated colorimetrically as above.

Analysis of 'barium' and 'alcohol' precipitates.—The inositol phosphates and orthophosphate present in the 'barium' and 'alcohol' precipitates were dissolved in hydrochloric acid. Determination of phosphate in the extract established the amount of orthophosphate occurring in these precipitates, while chromatographic examination and analysis of the spots determined the relative amounts of orthophosphate and inositol phosphates. The actual amount of the latter which occurred in the precipitate was then calculated by direct proportion. This gave more accurate results than the application of measured aliquot portions to a chromatogram.

In preliminary experiments, the phosphate was dissolved by treating the precipitates with hydrochloric acid, and filtering and washing the residues thoroughly with acid, and aliquot portions of the filtrate were analysed. Filtration was extremely slow, however, and an alternative very simple method in which the precipitate was treated with a measured volume of acid, centrifuged, and a small aliquot portion analysed, was found to give identical results.

Before chromatographic examination of the extracts, barium was removed either by precipitation as sulphate or by passing the extract through a cation-exchange resin. One-dimensional chromatograms were developed with methanol/aqueous ammonia, and the relative amounts of the inositol phosphate esters and of orthophosphate were measured. As an additional check, total inositol phosphate was determined in chromatograms developed with acetone/acetic acid, but in certain cases a discrepancy was found between the two sets of values, the alkaline solvent giving higher results. The organic phosphate unaccounted for in the acid-developed chromatogram was not located in a compact area, but in a uniform haze extending from the starting line to the inositol phosphate spot. Previous treatment of the extract with an exchange resin did not alter this, but it was found that if the alkaline solvent were used first, and the acid solvent at right angles, the haze disappeared and the amount of inositol phosphate correspondingly increased. This type of development seems to be the most satisfactory for the examination of such soil preparations, and a typical chromatogram is shown in Fig. 2.

Procedure.—Approximately 0·1 g. of the 'barium' or 'alcohol' precipitate was weighed accurately into a 15-ml. tapered centrifuge tube, 5 ml. of N-hydrochloric acid were added by pipette and the tube placed in a beaker of water at 30° for 15 minutes, with frequent stirring. After centrifuging, 2 ml. of the clear supernatant liquid were pipetted into a 50-ml. graduated flask and made up to the mark. Ten ml. of the solution were transferred by pipette to a 50-ml. beaker, warmed, treated with 1 ml. of hot N-sulphuric acid, and filtered through a Whatman No. 42 paper into a 100-ml. graduated flask, with thorough washing. Orthophosphate was determined colorimetrically in suitable aliquot portions.

For chromatographic examination, a further 1 ml. of the original supernatant liquid was transferred to another centrifuge tube, treated slowly with a small excess of hot N-sulphuric acid, and centrifuged until perfectly clear. Repeated applications of the supernatant liquid were made to Whatman No. 1 paper, purified as described above, each application consisting of about 5 μl. in one-dimensional and 1 μl. in two-dimensional work. The spots were allowed to dry after each application. Finally a single application was made of a 0·5% aqueous solution of ethylenediaminetetra-acetic acid (sodium salt). A chromatogram was developed in one dimension with methanol/aqueous ammonia, dried, sprayed with Hanes & Isherwood's reagent, and heated as already described. A number of extracts were examined simultaneously on one sheet of paper, alongside known standards. Usually it is sufficient to run only two standards, inositol hexaphosphate and orthophosphate. Secondly, a chromatogram was developed in two-dimensions

with methanol/aqueous ammonia, and acetone/acetic acid. Spots were located by the Wade & Morgan spray, and their identity confirmed by reference to a chromatogram of standards run at the same time. The spots of esters were cut out, moistened with 0·5 ml. of 12% magnesium acetate solution, evaporated to dryness on the water-bath and ignited at 600° for 15 minutes in a muffle furnace. Thereafter the residues were evaporated with hydrochloric acid and treated as in the determination of total phosphate. If the orthophosphate was thought to coincide with another phosphate, the spot was cut out, moistened with two drops of N-sulphuric acid, and eluted with 10 ml. of 0·1 N-sulphuric acid into a graduated flask. After pH adjustment, orthophosphate was determined colorimetrically. Blank determinations were carried out with each batch of analyses.

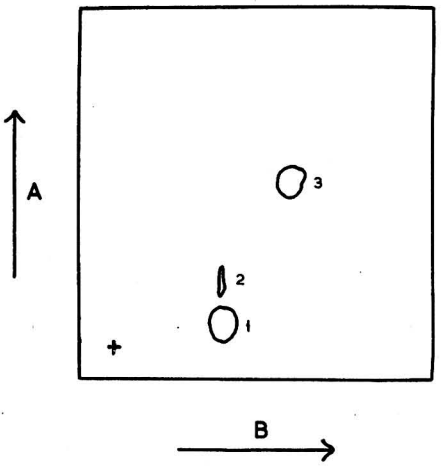

FIG. 2.—*Typical chromatogram of the phosphates present in the 'barium' precipitate obtained from a Scottish soil, developed A, upwards with methanol/aqueous ammonia, and B, upwards at right angles with acetone/acetic acid*

The compounds are: 1. inositol hexaphosphate, 2. inositol tetra/triphosphate, 3. orthophosphate

The results obtained for three typical agricultural top-soils of the Aberdeen area are presented in Tables II and III. Inositol hexaphosphate has been identified in appreciable amount, together with small quantities of the tetra- and/or tri-phosphates. No trace has yet been found of the di- or mono-phosphate.

Table II

Phosphate content of soils and precipitates
(mg. P_2O_5/100 g. of soil)

Soil (Location and parent material)	Organic phosphate: Total in soil* A	Organic phosphate: Total in soil* B	Organic phosphate: Total extracted by hot N-NaOH C	C as % of A	Orthophosphate: 'Barium' precipitate	Orthophosphate: 'Alcohol' precipitate
Lodie Newton, slate	204	208	139	68	36	67
Conveth Mains, old red sandstone	117	122	89	76	20	24
Newbigging, basic igneous drift	215	230	140	65	59	47

* A, Determined by HCl/NaOH extraction method
B, Determined by ignition method

Table III

Inositol phosphate content of soils and precipitates*
(mg. P_2O_5/100 g. soil)

Soil (Location and parent material)	Inositol hexaphosphate: 'Barium' precipitate	Inositol hexaphosphate: 'Alcohol' precipitate	Inositol hexaphosphate: Total	Inositol tetra-/tri-phosphate: 'Barium' precipitate	Inositol tetra-/tri-phosphate: 'Alcohol' precipitate	Inositol tetra-/tri-phosphate: Total	Total inositol phosphate	Total as % of soil organic phosphate (Table II, column A)
Logie Newton, slate	50	17	67	3	3	6	73	36
Conveth Mains, old red sandstone	39	1	40	4	trace	4	44	38
Newbigging, basic igneous drift	52	1	53	5	—	5	58	27

* No inositol monophosphate or diphosphate could be detected.

Discussion

Previous methods for the estimation of phytin involved precipitations of the ferric, calcium or barium salts under carefully controlled conditions, followed by purification and phosphate estimation. With such heterogeneous systems as soil extracts, the necessary purification involved considerable loss. In addition, such methods did not differentiate the inositol phosphates (with the exception of the monophosphate), which could only be separated by repeated fractional precipitation on a large scale.

The present method permits more accurate identification and estimation of inositol phosphates, even in very small amounts.

One-dimensional chromatography, developing upward with methanol/aqueous ammonia, resolves inositol mono-, di-, and hexa-phosphates, while the tetra- and tri-phosphates move together in a fourth spot. With acetone/acetic acid, or certain other acid solvents, all the inositol phosphates move in a single compact group. Two-dimensional chromatography with these solvents separates other phosphate esters which might cause confusion. By varying the nature of the acid solvent it is likely that any interfering phosphate could be removed, and this method of analysis should be applicable to most biological systems.

The technique has been applied in estimating the inositol phosphates of soil. The pioneer work of Wrenshall & Dyer,[1] Bower[2] and others on soil organic phosphorus compounds provided strong evidence that inositol hexaphosphate and other inositol phosphates were major constituents, although many of their conclusions were based on iron : phosphorus or inositol : phosphorus ratios of products which were either impure or had been subjected to such rigorous purification that loss or hydrolysis seemed likely. In particular, a hypobromite oxidation was carried out, succeeded in turn by boiling in acid medium to expel excess bromine, conditions which may have caused some breakdown.

Particular care was therefore taken in the preparation of soil inositol phosphates prior to chromatographic analysis, to ensure that hydrolysis was minimized and that they were precipitated quantitatively. Many organic and inorganic impurities were first separated by precipitations which did not bring down inositol phosphates. Purification of the final ' barium ' and ' alcohol ' precipitates was unnecessary as contaminants were separated during the chromatographic analysis. The ' barium ' and ' alcohol ' precipitations were carried out separately with the intention of fractionating inositol phosphates to a certain extent before examination. In fact no trace of the lowest phosphate esters has yet been found in the ' alcohol ' precipitate, but instead it contained a considerable proportion of the orthophosphate. Only about half, or less, of the orthophosphate present in the alkaline extract was precipitated by barium acetate, whereas most of the inositol hexaphosphate and tetra-/tri-phosphate was brought down. It was noteworthy that in the soils so far examined, inositol hexaphosphate was present in considerable quantity, making up about one-third of the total soil organic phosphate, whereas the tetra- and/or tri-phosphates only accounted for 2 or 3%, and no other inositol phosphate was detected.

Acknowledgment

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THE EFFECT OF POST-HARVEST STORAGE CONDITIONS OF RAW POTATOES ON THE STORAGE LIFE (AT TROPICAL TEMPERATURES) OF THEIR DEHYDRATED PRODUCTS

By E. G. B. GOODING, R. B. DUCKWORTH and J. M. HARRIES

The increase in reducing sugar content of potatoes stored in a field clamp after a spell of cold winter weather was sufficient to halve the storage life of the dehydrated product when held at 98·6° F. Conditioning of the potatoes after this cold spell for 2–3 weeks at 60° F immediately before dehydration lowered the reducing sugar content and led to some improvement in the product. The lowest reducing sugar contents and the products most resistant to deterioration during high-temperature storage were obtained by keeping the raw potatoes, treated with a sprout depressant, in a shed with a minimum temperature of 43° F.

Introduction

One of the main problems connected with dehydration of vegetables at the present time is deterioration of the product during storage at tropical temperatures. Storage life is limited in most cases by brown discoloration and a considerable weight of evidence supports the view that these pigments are the end-products of reactions of the Maillard type.[1–5] Factors affecting the rate of these deteriorative changes have received a great deal of attention, but in spite of this no major improvements in stability have been effected in recent years. The need for a reduction in moisture content to as low a level as possible during dehydration has been emphasized by many authors[1, 2, 6–9] and the protective action of sulphur dioxide[10–12] is also well established. Further reduction of moisture content by in-package desiccation[13, 14] has given useful results, but has a basic disadvantage in that it partially offsets the saving in bulk and weight achieved by dehydration.

A factor of importance in the discoloration of potatoes is the reducing sugar content of the material. Although this has been pointed out by several workers[2, 15] this aspect of the problem has not received adequate attention. It is possible to leach the reducing sugars out of potato strips or dice by soaking in water after the scalding treatment which normally precedes dehydration, and Dexter & Salunkhe[16] have attempted to control the colour of potato crisps by extracting reducing sugars differentially by subjecting the slices to special soaking treatments. Heavy leaching before dehydration, however, has certain undesirable features, since it results in loss of yield, nutritive value and flavour. It would seem preferable to start with a potato of initially low sugar content rather than to attempt to lower the sugar content during processing. It is obviously desirable to avoid using varieties which normally have high reducing sugar contents, but there appears to be little information about the reducing sugar content of the varieties used in Britain. In any case, many other features have to be considered when selecting potatoes for dehydration and the choice is very limited.

One factor, however, which influences sugar content, and about which there is some knowledge, is the temperature of storage of the raw potato. It has been established[17–20] that at low temperatures (below about 42° F) the sugar content of potatoes, both sucrose and hexose, rises, but that at higher temperatures (60–70° F) sugars which have accumulated during low-temperature storage diminish in quantity, and conditioning for 2–3 weeks at such temperatures has been recommended to, and is practised by, manufacturers of potato crisps in the U.S.A.[4, 21] More recently similar recommendations have been made to crisp manufacturers in this country[22] and to domestic users in India.[19]

Until recently it has been essential to store potatoes at low temperatures (with appropriate safeguards against freezing) in order to avoid early sprouting. The advent of sprout depressants has led to the possibility of storing potatoes at higher temperatures and thus probably avoiding the undesirable accumulation of sugars.

Vegetable dehydration is being studied at Aberdeen on a factory scale, and, *inter alia*, the relationships between the conditions of storage of raw potatoes, their suitability for dehydration and the high-temperature storage-life of the dehydrated product are being investigated. The present paper reports some of the results so far obtained.

A. The effect of the conditions of storage on the reducing sugar content of the raw potato

Experimental

(*a*) *Material*

The potatoes used in this work were of the variety King Edward, grown at Millhead Farm, Tarland, Aberdeenshire. They were lifted during October 1953 and put into storage as described below.

(*b*) *Conditions of storage*

Three different treatments were employed:

(i) *Clamped on the farm.*—Approximately 130 tons were clamped in the field in which they were grown. The clamp followed the normal construction for the district, with internal dimensions of approximately 9 feet in width by 5 feet in height. The clamp was about 240 feet in length and the long axis ran from north-east to south-west down a slight slope. The potatoes were covered with about 4 inches of straw and the external layer of earth was approximately 1 foot thick. Provision was made for collecting temperature records by inserting into the clamp 16 galvanized steel tubes each containing a brass-cased mercury thermometer on a string. The external openings of the tubes, which ended flush with the surface of the clamp so as to reduce conduction to a minimum, were closed with corks except when opened for partial withdrawal and reading of the thermometers. The thermometers were arranged at three levels on each side of the clamp, and along its length. The maximum and minimum temperatures of the external air were recorded by means of a screened thermograph alongside the clamp. Readings of all thermometers were taken at weekly intervals.

(ii) *Conditioned.*—At intervals (see schedule of sampling below), six-ton lots of potatoes were removed from the clamp and transferred to the Experimental Factory at Aberdeen for 'conditioning'. The potatoes were stacked in bags on slatted stillages in a small heated store for 15–17 days (longer in two cases) immediately before dehydration. The air in the store was circulated by means of a fan and thermostatically controlled at 60° F, although the capacity of the heaters was not always adequate to maintain this temperature.

(iii) *Stored in a warm shed.*—Twenty-four tons of potatoes from the same field as those in (i) and (ii) were stacked loose, 5 to 6 feet high, in a shed lined with straw bales at Craibstone, Aberdeenshire. The air was heated by means of a 3-kw convector heater set at one end of the shed and thermostatically controlled to cut in at temperatures below 50° F. The potatoes were dusted with technical grade TCNB (3% by weight of 2 : 3 : 5 : 6-tetrachloro-1-nitrobenzene in an inert carrier) at the rate of 10 lb. of the preparation per ton, and were covered with loose straw. A mercury thermometer was hung above the stack, and readings were taken at weekly intervals.

(c) *Schedule of sampling*

Potatoes subjected to the three storage treatments were dehydrated at intervals between January and May 1954. Twelve-ton lots were taken from the clamp after 10, 14, 18 and 26 weeks; half of each consignment was dehydrated in two three-ton lots as soon as they were received at the factory; the other half of each consignment was put into the conditioning store and subsequently dehydrated in two three-ton lots. Three tons of material from the warm shed at Craibstone were dehydrated at fortnightly intervals (see Table I).

(d) *Determination of sugar in the raw potatoes*

Reducing sugar and sucrose determinations were carried out on extracts prepared from samples from each three-ton lot of potatoes as it was withdrawn from storage for dehydration, using Nelson's modification of Somogyi's method[23] checked at intervals by a modified Schaffer–Hartmann technique (after Narain & Maskell, described by Wager[24]). The preparation of the extract, however, differed from the standard techniques, and details are given below.*

Results

(a) *Temperature changes during storage*

(i) *In the clamp.*—The readings of thermometers inserted at different points in the clamp showed that the temperature in the upper part might be 5–6° F higher than that in the lower part, while differences along the length of the clamp were negligible. The average of the readings of thermometers in the clamp remained generally rather similar to the maximum external air temperature throughout the period of storage, as can be seen from Fig. 1. The minimum average temperature at the lower level (representing the large majority of the total weight of potatoes) was 39° F. The average temperature for all thermometers in the clamp at this time was 41° F. During January and the first week of February, the thermograph was deeply buried in snow and the readings during this time do not therefore represent true external air temperatures.

(ii) *In the conditioning store.*—The intended temperature of 60° F was not always attained in the conditioning store and on occasion the thermometer readings fell as low as 55° F. Towards the end of the season attempts were made to compensate for this by prolonging the period of treatment (see Table I).

(iii) *In the warm shed.*—A graph of the temperature of the air above the potatoes is given in Fig. 2 and although the temperature of the atmosphere actually within the stack of potatoes was not obtained, there is no doubt that it would have been higher than that of the surrounding atmosphere.[26] Even during the coldest period of the winter it did not drop below 43° F, and in all probability remained some degrees above this level.

(b) *Sugar contents on storage*

The results of the sugar analyses for potatoes subjected to the three different storage treatments are given in Table I. It is seen that as the duration of storage in the clamp increased from 10 to 18 weeks, the reducing sugar content of the potatoes rose about four-fold, the highest reducing sugar content occurring at about the time of the lowest temperature in the clamp (Fig. 1). Subsequently, the sugar content of the clamped potatoes fell almost to its original

* One tuber was taken from each fifth sack of a consignment to provide as large and representative a sample as could be dealt with in the laboratory. Each tuber was quartered, a thin slice removed, and duplicate samples of 25 g. of the slices weighed out for extraction. 25 g. of material were rapidly homogenized to a slurry with 65 ml. of boiling 95% alcohol and transferred to a conical flask with another 40 ml. of 95% alcohol; these proportions gave approximately 80% alcohol in the final extract. The material was then refluxed on a water-bath for one hour. (The extract at this stage was alkaline to methyl red and acid to phenol red and it was not treated with $CaCO_3$, as recommended in the AOAC method.[25]) After refluxing, the extract was filtered through Celite on a No. 3 sinter pad and the residue washed with 80% alcohol. The filtrate was evaporated under vacuum (temperature maintained below 131° F) to remove alcohol and the syrupy liquor remaining again filtered through Celite. The Celite was thoroughly washed with distilled water to bring the volume of the filtrate to 200 ml. and the filtrate was then made up to 250 ml.

Duplicate aliquot portions were taken from this extract. For glucose determination each portion was neutralized to phenol red with 0·1N-sodium hydroxide and made up to 100 ml. For determination of 'total sugars' each aliquot portion was neutralized to methyl red with 5% acetic acid; 2 ml. of invertase concentrate diluted 1 : 20 were added, and the solution made up to 100 ml. These solutions were incubated at 98·6° F for one hour, and determination of reducing sugar again made.

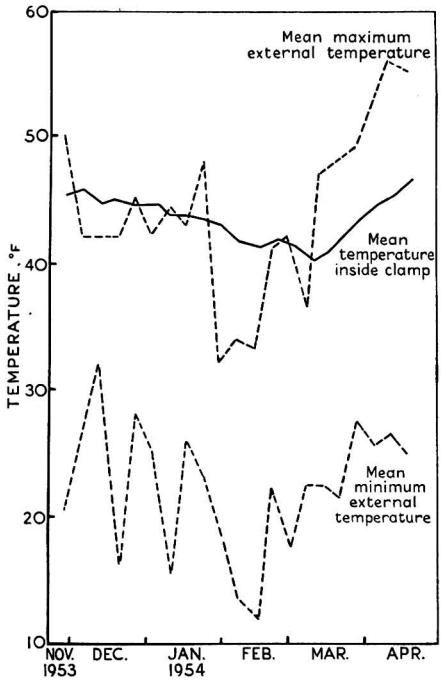

FIG. 1. (*left*)—*Mean temperatures of atmosphere in clamp at Tarland and weekly maximum and minimum temperatures of external air*

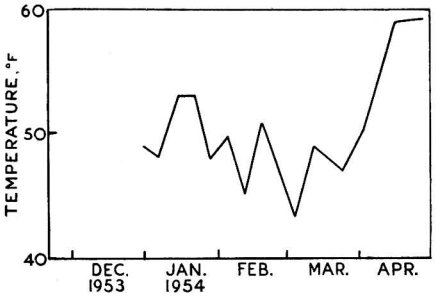

FIG. 2.—*Changes in the temperature of the air above the stack of potatoes in the shed at Craibstone*

level. Conditioning brought about a marked reduction in sugar content only in the one case in which the initial level was exceptionally high. There was little change in sugar content in the material stored in the warm shed, the value remaining consistently low throughout the period of storage.

B. The effect of reducing sugar content of the raw potato on the storage behaviour of the dehydrated product

Experimental

(*a*) *Method of dehydration*

The procedure followed conventional lines with only slight modifications.[27] After peeling and trimming, the potatoes were cut into strips of cross-section $\frac{3}{16} \times \frac{5}{16}$ in. The strips were washed, scalded for $2\frac{1}{2}$ minutes in water containing a small concentration of sodium metabisulphite (350–500 p.p.m. as SO_2), cooled and dried on trays in a three-stage cross-flow drier. The product was packed in A10 tall cans (603 × 904) in an atmosphere of nitrogen.

The resulting material, taking the season as a whole, showed variations in moisture content from 5·0 to 7·4%, and the SO_2 content varied over the range 277–444 p.p.m.

(*b*) *Selection of material for storage tests*

It is not possible when working on the factory scale to obtain material differing only in respect of one factor, but batches, representative of the different treatments to which the raw material was subjected, could be selected with relatively small differences in moisture content and sulphur dioxide content. Possible differences due to leaching during scalding were reduced to a minimum by using, in each case, material processed at the end of a run, by which time the soluble solids in the scald liquor had had time to build up to the equilibrium level,[28] a condition that would normally obtain in commercial practice.

In the final selection there were nine types of material, obtained from potatoes subjected to each of the three storage treatments prior to dehydration and dehydrated at three different times

during the season—early (January-February), immediately after the coldest spell (March), and towards the end of the season (May). Analytical data for the selected material are included in Table I.

Table I

Conditions and duration of storage and analyses of potatoes and dehydrated material

Treatment	Duration of storage (weeks)	Duration of conditioning (days)	Run	Code	Raw potato		Dehydrated potato	
					Reducing sugar % (as glucose) (moisture-free basis)	Sucrose % (moisture-free basis)	Moisture %	SO_2 p.p.m.
Clamped on the farm	10		100		1·60	0·85		
	10		101		1·93	1·90		
	14		106	A	2·48	0·42	6·73	418
	14		107		4·21	0·46		
	18		112	D	6·43	3·65	6·64	474
	18		113		6·25	0·75		
	26		121	E	2·15	0·46	7·04	504
	26		122		2·05	0·50		
Clamped on the farm, subsequently conditioned	10	15	103	B	1·95	0·51	7·34	339
	10	17	104		1·91	0·76		
	14	15	109		2·77	0·19		
	14	17	110		2·79	0·21		
	18	15	117		5·90	1·91		
	18	24	119	F	2·40	1·04	6·92	·472
	26	15	124	G	1·89	0·10	6·52	389
	26	21	125		1·93	—		
Stored in warm shed (TCNB treated)	11		102		1·67	0·90		
	13		105	C	0·82	0·48	7·60	348
	15		101		1·68	0·40		
	17		111		1·48	0·78		
	19		115		1·34	1·12		
	21		118	H	1·45	1·62	6·42	398
	27		123		1·16	0·28		
	29		126	J	2·05	—	6·21	400

(*c*) *Packing, storage and sampling*

The material, after thorough mixing within each batch, was re-packed in nitrogen in A2½ cans (401 × 411). Extra material of two kinds (H and D in Table I) was packed for control purposes. For the high-temperature storage test the samples were stored at an even temperature of 98·6° F, and the control samples (H and D) at 23° F. Duplicate cans of each type of material were removed from storage at 98·6° F after three, four, five and six months. On each occasion, three cans of each type of control material were also removed. The samples were submitted to an experienced tasting panel for assessment of colour, flavour and texture, and were also analysed in the laboratory.

(*d*) *Tasting panel technique*

After reconstitution in the normal way,[29] each type of material was tasted in duplicate by a trained panel. Six panels were held at each sampling time, one in the morning and one in the afternoon for three consecutive days. On the first day, samples of material dehydrated during the early part of the season were examined, one sample of each kind of material in the morning and the duplicates in the afternoon. On the second day, the material dehydrated immediately after the coldest spell was tasted in the same manner and on the third day the material dehydrated towards the end of the season.

Marking for colour and texture was made in each case on an arbitrary six-point scale. For flavour, two marks were allotted to each sample, one for the strength of any ' earthy ' taint and one for natural flavour. The purpose of this dual scoring for flavour was to allow for any taint

present in the material stored in a warm shed because of its treatment with technical grade TCNB[30] and to measure any effect of the different storage conditions on the flavour of the dehydrated product independently of such taint. Each of these was based on an arbitrary six-point scale. The details of the score sheet used are shown in Fig. 3.

(1) *Colour*

Typical white or cream of freshly boiled potato	6
Just discernible off-colour—greyish tinge	5
Just discernible off-colour—beige tinge	4
Definite but slight browning	3
Marked browning	2
Extreme overall browning	1

(2) *Flavour* (Two marks must be accorded each sample)

(*a*) Give each sample a mark from 1 to 6 according to the strength of any earthy taint (6 would represent a complete absence of earthy flavour, 1 a condition in which the taint is so strong as to render the material inedible)

(*b*) Full natural flavour 6
Slight loss of natural flavour 5
Very weak but natural flavour 4
Off-flavour (other than earthiness) detectable but slight 3
Marked off-flavour 2
Inedible 1

(3) *Texture*

Typical texture of freshly boiled potato	6
Slight divergence from above	5
A few pieces tough	4
Whole sample slightly tough	3
Whole sample distinctly tough	2
Whole sample extremely tough	1

FIG. 3.—*Score sheet used by taste panel in assessing the culinary quality of the dehydrated material during storage*

One control sample was included in each panel, the sample used in the afternoon on any particular day being different from that used in the morning, e.g. if the control sample for the morning panel was TCNB-treated, that for the afternoon panel was untreated.

(*e*) *Analytical methods*

For laboratory work, a sample, including strips from each of the duplicate cans, was ground in a Christy & Norris mill until 95% would pass a 40-mesh sieve. Moisture content,* sulphur dioxide content† and browning extinction values‡ were determined.

(*f*) *Visual assessment of browning*

An attempt was made during the course of the test to measure deterioration visually by comparing test samples with a series of standards showing different degrees of browning. To obtain standards, dehydrated potato strips were kibbled by coarse grinding in a coffee mill, the fraction held by a 10-mesh sieve being retained, although pieces exceeding $\frac{1}{4}$ in. in length were discarded. The kibbled material was gas-packed in Kilner jars and held at 131° F for different periods to induce a range of degrees of browning. An arbitrary selection of 8 degrees of browning was made, standard 8 being completely free from browning and standard 1 very severely browned. After selection the standards were stored at 23° F.

* Duplicate 3-g. samples of the ground material were heated in a shallow metal dish, 65 mm. diameter × 15 mm. deep, at 158° F for five hours under an absolute pressure of 2 mm. Hg. The percentage loss in weight (average of duplicates) was taken as the moisture content.

† Determined by a modified Monier-Williams technique, the values given being averages of duplicate determinations.

‡ 5 g. of the ground samples were extracted with 100 ml. of 60% ethyl alcohol by standing overnight with occasional shaking. The extract was filtered and the extinction value (optical density) of the filtrate at a wavelength of 400 mμ was read on a Unicam Spectrophotometer (S.P. 350) using a 60% alcohol blank.

Samples of each type of material under test were kibbled and gas-packed in 1-lb. Kilner jars in the same way as the standards, and stored at 98·6° F. At monthly intervals, the samples were compared visually with the standards by three observers independently, and marked to the nearest half mark on the standard scale.

Results

1. *Tasting panel scores*

The average marks accorded each type of material initially and after storage for various times are given in Table II. Analyses of variance were used to examine the significance of these scores and these are shown in Table III, separately for colour, flavour and texture. Although objections can be raised to the use of this technique with arbitrarily assigned marks, its advantages are such that it has been used by a number of other workers in this context (see [31, 32]).

Table II

Tasting panel marks during storage

	Code	Reducing sugars as % glucose in raw potato	Marks (maximum 6) Storage in months				
			0	3	4	5	6
					COLOUR		
Clamped on the farm	A	2·48	5·9	4·3	4·5	4·5	2·5
	D	6·43	5·7	1·8	1·9	1·4	1·2
	E	2·15	5·8	4·1	3·6	2·4	1·8
Conditioned	B	1·95	5·6	3·9	3·5	2·9	1·9
	F	2·40	5·8	3·3	2·4	3·0	1·9
	G	1·89	5·7	4·3	3·5	3·0	1·8
Stored in warm shed	C	0·82	5·8	4·4	4·5	4·2	2·7
	H	1·45	5·8	5·1	4·3	4·0	3·4
	J	2·05	5·7	3·8	4·0	4·3	3·3
Control material at − 5° c	D		5·8	5·5	5·5	5·4	5·5
	H		5·7	5·2	5·5	4·9	4·7
					FLAVOUR (Natural)		
Clamped on the farm	A	2·48	4·8	4·1	3·8	3·9	2·8
	D	6·43	5·2	2·7	2·9	2·7	1·7
	E	2·15	5·0	4·5	3·0	2·4	2·0
Conditioned	B	1·95	5·0	3·9	3·9	3·2	2·0
	F	2·40	5·1	3·7	3·1	3·0	2·0
	G	1·89	5·1	4·5	3·3	2·8	2·2
Stored in warm shed	C	0·82	4·5	3·9	4·2	3·0	2·4
	H	1·45	4·1	3·8	4·3	3·5	3·1
	J	2·05	4·2	4·0	3·8	3·8	2·5
Control material at − 5° c	D		5·0	4·1	4·7	4·4	4·6
	H		4·7	3·9	4·7	4·1	3·8
					TEXTURE		
Clamped on the farm	A	2·48	5·1	4·1	4·5	4·1	2·7
	D	6·43	5·2	3·6	2·9	2·6	2·3
	E	2·15	5·3	4·1	4·0	2·6	2·2
Conditioned	B	1·95	5·1	3·6	3·8	3·0	2·2
	F	2·40	5·5	4·4	3·2	3·2	2·8
	G	1·89	4·9	4·4	3·6	3·2	2·8
Stored in warm shed	C	0·82	5·1	3·6	4·8	3·5	1·7
	H	1·45	5·2	4·2	4·0	4·3	2·7
	J	2·05	4·8	3·7	4·0	2·8	2·4
Control material at − 5° c	D		5·1	5·1	5·3	4·7	5·0
	H		5·3	4·6	5·0	4·4	4·2

Table III

Analyses of variance (morning tasting only)

Source of variance	Degrees of freedom	Colour		Flavour		Texture	
		Mean square	Significance	Mean square	Significance	Mean square	Significance
Between types of pre-storage (A)	2	11·61		7·48	**	1·30	
Between times of pre-storage (B)	2	2·87		1·92	n.s.	0·23	
Between times of storage (dehydrated) (C)	4	29·62		22·07	***	28·35	
Interactions							
A × B	4	7·26	***	0·58	n.s.	1·85	***
A × C	8	1·42	*	0·44		0·52	*
B × C	8	1·21	*	1·14		2·08	***
A × B × C	16	0·92	n.s.	0·62		2·08	***
Residual	90	0·48		0·88		0·22	
Total	134	—		—		—	

n.s. not significant
* significant $P < 0{\cdot}05$
** ,, $P < 0{\cdot}01$
*** ,, $P < 0{\cdot}001$

In this investigation, since all tasters were not present on all occasions, the analyses have been computed using the maximum possible number of tasters selected at random as replications. This means that the average results to which the analyses of variance refer are not identical with the average results of the experiment as a whole, which are given in Table II. Care has been taken to see that the average results of the readings used in the analyses of variance do not differ markedly from the overall averages. In fact the agreement is remarkably good.

In order to include times of pre-storage as a factor in a factorial analysis of variance, it is assumed that there was a one-to-one correspondence between these times for each type of material, though, in fact, this is not strictly true.

Deterioration in colour.—As shown by the significant interaction in Table III, the effect of length of pre-storage on the colour of the material varied for the different types of pre-storage, and separate analyses of variance for each type of pre-storage are shown in Table IV. It is clear that for material stored in the clamp on the farm, length of pre-storage had a consistent and important effect on colour, the worst material at all times being that stored for 18 weeks—viz., the material with a high initial content of reducing sugars. Potatoes stored in a warm shed clearly kept much better colour than those stored in any other way, and the time for which these potatoes had been pre-stored did not seem to affect their keeping quality after dehydration. Potatoes conditioned in the factory showed no significant differences due to time of pre-storage, but those with a high percentage of reducing sugars again showed more rapid deterioration.

Deterioration in flavour.—As will be seen from the attached score scale, tasters were instructed to allot two scores for flavour, one for natural flavour and the other for an ' earthy ' off-flavour which was expected to occur in the material treated with technical grade TCNB. The latter was characterized in the instructions as ' earthy '. The results for the intensity of this ' earthy ' flavour do not show much fluctuation with any of the main factors involved in the experiment and therefore no results have been quoted for this characteristic. In fact there was no difference between the TCNB-treated material and other materials in the score given for ' earthy off-flavour ', but it will be seen from Table II that there was a difference in the score given for natural flavour ; so it may well be that the lack of initial difference was due not so much to an absence of off-flavour but to a mistake made in characterizing the off-flavour in the instructions as ' earthy '.

It is clear from Tables II and III that the material stored in a warm shed did not deteriorate in flavour as rapidly as the other material. Length of pre-storage did not seem to have any effect

Table IV

Analyses of variance of colour scores, separately for type of pre-storage (morning tasting only)

Source of variance	Degrees of freedom	Clamp-stored		Factory conditioned		Warm shed	
		Mean square	Significance	Mean square	Significance	Mean square	Significance
Between times of pre-storage	2	15·56	*	0·63	n.s.	1·15	n.s.
Between times of storage (dehydrated)	4	14·80	**	12·52	***	4·86	*
Interaction	8	1·84	**	0·54	n.s.	0·85	*
Residual	30	0·51		0·58		0·35	
Total	44	—		—			

n.s. not significant
* significant $P < 0.05$
** ,, $P < 0.01$
*** ,, $P < 0.001$

either on the conditioned material or on material kept in a warm shed, but affected the rate of deterioration of material stored in clamp in that deterioration was most rapid in the material which had a high percentage of reducing sugar.

Deterioration in texture.—The same effects as for colour and flavour are apparent in the texture scores, though to a lesser degree. In general, material with a high initial content of reducing sugars shows more rapid textural deterioration.

2. *Analytical*

Determinations of moisture content carried out initially and on material stored for six months at 98·6° F showed no significant change in this value during storage (see Table V). The rate of loss of sulphur dioxide was high and closely parallel with the rate of formation of browning pigments (compare Tables V and VI).

3. *Visual assessment of browning*

Close agreement was shown between the marks accorded to individual samples by different observers and very good overall correlation was shown between the average visual marks and the browning extinction values obtained for similar samples.

Table V

Moisture and SO_2 changes during storage

Treatment	Code	Reducing sugars (as % glucose in raw potato)	Moisture content (%)		SO_2 content (p.p.m.) after months at 98·6° F			
			Initially	After 6 months at 98·6° F	0	3	4	6
Clamped on the farm	A	2·48	6·73	6·81	418	—	150	92
	D	6·43	6·64	7·19	474	99	51	—
	E	2·15	7·04	7·00	504	201	155	91
Conditioned	B	1·95	7·34	7·25	339	203	80	49
	F	2·40	6·92	6·92	477	149	83	65
	G	1·89	6·52	6·44	389	140	116	100
Stored in warm shed	C	0·82	7·60	6·89	348	—	177	150
	H	1·45	6·42	6·38	398	255	214	190
	J	2·05	6·21	6·46	400	243	216	—

The corresponding extinction values at 400 mμ of 60% alcohol extracts from these samples are given in Table VI

The average marks accorded to each type of material during storage for six months at 98·6° F are shown in the form of graphs in Fig. 4. The comparatively rapid deterioration in colour of the material containing a high content of reducing sugars is strikingly illustrated.

Table VI

Increase in browning extinction value during storage at 98·6° F

Treatment	Code	Reducing sugars (as % glucose in raw potato)	Extinction value of extract after months at 98·6° F			
			0	3	4	6
Clamped on the farm	A	2·48	0·057	0·084	0·087	0·223
	D	6·43	0·064	0·249	0·400	0·662
	E	2·15	0·067	0·118	0·192	0·338
Conditioned	B	1·95	0·092	0·111	0·135	0·268
	F	2·40	0·051	0·142	0·247	0·406
	G	1·89	0·074	0·118	0·155	0·280
Stored in warm shed	C	0·82	0·063	0·070	0·095	0·175
	H	1·45	0·063	0·066	0·070	0·144
	J	2·05	0·064	0·083	0·106	0·192

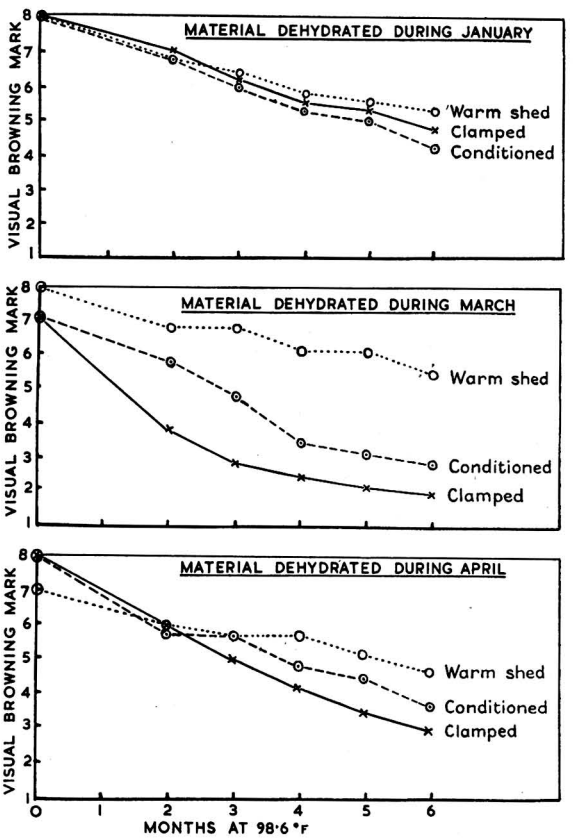

FIG. 4.—*Deterioration in colour during storage at 98·6° F as assessed by visual comparison with kibbled standards*

Discussion

The present results confirm that high reducing sugar content in the raw potato diminishes the life of the dehydrated product when stored at high temperature, and that this can to some extent be avoided by controlling the conditions of storage of the raw material. The magnitude of the effect under normal British conditions is striking : a fall to 40° F (a far from uncommon temperature in clamps[33]) led to a rise in reducing sugar content from about 2·5% to over 6%. This, in

turn, affected the storage life of the dehydrated potato to such an extent that after 3 months at 98·6° F the material with the higher reducing sugar content scored lower marks for colour and flavour, and showed more browning than did the product with lower reducing sugar after 6 months. The latter remained acceptable after 5 months' storage; the former was quite unacceptable at the first sampling, after 3 months' storage.

It appeared that some improvement could be made by 'conditioning' tubers which had developed excessively high reducing sugar contents, but the best results by far were obtained with the potatoes stored in the heated shed. It may be that storage of the crop, or of that part of the crop which is to be used after, say, December or January, under conditions which maintain the minimum temperature above 45° F, would be the most suitable way of providing potatoes of low reducing sugar content for processing.

There are certain other points of interest which merit further consideration.

(*a*) *Effect of conditioning*

Conditioning was effective only on potatoes of high reducing sugar content, when a decrease from 6·43 to 2·40% was obtained (D and F in Table V). In the other cases (A and B, E and G) the decrease in reducing sugar content was very small. Similar results have recently been reported from the U.S.A.[3,4] and there is little reason to believe that if the higher temperatures of conditioning (approx. 70° F) usually recommended[4] had been used the results would have been very different, though the period of conditioning could probably have been reduced.

(*b*) *Relationship between reducing sugar content and browning*

There was a general correspondence between the reducing sugar content of the raw potato and the browning of the dehydrated product stored at 98·6° F, but it was far from precise. Deficiencies in sampling (the relatively small number of tubers to which a sample had to be confined) may have contributed to this, but there have been indications in the literature[4] that there is no exact correlation between browning and reducing sugar content, and it has been postulated that other factors, besides the known ones of moisture content and sulphur dioxide content, may operate.

In this connexion two rather striking anomalies appeared in the present work. Although the data are too scanty and the possibility of sampling error too great for conclusions to be drawn it seemed desirable to record the following:

(i) Although by the end of the season the potatoes stored in the warm shed had accumulated reducing sugars to the extent of 2·05% (J in Table VI), the dehydrated product showed substantially less discoloration than that made from potatoes stored in a clamp and taken at the same time (E), with almost the same proportion of reducing sugar, or than either of the two lots of conditioned material (B and G) which had rather lower reducing sugar contents. The question arises whether some factor other than reducing sugar content, perhaps related to the conditions of storage, might be playing a part.

(ii) In the one case in which a marked decrease in reducing sugar content resulted from conditioning (F), the resistance of the product to browning was not as great as might have been expected from the figure shown for the reducing sugar content, again suggesting the possibility that some factor other than reducing sugar content might be involved.

No data has so far been published on the effect of amino-acid content on browning in dehydrated potato and it may be that in certain circumstances the rate of the reaction is limited by this factor.

(*c*) *Behaviour of sulphur dioxide*

It is well known[7] that the disappearance of sulphur dioxide from dehydrated vegetables stored at elevated temperatures is related to the moisture content of the material; the higher the moisture content, the more rapid the loss of sulphur dioxide. Similarly, the higher the moisture content, the more rapid the browning of the dehydrated vegetable.

In the present work it was found that when dehydrated potatoes of similar moisture content were stored at high temperatures, the loss of sulphur dioxide was more rapid in those samples

that had high reducing sugar contents—i.e., the samples which had the highest rates of browning. Similar observations (unpublished) have been made in this laboratory, that when sulphited dehydrated potatoes are subjected to the alternatives of severe or light leaching during scalding, then, other things being equal, the lightly-leached material loses sulphur dioxide more rapidly, and browns more readily, during storage at 98·6° F, than does the heavily leached material.

There is ample evidence[7, 10] that sulphur dioxide exercises a protective effect against browning and it is generally assumed that the 'loss' of sulphur dioxide from dehydrated vegetables during storage at high temperature is a major factor in allowing the brown discoloration to develop.

The belief that the browning reaction involves the formation of colourless intermediate compounds, has recently been supported experimentally, and the observations noted above strengthen the view that disappearance of sulphur dioxide is necessarily linked with the progress of the chain of reactions. It seems reasonable to conclude that sulphur dioxide reacts with some intermediate compound and exercises its protective action by blocking or diverting the course which the reaction would otherwise take.

Appendix

Note on time of tasting

There has been considerable discussion in the literature of the advantages to be gained by holding tasting panel sessions before lunch, different workers disagreeing on the need for this. Since in the present work, samples of the same material were tasted in the morning and in the afternoon, analyses of variance could be carried out separately for each set of data. It has thus been possible to compare in an extensive experiment the performance of tasters at different times of day using the same criteria as Overman & Li.[35] Table VII shows the direct comparison which can be made by means of the analysis of variance, where the ratio of the variation between treatments to the residual variation is an indication of the discrimination of the judges considered as a panel, and the magnitude of the residual variation can be used as an indication of the

Table VII*

Comparison of morning and afternoon tasting

	Colour				Flavour				Texture			
	Variance ratio		Residual or error		Variance ratio		Residual or error		Variance ratio		Residual or error	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Clamp-stored	3·6	1·4	0·51	0·44					1·8	1·5	0·64	0·40
Conditioned	<1·0	2·1	0·54	0·60					4·5	<1·0	0·13	0·58
Shed-stored	2·4	1·4	0·35	0·44					2·6	<1·0	0·45	0·71
Types of pre-storage					9·0	1·3	0·83	0·74				
Times of pre-storage					2·3	1·7						
Time of storage (dehydrated)					26·6	39·8						

accuracy with which comparisons were made. It is unfortunate that no comparison can also be made of the biases shown by the different tasters, as would be reflected in a source of variation between tasters. It will be seen from Table VII that generally greater discrimination and fewer tasting errors were shown in the morning, although differences are not very great, and it is for this reason that Table III was based on the morning results only (although Table II, the average

* For colour and texture, the comparisons are drawn from the separate analyses of variance, as in Table IV, when the variance ratio shown is that between the interaction and the residual (since the interaction reflects *differential* storage life amongst the three times of pre-storage, within types). For flavour, the comparisons are drawn from the main analyses, as shown in Table III, where the variance ratios shown are those between the main factors and the estimate of error obtained by pooling the (non-significant) interactions with the residual.

results of the experiment as a whole, was based on all the results). This difference between morning and afternoon tasting was apparent in the scores allotted for colour as well as in those allotted for flavour and texture. Similar results were obtained in a previous investigation with fresh potatoes.[36] It is also noteworthy that if both sets of data (morning and afternoon) are considered separately, the same effects of the treatments are apparent, and the agreement between the two sets of averages is particularly good, although these effects are demonstrable to a higher level of significance in the morning results.

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The authors are indebted to Mr. A. Blackhall, Tarland, for keeping temperature records of the clamped potatoes; to the North of Scotland College of Agriculture, for the use of the shed at Craibstone; to Mr. T. Hay who carried out the sugar analyses and to other members of the staff of the Experimental Factory, Aberdeen, with whose assistance the remainder of the work was carried out.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JUNE, 1956

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

INDEX OF AUTHORS' NAMES

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JUNE, 1956

I.—AGRICULTURE AND HORTICULTURE

General : Soils and Fertilisers

Annual Report for 1954 of the National Institute of Agronomic Research, Paris. Anon. (*Ann. Agron.*, 1955, **6**, 683—958).—The Annual Report of the Institute for 1954 is presented under the main headings: climatological observations, methods and techniques, studies of soils, plants and fertilisers and miscellaneous studies. A. H. CORNFIELD.

Soil survey: Territory of Hawaii. M. G. Cline *et al.* (*U.S. Dep. Agric. & Hawaii agric. Exp. Sta.*, 1955, Series 1939, No. 25, 644 pp.). —A survey is made, with many maps, of the different types and agricultural uses of soils found in the six major islands of the Hawaii territory. H. S. R.

Soil survey. A. **Dutchess Co., N.Y.** W. Secor, L. F. Koehler, D. F. Kinsman, W. E. Benson, M. G. Cline, W. J. Moran, R. G. Leighty, G. A. Johnsgard, I. L. Martin, H. L. Donner, J. S. Hardesty, J. D. Ruffner, J. D. Sheetz, L. P. Kelsey and W. J. Latimer. B. **Mendota Area, Cal.** F. C. Harradine, R. A. Gardner, L. G. Rooke, E. A. Knecht and Ray C. Roberts (*U.S. Dep. Agric., Soil Conserv. Service*, 1955, Series 1939, No. 23, 178 pp.; 1956, Series 1940, No. 18, 96 pp.).—A survey is given of the various types, uses, management and productivity of the soils found in the areas named. H. S. R.

Soils of South-West Aquitainia, France. J. Delmas and G. Theiller (*Ann. Agron.*, 1955, **6**, 569—582).—A preliminary communication on characteristics of the soils of the region, with particular reference to textural characteristics. A. H. CORNFIELD.

Climate of Bombay State. J. A. Daji (*J. Indian Soc. Soil Sci.*, 1955, **3**, 133—152).—Rainfall, temp., R.H. and evaporation values in Bombay State are presented. Four systems of classifying climatic zones based on these values indicate that the state can be divided into three more or less well-defined zones. A. H. CORNFIELD.

Brown forest–grey wooded soil sequence in the Temiskaming district of Ontario. B. C. Matthews, D. W. Hoffman and D. J. Eagle (*Canad. J. agric. Sci.*, 1955, **35**, 538—551).—Profile characteristics, physical and chemical data from four sites are recorded. The soils probably represent segments in a hypothetical development sequence from brown forest through grey wooded to podsolised grey wooded soils. Decrease in the clay content of the parent material is associated with increase in thickness of horizons and depth of carbonates below the surface and with decrease in base saturation (%). A. G. POLLARD.

Evaluation of the stability of soil structure. S. Henin, O. Robichet and A. Jongerius (*Ann. Agron.*, 1955, **6**, 537—557).—The mechanism of the degradation of soil structure by slaking and dispersion are discussed. Methods of assessing aggregate structure are described briefly and results obtained with typical Dutch soils are presented. A. H. CORNFIELD.

Effect of synthetic soil conditioners on soil structure. R. V. Tamhane and R. K. Chibber (*J. Indian Soc. Soil Sci.*, 1955, **3**, 97—108).—Addition of 0·05—0·10% of conditioners (three formulations of hydrolysed polyacrylonitrile) to clay soils increased the size and % of water-stable aggregates and the percolation rate in the soils. Water-holding capacity was increased only slightly by the treatments. Addition of 0·1% of soil conditioner to alkali and alkali-saline soils reduced their tendency to disperse under the action of water and increased the % of water-stable aggregates present. A. H. CORNFIELD.

Effect of a synthetic polyelectrolyte on the chemical, physical, biological and agronomic aspects of some Punjab soils. A. Wahhab, A. Khabir, M. N. Azim and F. Uddin (*Soil Sci.*, 1956, **81**, 139—150). —The effect of Krilium on the chemical and physical properties of eight Punjab soils has been examined. Krilium had no effect on the quantity of sol. salts, pH level, total N, or the amounts of CO_3'', HCO_3', Cl', SO_4'' and Ca^{++}. Available P_2O_5 was increased in all soils especially in those with initially low values. Similarly, K_2O was increased in soils initially low in K, but decreased in those with high initial values. Rates of percolation were greatly increased in all treated soils, especially sandy loams. Krilium also tended to increase water-holding capacities. The rates of evaporation were little affected. Sorghum grown in Krilium-treated soil was superior in all respects to that grown in untreated soil. Highest yields were obtained when $(NH_4)_2SO_4$ was used with Krilium, but Krilium alone did not affect yields. Plants on treated soil needed more water than those on untreated, especially if the soil was coarse. When the water supply was restricted Krilium, alone or with $(NH_4)_2SO_4$, increased grain yields. On a saline soil germination of sorghum was nil on controls but good on Krilium-treated soil. Microbiological activity was increased by Krilium, especially in the presence of org. matter, but *Azotobacter* counts and nitrification of $(NH_4)_2SO_4$ were unaffected. T. G. MORRIS.

Effect of soil conditioners on crop yields. R. E. Wester (*Agric. Chemicals*, 1955, **10**, 44—46, 99).—Application of hydrolysed polyacrylonitrile or vinyl acetate–maleic acid copolymer (2000 lb. per acre) to a silt loam significantly increased yields of lettuce, carrots, lima beans and tomatoes and also increased infiltration rates. Application of carboxymethylcellulose (2500 lb. per acre) had no effect on yields and resulted in puddling of the surface inch of soil. A. H. CORNFIELD.

Diffusion of water vapour and its exchange between condensation and evaporation in soil. H. Fukuda (*Soil Sci.*, 1956, **81**, 81—85).— A theoretical discussion of the process of water vapour diffusion in soil is followed by experimental results. During a dry period of summer, air temp., R.H. and vapour pressure at different heights above the surface of bare loam and sandy soil were recorded, as well as soil temp., moisture and R.H. at different depths. Results show that R.H. in soil pores, if below 100%, shows fluctuations comparable with those of soil moisture. The quantity of water vapour in soil pores depends on exchange between diffusion, evaporation and condensation. Evaporation and condensation zones in the soil move downward from the surface in daytime and night-time respectively. T. G. MORRIS.

Factors influencing run-off. O. E. Hays (*Agric. Engng*, 1955, **36**, 732—735).—Run-off from sloping land was less where bluegrass was allowed to grow without being cut than where it was mowed. Run-off from eroded land was greater than from non-eroded land. Where annual crops were grown greater run-off occurred during the winter period than during the growing period. In a five-year rotation of maize–oats–hay (three years), soil losses were greatest whilst maize and oats were growing and were negligible under hay. Contour ploughing was much more effective than was terracing or strip-cropping in reducing soil losses. Extent of run-off was of the same order from all systems. A. H. CORNFIELD.

Determining the water needs of crops from climatic data. N. A. Halkias, F. J. Veihmeyer and A. H. Hendrickson (*Hilgardia*, 1955, **24**, 207—233).—Factors involved in determining the water requirement of crops by direct measurement of changes in soil moisture content during a rainless season are examined. Reproducible results may be obtained by this means. The difference in water evaporation from black and white Livingston atmometers is highly correlated with the use of water by crops and also with solar radiation as measured by an Eppley pyrheliometer. Date recorded illustrate the use of the atmometer method in assessing the water requirement of a no. of crops. A. G. POLLARD.

Storage pond design. D. D. Smith (*Agric. Engng*, 1955, **36**, 743—746).—Evaporation and seepage losses from two experimental irrigation water storage ponds are presented. The application of the results to the design of storage ponds is discussed. A. H. CORNFIELD.

Amperometric titration for cation-exchange capacity of soils. R. F. Holt (*Soil Sci.*, 1956, **81**, 121—124).—In the method described 0·5 to 1·0 g. of soil is placed in a Gooch crucible fitted with a disc of Whatman 542 paper; 25 ml. of 0·05N-HNO_3, 75 ml. of 0·75% aq. silver acetate, 25 ml. of 2·5% aq. $AgNO_3$ and 50 ml. of 95% ethanol are added in that order to the soil, gentle suction being applied. The soil and filter paper are transferred to a beaker, diluted with 100 ml. of water, and then treated with 0·5 g. of NH_4NO_3, 5 ml. of "conc. ammonia", and 50 ml. of 95% ethanol; 25 ml. of standard thiourea (0·005M) are added and the excess thiourea titrated with standard $AgNO_3$ (0·01M), using a rotating platinum electrode to determine the endpoint amperometrically. Results obtained correlate well (0·9945) with those obtained using the ammonium acetate method of Peech. T. G. MORRIS.

Sodium/potassium reversibility on clay membrane electrodes. S. K. Bose (*J. Indian Soc. Soil Sci.*, 1955, **3**, 109—112).—The mobility ratio of Na and K was determined using clay and resin membrane electrodes. A. H. CORNFIELD.

Adsorption of nucleic materials by montmorillonite. W. Flaig, H. Kuron and R. Kaul (*Z. PflErnähr. Düng.*, 1955, **71**, 141—154).—At pH 3·2 nucleic acid was adsorbed by montmorillonite to a somewhat greater extent than was a nucleotide. At const. pH, adsorption of adenylic acid was increased by the addition of Ca^{++} to the suspension. The adsorption of adenosine and adenylic acid by montmorillonite decreased from pH 2 to very low values at pH 6–7. The cation-exchange capacity of montmorillonite increased slightly as the amount of nucleic acid (pH 3·7) adsorbed on the clay increased. The exchange capacity of the clay decreased as the amount of neutralised nucleic acid adsorbed on the clay decreased. A. H. CORNFIELD.

New method for preparation and treatment of oriented-aggregate specimens of soil clays for *X*-ray diffraction analysis. E. B. Kinter and S. Diamond (*Soil Sci.*, 1956, **81**, 111—120).—In the method reported the film or aggregate is deposited from an aq. suspension (1—2%) by centrifuging on to a flat porous ceramic plate, 1·125 by 2·125 in. by 0·25 in. thick. The plates can be washed, or treated with reagents by suction in a suitable holder (dimensions given), and the *X*-ray diffraction patterns recorded after each treatment. T. G. MORRIS.

Some effects of irrigation water quality on soil characteristics. G. C. Lewis and R. L. Juve (*Soil Sci.*, 1956, **81**, 125—137).—Chemical analyses of irrigation waters, and irrigated and non-irrigated soils are reported. Irrigation reduced the total salt concn. and sol. and exchangeable Na % in the soils except where these values were low initially. Sol. salts were evenly distributed throughout the irrigated profiles, but in the non-irrigated profiles there was an accumulation in the lower horizons. Sol. Ca and Mg decreased with depth in 90% and 77% of the profiles respectively, but sol. Na increased in 70% with depth. In the top 36 in. of soil there was a fair correlation between the Na absorption ratio $(Na/\sqrt{(Ca+Mg)/2}$ in mequiv. per l.) of the irrigation water and the saturation extract of the soil. Good correlation was found between the chemical composition of the irrigation water and the saturation extract provided no residual Na_2CO_3 was present in the water. Increased levels of soil $CaCO_3$ were found where the irrigation water contained much Ca and bicarbonate. T. G. MORRIS.

Manuring trials on intractable recently-cleared soils. E. Bovay (*Landw. Jahrb. Schweiz*, 1955, **69**, 725—740).—Failures of various crops on a light, incoherent calcareous soil were due to poor water-retention, and deficiencies of K and (available) Mn. The adverse conditions were remedied by applications to the soil of farmyard manure and K, and by spraying the plants with aq. $MnSO_4$, the proportions of the fertilisers being based on the results of pot experiments. P. S. ARUP.

Nutrient status of some West Bengal soils as determined by rapid chemical tests. S. N. Chakravarti (*J. Indian Soc. Soil Sci.*, 1955, **3**, 83—86).—The pH and NH_4', K, P, Ca, Mg, Al, Mn and Fe status of low- and high-lying paddy soils were determined in the Morgan extracts of the soils. In general the K, Ca and Mg status of the soils were satisfactory whilst P and NH_4 status were low. The Al and Fe status were highest in the most acid soils. In general the NH_4, Mg, Al and Fe status were higher, whilst the P and Mn status were lower, in low- than in high-lying paddy soils. A. H. CORNFIELD.

Nitrogen fertilisation of crop residues. W. V. Bartholomew (*Agric. Chemicals*, 1955, **10**, 38—40, 97—99).—A critical review of the question as to whether or not N should be applied to crop residues in the field. Application of N sometimes results in increased and sometimes in reduced rate of rotting of the residues. Addition of N generally did not help to conserve org. C during decomposition of the residues. Application of N appears economically justifiable only when large quantities of plant material of low N content are ploughed in. A. H. CORNFIELD.

Liberation of amino-acids and reducing compounds by plant roots. H. Katznelson, J. W. Rouatt and T. M. B. Payne (*Plant & Soil*, 1955, **7**, 35—47).—Appreciable amounts of amino-acids and detectable amounts of reducing compounds were released from the roots of plants grown in sand or sand-soil cultures which had been allowed to dry and then remoistened a no. of times. Small amounts of amino-acids were released even under normal conditions of growth. Wheat and barley liberated considerably more amino-N than did peas, soya-beans or tomatoes. Results are discussed in relation to the incidence of amino-acid-requiring bacteria in the rhizosphere. A. H. CORNFIELD.

Effect of irrigation on the microbiological changes of the black soils of Rayalaseema. M. S. Raju and S. Varadarajan (*J. Indian Soc. Soil Sci.*, 1955, **3**, 77—82).—Microbiological no. (plate count) of and CO_2 evolution from black soils were greater in the year of irrigation than in the previous non-irrigated year. In further (irrigated) years microbiological no. fell off where no manure was applied but were generally maintained at fairly high levels by manure applications. Higher no. were maintained where dry cultivation and moderate irrigation than where wet cultivation and heavy irrigation were practised. The increase in CO_2 production after addition of glucose was inversely related to the org. matter content of the soils. Microbiological no. in fallow soil were higher than in comparable cropped soil. A. H. CORNFIELD.

Growth of mixed cultures of *Nitrosomonas* and heterotrophic soil bacteria. K. Gundersen (*Plant & Soil*, 1955, **7**, 26—34).—Observations on mixed cultures of *Nitrosomonas* and heterotrophic bacteria from enrichment cultures of the former are reported. *Nitrosomonas* and the heterotrophs grew and multiplied in close association in inorg. liquid and silica gel media and formed characteristic mixed colonies. The possibilities of symbiotic association are discussed. A. H. CORNFIELD.

Relationship between the mechanical properties of soil and the performance of simple cultivation implements. P. C. J. Payne (*J. agric. Engng Res.*, 1956, **1**, 23—50).—The mechanical strength of soil is related to the stresses on and performance of cultural implements. The cohesion of soil is a useful basis of comparison in studies of the effects of certain soil conditions on the behaviour of soil during cultivation. The theoretical aspects of such problems are considered mathematically and possible applications to implement design are indicated. Detailed consideration is given to the operation of a vertical plate line. A. G. POLLARD.

Oscillation of tillage implements. J. T. Gunn and V. N. Tramontini (*Agric. Engng*, 1955, **36**, 725—729).—Oscillation of the soil-working parts of tillage implements reduces draft and permits the use of lighter tractors. The theory behind the principle and results obtained with an experimental tractor are presented. A. H. CORNFIELD.

Soils and fertilisers. E. W. Russell (*J. roy. agric. Soc. England*, 1955, **116**, 111—125).—A short review covering the soil survey of England, top dressing of cereals with N, manuring leys and pastures with K, placement of fertilisers and the spray application of fertilisers. A. G. POLLARD.

Improving the efficiency of fertiliser dressings. F. V. Widdowson (*Agric. Rev.*, 1956, **1**, No. 11, 20—26).—A review of recent trials of the placement of fertilisers for a no. of crops. A. G. POLLARD.

Rock phosphates from various sources. L. Gisiger and H. Pulver (*Landw. Jahrb. Schweiz*, 1955, **69**, 941—960).—Solubilities in 2% citric acid of rock phosphates decrease with increasing sp. gr. (2·80—3·23), and increase with increasing contents of CO_3'', Mg or Na. All samples show (by *X*-ray microscopy) a cryst. structure, the fineness of which is probably a factor favouring solubility. The fertilising value of the samples (seven, ground to the same degree of fineness) varies very widely. Solubilities can be determined in 2% citric acid or 2% formic acid; reasons are given for preferring the former solvent. Samples can be classified according to their % yield of citric acid-sol. P, viz. >35 (soft), 35—25 (semi-hard), 25—15 (hard, and <15% (very hard). The % availability of the total P of the first three of these groups is approx. $\lessapprox$80, 60—80, and $\gtrapprox$50%, respectively. For use on acid soils, the % availability can generally be taken as double the % solubility, in 2% citric acid. With 2% formic acid as solvent, the available P in the harder samples is underestimated. P. S. ARUP.

Mixed nitrogen-phosphorus fertiliser. G. T. Gadre and J. Gupta (*J. sci. industr. Res.*, 1956, **15A**, 82—84).—A process (Indian Pat. 47,439) is described for the manufacture of mixed NP fertiliser from rock phosphate, HCl and NH_4 sulphate. The product is a free-flowing powder containing 15% of P_2O_5 (90% of which is citrate-soluble) and 7·4% of N as NH_4Cl. Results of pot culture tests with the fertiliser were satisfactory. E. M. J.

Mixing superphosphate and manure. K. H. S. Haasies and L. J. Carpentier (*Agric. Chemicals*, 1955, **10**, 49—51).—Methods of incorporating superphosphate with farmyard manure are described. The presence of superphosphate reduces losses of N during fermentation and improves the NPK balance of the rotted material. A. H. CORNFIELD.

Sewage sludge for soil improvement. M. S. Anderson (*U.S. Dep. Agric.*, 1955, *Circ.* 972, 27 pp.).—Chemical compositions of sludges variously prepared in different parts of the U.S. are reported. Only 18 to 25% of the N present in digested sludges is normally nitrified during a period of 16 weeks; activated sludges show nitrification values of 50 to 60% for a similar period. (41 references.) E. G. BRICKELL.

Plant Physiology, Nutrition and Biochemistry

Protection against frost in agriculture. J. Jenny (*Industr. aliment. agric.*, 1955, **72**, 789—794).—The discussion of crop pro-

tection against spring frosts in the earlier part of the paper (cf. J.S.F.A. Abstr., 1956, i, 249) is concluded with an account of the use of irrigation for this purpose and a comparison of the various methods now in use. J. S. C.

Effects of light quality on growth and development of plants. I. Apparatus for growing plants under controlled light and temperature conditions. D. Vince, M. G. Clarke, H. R. Ruff and R. H. Stoughton. **II. Formative effects in *Lycopersicon esculentum* and *Pisum sativum*.** D. Vince (*J. hort. Sci.*, 1956, **31**, 8—15, 16—24).—I. The design and equipment of rooms is described in which large no. of plants can be grown under controlled conditions with provision for illumination under the three main ranges of λ within the visible spectrum, and under artificial daylight. Equipment for measuring the intensity of radiation, and devices for securing uniform radiation are described.

II. On comparative cultivation of tomato (and several other) plants in red, green and blue light, total stem-, internode-, and leaf-length increase with increasing λ. Under the same conditions, *P. sativum* var. Meteor shows decreases in stem- and internode-length, and increases in leaf-length, whilst the var. Alaska shows slight response. Tomato and other plants develop leaf epinasty in completely filtered red light, but the energy of the blue light in the incompletely filtered light from the red Hg-lamp is sufficient to prevent this occurrence. The total dry matter formed is greater in red light or artificial daylight, than in green or blue light. P. S. ARUP.

Effects of electromagnetic energy on plants and animals. V. H. Baker, D. E. Wiant and O. Taboada (*Agric. Engng*, 1955, **36**, 808—812).—A general account. A. H. CORNFIELD.

Cation-exchange capacity of roots and its relation to calcium and potassium content of plants. R. L. Smith and A. Wallace (*Soil Sci.*, 1956, **81**, 97—109).—The cation-exchange capacity (CEC) of the excised roots of avocado, citrus, beans, barley and peach has been determined on an area and wt. basis. On an area basis better agreement was obtained between large and small roots. On an area basis all the citrus species examined had similar CEC values; peach and avocado had similar values but less than citrus. Two-weeks-old barley had much lower values than any other plant tested, while the CEC of 12-day-old beans was similar to that of avocado. The CEC was the same whether the roots were active or inactive metabolically. When H-saturated roots were immersed for 2 min. in amounts of either 0·002N-$CaCl_2$ or -KCl equivalent to the CEC as regards Ca or K content, the ratio of the amount of Ca adsorbed to that of K was less for citrus than for beans and cereal; in all cases more Ca than K was adsorbed. There was no correlation between the Ca/K ratio and the Ca or K content of the plants. T. G. MORRIS.

Uptake of anions and cations by sunflower grown in sand cultures. K. Scharrer and J. Jung (*Z. PflErnähr. Düng.*, 1955, **71**, 97—113).—An extensive paper reporting the effects of varying the nutrient supply on the uptake of anions and cations by sunflower grown in sand culture. A. H. CORNFIELD.

Uptake of nutrients by mature forest growth. P. J. Rennie (*Plant & Soil*, 1955, **7**, 49—95).—Estimates of the uptake of Ca, K and P by one acre of timber-exploited "hardwood", "other conifer" and "pine" forests after periods of 50 and 100 years are presented. The pedological and silvicultural significance of the results are discussed. On nutrient-poor (e.g. moor) soils the relatively large uptake of nutrients in comparison with the nutrient status of the soils will result eventually in diminished site-productivity and overall soil degradation. A. H. CORNFIELD.

Moisture content and enzyme activities in barley seedlings in relation to their growth rate. G. Jansson (*Ark. Kem.*, 1956, **9**, 139—145).—The seeds grown at room temp. for four days in moist sand in darkness were examined for moisture content, respiration capacity, activities of catalase, dehydrogenase and β-amylase and for growth rate (defined as the wt. of coleoptile formed per mg. of kernel dry matter per four days). The water-absorbing capacity was in high correlation with the growth rate. Of the enzyme activities the dehydrogenase activity was in strongest correlation with growth rate, followed by catalase, respiration and β-amylase, in that order. E. M. J.

Lactones and metabolism of certain plant tissues. Influence of δ-hexenolactone and α, β-angelicalactone on some phases of metabolism of (*i*) pea stem sections, (*ii*) potato slices. H. W. Buston and C. M. Mehta (*J. Maharaja Sayajirao, Univ. Baroda*, 1955, **4**, 119—123).—(i) Sections 15 mm. long, cut from the most apical part of the third internode of pea seedlings 10—12 days old, blotted dry and weighed, were grown in water, and the following solutions each of hexenolactone and angelicalactone: 5×10^{-5}M; 5×10^{-4}M; 5×10^{-3}M and 5×10^{-2}M. Hexenolactone inhibited growth more actively than did angelicalactone. Respiration was inhibited by each lactone. (ii) Using potato discs of 0·75 mm. thickness and 10 mm. diameter, hexenolactone had a greater inhibitory action on growth than had angelicalactone and there was indication that the substrate was used for respiration. In pea stems there was decrease in tissue metabolism during periods of 1—4 hr. and of 16—20 hr., more marked with hexenolactone than with angelicalactone. With potato the equilibrium fat $\rightleftarrows$ carbohydrate or acid is shifted to the right at high concentrations of the lactones by disturbance of the enzyme systems involved; soluble compounds formed diffuse away. (20 references.) E. M. J.

Vitamin C and other reducing agents in vine leaves during the period of vegetation. A. F. Damanski and S. G. Stanimirović (*Bull. Soc. chim. Belgrade*, 1955, **20**, 133—139).—A new procedure for determining reducing agents is described and applied to the analysis of vine leaves at various stages of development. The level of reducing matter in the mesophyll depends primarily on the stage of growth. It is highest during fruit formation and the values attained cannot be explained by the action of catalytic compounds alone. The ratio of reducing agents in the veins of leaf and stem is proportional to that in the mesophyll; this depends on the intensity and duration of solar light and varies in accordance with the contents of L-ascorbic acid. The level of reducing matter is also considerable in the final growth stage, especially during senescence. Appreciable quantities of dehydroascorbic acid are found only during initial and final vegetative phases and towards the end of flowering. Some dehydroascorbic acid is always present in the lower leaves, while its appearance in the higher parts is sporadic. The correlation between dehydroascorbic and L-ascorbic acid depends not on the redox system, but on a direct reduction of dehydroascorbic acid, which, in turn, depends on the intensity and duration of sunlight. The level of reducing sugars in the mesophyll is higher during flowering than during the ripening periods of fruits. L. S.

Influence of high concentrations of ammonium and sodium molybdates on flax, soya-bean and peas, grown in nutrient solutions containing deficient or excess iron. K. Warington (*Ann. appl. Biol.*, 1955, **43**, 709—719).—Ammonium molybdate (Mo, 20—40 p.p.m.) in the nutrient solution prevented chlorosis caused by low Fe supply in young flax plants, whilst Na molybdate was effective in preventing chlorosis only at concn. of 40 p.p.m. (Mo). Symptoms of Mo toxicity always developed at levels of 40 p.p.m., whether or not the intensity of chlorosis was reduced. A reduction in Mo toxicity symptoms occurred in soya-beans and peas with increasing Fe supply. In flax the higher level of Fe was excessive unless combined with Mo (40 p.p.m.). High Mo counteracted both Fe deficiency and toxicity in flax. High Fe reduced the Mo content of both shoot and root in soya-beans and peas and also in flax providing Fe was not excessive. High Mo supply usually reduced the Fe content of the shoot and markedly increased it in the root. A. H. CORNFIELD.

Incidence of trace element supply on crop growth. N. H. Pizer (*J. roy. agric. Soc. England*, 1955, **116**, 68—78).—A summary of problems associated with deficiency and/or excess of Fe, Mn, Cu, Zn, B and Mo. A. G. POLLARD.

Rapid colorimetric estimation of soil copper employing a sodium diethyldithiocarbamate–salt mixture. S. N. Edson and L. E. Watson (*Chemist Analyst*, 1955, **44**, 94—95).—The colorimetric method described for estimating Cu in soils is rapid and simple. Extracts of the soil in Na acetate solution are tested with a dry Na diethyldithiocarbamate–NaCl mixture in the presence of a buffer solution which prevents interference from Fe and Ca. The colour developed is compared against standards or the colour density is obtained by a photometer with a blue filter, the colour following Beer's law for 0·2 to about 10 p.p.m. of copper. The blue filter prevents interference from Mn in alkaline solution. Minute amounts of Ni, Co and Bi in soil extracts scarcely interfere. O. M. WHITTON.

Manganese content of phanerogams. G. Bertrand and L. Silberstein (*Ann. Agron.*, 1955, **6**, 523—535).—The Mn contents of the above-ground portion of 501 samples representing 462 species of phanerogams are presented and discussed. A. H. CORNFIELD.

Iron chelates in agriculture. F. E. Bear (*Agric. Chemicals*, 1955, **10**, 34—35, 107—109).—A general account of the use of chelated trace elements, with special reference to Fe, for the control of deficiencies of these elements in plants. A. H. CORNFIELD.

Growth-phenomena of *Proteus vulgaris*, Hauser under influence of penicillin in high concentration. F. Radler and A. Rippel-Baldes (*Arch. Mikrobiol.*, 1956, **23**, 400—411).—A morphological study is made of the transition of the normal into a spherical form of *P. vulgaris*, under the influence of penicillin (500 i.u. per ml.) in a simple (serum-free) liquid medium. The spherical cells show no form of viability. All the evidence obtained indicates the transition to be degenerative in character. (33 references.) P. S. ARUP.

Physiology of rhodomycin production. W. Frommer (*Arch. Mikrobiol.*, 1956, **23**, 385—399).—The fermentation of a medium containing glycerol, $NaNO_3$ and nutrient salts by *Streptomyces purpurascens* is accompanied by a rise in pH; this is followed by a decrease after the exhaustion of the $NaNO_3$, provided that sufficient glycerol remains available. The formation of rhodomycin and *iso*rhodomycin is appreciably affected by the C : N ratio, the optimum value for which varies with each of the four strains under examination. Production of *iso*rhodomycin (unlike that of rhodomycin) is promoted by lowering the pH of the medium. Symptoms of degeneration are described, and possible methods for achieving regeneration are discussed. (22 references.)
P. S. Arup.

Growth of mucus-forming bacteria in drip-feed irrigation lines. R. B. Sharp (*J. agric. Engng Res.* 1956, **1**, 83—88).—Tubing of polyvinyl chloride used to distribute nutrient solutions favoured the growth of mucus-forming organisms. The jelly-like material formed restricted or blocked the pipe-line nozzles in a few weeks. Addition of NaClO to the nutrient (Cl = 0·5 p.p.m. initially to give traces at the end of the line) maintained the irrigation system in working order but ClO_3' formed by decomposition of $NaClO_3$ was deleterious to tomato plants. Sterilisation of the nutrient by the Catadyn process (finely divided Ag) gave good results. A. G. Pollard.

An integrating photometer. E. S. Trickett and L. J. Moulsley (*J. agric. Engng Res.*, 1956, **1**, 1—11).—The construction and operation of a simple and cheap photometer are described. Light intensities and their integration with time are determined more effectively by this means than by the Campbell Stokes Sunshine Recorder.
A. G. Pollard.

Disintegrator for small quantities of fresh leaves. N. W. Pirie (*J. agric. Engng Res.*, 1956, **1**, 81—82).—Batches of leaves up to 40 g. are placed in a cylindrical vessel and pressure is applied to a plunger sufficient to force the material through a fine annular slot, the size of which can be adjusted. No fluid addition to the leaves is necessary and the material can be recovered quantitatively.
A. G. Pollard.

Effect of fruit thinning on quality of apples and biennial fluctuations in bearing. W. Wurgler and P. Aubert (*Landw. Jahrb. Schweiz*, 1955, **69**, 809—814).—A thinning of the fruit of Belle de Boskoop at petal-fall by the use of 1-naphthylacetic acid (at 8 g. per 100 l.) prevents biennial fluctuation in bearing during the subsequent three seasons. Thinning of Golden Delicious (using 5 g. of the same substance per 100 l.), 10 days after petal-fall increases the size of the fruits without detriment to the foliage or the keeping quality of the apples on cold storage. P. S. Arup.

Crops and Cropping

Response of wheat to nitrogen fertilisation. J. J. Chandnani and A. G. Kavitkar (*J. Indian Soc. Soil Sci.*, 1955, **3**, 123—131).—Wheat yields increased with the amount of N applied (10—100 lb. per acre), but the extent of increase varied with season. The responses to N were somewhat greater where P + K was applied. The N content of the grain increased with N application up to 80 lb. of N per acre. The N content of the grain varied considerably from year to year. Net financial returns increased with the amount of N applied and were greater where P + K was not applied.
A. H. Cornfield.

Effects of heat treatment on the viability of rice. V. H. McFarlane, J. T. Hogan and T. A. McLemore (*U.S. Dep. Agric.*, 1955, *Tech. Bull.* 1129, 51 pp.).—*Oryza sativa* [both medium-grain (Sel. 61-25-12 and Zenith) and long-grain (Rexoro, Bluebonnet and Century 52)] was tested for the effects of time and temp. and of moisture content on its viability. The range between the temp. at which heat damage to viability just starts and the temp. at which it is complete, is a narrow one (approx. 8—12°). This range is unaffected by moisture content but is higher on the temp. scale the lower the moisture content of the rice. Resistance to heat varies inversely as moisture content. (91 references.) E. G. Brickell.

Maize yields in relation to the preceding crop in the rotation in the Pusa permanent manurial experiment (new series). S. Sen and A. G. Kavitkar (*J. Indian Soc. Soil Sci.*, 1955, **3**, 113—121).—Maize yields as affected by fertiliser treatment and the nature of the preceding crop in four-year eight-course rotations over 20 years are reported for this calcareous alluvial soil. Rape cake supplying 40 lb. of N per acre gave the highest yields in all years and was more effective than were farmyard manure or $(NH_4)_2SO_4$ supplying the same amount of N. Farmyard manure, inorg. N + P + K, and inorg. N + P gave significantly better yields than did P, whilst K had no effect. Higher yields were obtained when maize was grown after gram than when grown after oats, wheat or peas. Yields of maize over the 20 years deteriorated with time under all systems of fertilisation.
A. H. Cornfield.

Harvesting maize by combine. L. W. Hurlbut (*Agric. Engng*, 1955, **36**, 791—800, 802).—Results of laboratory and field trials in adapting the grain combine to the harvesting of maize are presented.
A. H. Cornfield.

Applicability of the cultural precontrol (Florida) test for potatoes in Switzerland. J. Münster (*Landw. Jahrb. Schweiz*, 1955, **69**, 741—751).—The breaking of dormancy immediately following harvesting is best achieved by the Rindite treatment, followed by steeping during 1 hr. in aq. 0·8% NH_4SCN; the subsequent culture of the tubers is, however, impracticable in Switzerland, due to the occurrence of early autumn frosts. P. S. Arup.

Frost damage to potatoes. II. T. Voss (*Phytopath. Z.*, 1956, **25**, 225—254; cf. J.S.F.A. Abstr., 1956, i, 252).—Resistance to frost damage depends on the capacity of the sap for supercooling, which can be measured by means of the "striking test," viz. the no. of min. elapsing between the striking of the tuber and the freezing of the sap. No relation is found between the capacity for supercooling and the size, ripeness or sp. gr. of the tuber, but varietal differences are observed in this respect. The capacity for supercooling is promoted by K-manuring, and depends directly on the org. and inorg. contents of the sap, which can be estimated refractometrically. Locally damaged tubers can be stored without the appearance of further symptoms, but show diminished sprouting and subsequent growth. Damaged vascular tissue is sealed off by cork-formation. (51 references.) P. S. Arup.

Grass for conservation in the Southern Great Plains. B. W. Allred and W. M. Nixon (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 2093, 30 pp.).—Advantages, kinds of grasses, seed supplies, seeding crop lands and range lands, and management of grasslands are discussed.
E. G. Brickell.

Seeding summer ranges in E. Oregon and Washington. R. S. Rummell and C. E. Holscher (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 2091, 34 pp.).—Choice of site, selection of species, mixtures and rates of seeding, preparation of seed beds, planting and grazing are discussed. Details are given for seeding particular range types, e.g., sage brush-grass, pine zones, mountain meadows, subalpine grasslands. E. G. Brickell.

Growing fruits and nuts in the Southern Great Plains. L. F. Locke (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 2087, 28 pp.).—Climatic effects, orchard location, irrigation, planting, cultivation, training and pruning, diseases and insects and their control, are discussed, together with details of the kinds and varieties of both fruits and nuts. E. G. Brickell.

Foliar analysis results from 40 Connecticut orchards. F. H. Emmert (*Conn. agric. Exp. Sta.*, 1955, *Bull.* 17, 18 pp.).—The total N, P, K, Ca and Mg contents of leaf samples from 40 apple orchards are reported. Results indicated that N and P nutritional levels were generally satisfactory. About 25% of the trees showed low and about 25% high K nutritional level. About 50% were low in Mg and Ca. Results of soil analyses are also presented.
A. H. Cornfield.

Nitrogen content of Cox's Orange Pippin apples in relation to manurial treatment. A. C. Hulme (*J. hort. Sci.*, 1956, **31**, 1—7).—Total- and protein-N contents of the fruit are increased by $(NH_4)_2SO_4$ manuring, and further increased by additional K_2SO_4 manuring. The N content is lower from trees on grassed-down than from trees on cultivated plots. Total N contents vary widely, but with N contents of 30 mg. per 100 g. of fresh wt., most of the N is generally present in the form of protein; with increasingly higher N contents, the sol. N increases ~ twice as rapidly as the protein-N.
P. S. Arup.

Effects of controlled root temperatures on growth of East Malling rootstocks in water culture. S. H. Nelson and H. B. Tukey (*J. hort. Sci.*, 1956, **31**, 55—63).—In experiments with five rootstocks of clonal and one of seedling origin, the temp. of the nutrient solution, but not the air temp. was varied. The onset of shoot and root growth was greatly accelerated in all cases by a rise in temp. from 6·7 to 25°, but three of the stocks failed to tolerate or survive temp. >12·8°. This group showed max. root growth at 12·8°, and severe browning and rupturing of the cortex at 18·9° or 25°. The other three stocks showed max. root growth at 25°, with considerably less damage to the cortex. Further morphological effects of variation in root temp. are described. High soil temp. are probably the cause of failure of some of the rootstocks in parts of U.S.A. (30 references.)
P. S. Arup.

Utilisation of poultry manure in strawberry production. C. W. Hitz (*Delaware agric. Exp. Sta.*, 1955, *Tech. Bull.* 312, 29 pp.).—Strawberries responded well to poultry manure applied the autumn before spring planting. Applying the manure just before planting sometimes caused excessive plant mortality and yield reductions. Autumn application (5—10 tons per acre) gave yields superior to those obtained with recommended applications of commercial

fertilisers. Application of fertiliser after planting had little effect on yields. Rainfall was the most important factor influencing yields from one year to another and variable responses to manuring were considered to be due to the variation in rainfall.
A. H. CORNFIELD.

Chemical composition of stone cells present in guava. R. Santini, jun. and N. Nadal (*J. Agric. Puerto Rico*, 1955, **39**, 172).—The stone cells present in guava fruit contained ash 0·45—0·66, protein 3·39—4·66, lignin 37·42—42·72, fat 0·24—0·55, cellulose 52·42—59·45 and crude fibre 55·00—60·70%. A. H. CORNFIELD.

Fourteen-year study of vegetable crop rotations on Merrimac fine sandy loam soil in Connecticut. B. E. Janes, W. O. Drinkwater, G. Beall, J. Scarchuk and J. M. Lent (*Conn. agric. Exp. Sta.*, 1955, *Bull.* 319, 55 pp.).—Results obtained with a variety of rotations involving 10 vegetable crops and three green manure crops are reported. Lowest yields were obtained where two vegetable crops were grown in one season without a green manure crop. Best yields were obtained when a winter cover crop was ploughed in every year and a summer crop of soya-beans incorporated every fourth year. A crop of sweet maize followed by a winter cover crop of vetch had a profound inhibitory effect on the growth of crops of lettuce and onions in the following spring. A slight inhibitory effect was noted on the growth of beets. The inhibitory effect disappeared as the season advanced. The org. matter content of the soils declined to about the same extent under all rotations. A. H. CORNFIELD.

Improved cucumber yields resulting from soil aëration. H. Fröhlich (*Dtsch. Gartenb.*, 1955, **2**, 21—23).—An increase (>16%) in yields of glasshouse cucumbers was obtained by aërating the soil with straw. Addition of stable manure produced only the same increase as did aëration with straw in absence of manure.
HORT. ABSTR. (A. G. P.).

Increasing the number of flowers in beans and tomatoes by means of 2 : 3 : 5-triiodobenzoic acid. C. J. Gorter (*Meded. Dir. Tuinb.*, 1955, **18**, 35—41).—Watering bean plants with 2 : 3 : 5-triiodobenzoic acid of various concn. increased flower production, wt. and no. of beans and (slightly) the total leaf area. With tomato plants increased flower production was obtained at the expense of vegetative growth. HORT. ABSTR. (A. G. P.).

Growing pumpkins and squashes. R. C. Thompson, S. P. Doolittle and D. J. Caffrey (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 2086, 30 pp.).—Climatic and soil requirements, varieties, culture, curing and storing, insects and control, diseases and control, are discussed.
E. G. BRICKELL.

Boron nutrition of hop. E. G. Cripps (*J. hort. Sci.*, 1956, **31**, 25—34).—The B content of hop leaves increases with maturity. Cutting of the bines at harvesting time does not affect the future nutrient or cropping status of the plant. In sand cultures, a supply of 0·5 p.p.m. of B is adequate, but the plants tolerate 2 p.p.m. Deficiency symptoms are accentuated by high Ca-treatment. Toxicity symptoms occur with 10 p.p.m. of B. The above symptoms are described A complete account is given of a field trial in which deficiency symptoms were remedied by treatment with borax. P. S. ARUP.

Mushroom growing in the U.S. E. B. Lambert (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 1875, 12 pp.).—Sites for mushroom growing preparation of compost, pasteurising, growing procedure, harvesting and marketing, drying and freezing, costs, and returns are discussed.
E. G. BRICKELL.

Influence of water supply on the flax plant. E. J. Schobert and K. C. Menzel (*Faserforsch. u. Textiltech.*, 1955, **6**, 539—545).—A study is made of the effects of water shortage at various stages of plant growth on the relative extents of fibre, rind and wood formation in the flax stem, the results of the studies being illustrated by photomicrographs of the plant stem and by tables which include the fibre properties of the ripe flaxes. The following main conclusions are reached. Water deficiency has the greatest effect between germination and the onset of flowering. Stunting of growth by water deficiency is more marked with oil-fibre flax than with fibre flax plants. Lack of water during growth increases the wood sheath, lessens the number of fibre cells in the stalk and in the fibre bundle, enlarges the cross-sectional area of the fibre cell, and reduces the fibre yield. Drought conditions result in a weak wood sheath, small fibre cells, strengthened cuticula, and greatly lowered fibre yield. Lack of water up to the onset of flowering with ample water from onset of flowering up to ripening lengthens the flowering period. Low water supply after flowering (with ample water up to flowering) probably lessens the wood sheath and increases the fibre yield. The behaviour of different flax plants (oil-fibre type and fibre types) to water deficiency during growth follows the same pattern but oil-fibre types of plant are more affected by water shortage than is the fibre type of flax plant. H. L. WHITEHEAD.

Co-operative Hevea rubber development programme in Latin America. R. D. Rands and L. G. Polhamus (*U.S. Dep. Agric.*, 1955, *Circ.* 976, 79 pp.).—A 14-year summary of major activities and results from a co-operative research and development programme to establish a rubber-producing industry in Bolivia, Brazil, Colombia, Costa Rica, Dominican Republic, Guatemala, Haiti, Mexico and Peru, is presented. (101 references.) E. G. BRICKELL.

Hardwood tree planting experiments on strip mine spoil banks of Indiana. D. DenUyl (*Purdue agric. Exp. Sta.*, 1955, *Bull.* 619, 16 pp.).—Tests with 10 species of hardwood over five years at a no. of locations are reported. Green ash showed good survival and growth on all but the most acid or toxic spoil, whilst sweet gum also grew well on acid spoil. Cottonwood showed the best initial growth on calcareous spoil. Sycamore survived and grew best on calcareous and slightly acid spoil. Tulip poplar and black walnut were satisfactory only for calcareous spoil, whilst ailanthus and red maple were unsatisfactory for spoil bank planting. A. H. CORNFIELD.

Pest Control

Climate host and parasite in crop disease. J. Grainger (*Quart. J. roy. met. Soc.*, 1955, **81**, 80—88).—Significant or near-significant correlations show that a low incidence of disease is associated with a relatively high soil temp. in July, relatively high amounts of bright sunshine in June, July and Sept., relatively low rainfall in Sept. and relatively high rainfall in Oct.
HORT. ABSTR. (A. G. P.).

Effects of insecticides on flavour and quality of food products. V. R. Boswell (*J. econ. Ent.*, 1955, **48**, 495—499).—A review.
A. A. MARSDEN.

Fungicide tests, 1954. D. A. Roberts (*Agric. Chemicals*, 1955, **10**, 53—59, 125, 127).—An annual summary of a large no. of reports of tests of chemicals for the control of fungus diseases of ornamental and vegetable plants. A. H. CORNFIELD.

Insect life in the soil. H. W. Miles (*J. roy. hort. Soc.*, 1956, **81**, 127—135).—A review. E. G. BRICKELL.

Effect of seed-dressing materials against soil fungi. I. K. H. Domsch (*Phytopath. Z.*, 1956, **25**, 311—322).—The formation of inhibition zones against *Rhizoctonia solani* from seeds coated with methoxyethyl-Hg silicate containing 20% of γ-$C_6H_6Cl_6$ is observed by the author's technique for cultivating soil fungi on soil-like media (cf. *Arch. Mikrobiol.*, 1955, **23**, 79). Effective seed protection with the use of a fungicide (such as the above) which becomes active only after dissolution in the aq. phase of the soil, depends on the relative freedom of the soil from adsorbent material, the size of the seed (peas must be <3—4 mm. in diameter), and the use of the fungicide in quantities more than sufficient for surface sterilisation of the seed. The fungicidal effect is independent of soil temp. or moisture. A simple method is described for comparing the adsorption of fungicides based on Hg by various soils. In some cases, the fungicide may diffuse from the seeds in concn. insufficient to destroy *R. solani*, but sufficient to destroy less resistant fungi. The effectiveness of the above and other fungicides is much greater in sand than in compost.
P. S. ARUP.

Metabolism of *Fusarium lycopersici*, Sacc. investigated with the aid of radioactive carbon. B. D. Sanwal (*Phytopath. Z.*, 1956, **25**, 333—384).—Reduction by 50% of the concn. of glucose and of NH_4NO_3 in Richard's medium causes a virulent strain of the fungus to produce toxins in greater amounts than those produced by a non-virulent strain, thus reversing the effects produced in the normal medium. With glycine (2·5%) as the sole source of C, the fungus produces all the three known toxins. In the biosynthesis of fusaric acid, certain amino-acids probably play an important part. After labelling the fungus by cultivation in a medium containing 90 μg. of 2-^{14}C-glycine per ml., ~40% of the cells become non-viable, but the fungus retains its virulence. The labelled mycelium when inoculated into tomato plants, secretes certain radioactive toxins which cause vein-clearing and vascular browning. The labelled virulent and non-virulent mycelia secrete, *in vivo*, compounds of various types, the amount of proteins secreted by the former being more than four times that secreted by the latter. In tomato cuttings, ^{14}C-fusaric acid is decarboxylated (with the formation of $^{14}CO_2$) and otherwise metabolised into compounds of various types, which are chromatographically examined. A proportion (depending on the amount introduced) of the acid remains unchanged in the plant. Probable modes of action of the toxins produced by the fungus in the plant are discussed in detail. (97 references.) P. S. ARUP.

Chelating action of some wilt-toxins. IV. Changes in toxicity due to increasing saturation with various heavy-metal ions. E. Gäumann and St. Naef-Roth (*Phytopath. Z.*, 1956, **25**, 418—444; cf. *ibid.*, 1955, **24**, 373).—Metallic ions are added in increasing concn. to solutions of Complexone III (**I**) and of lycomarasmin (**II**) before administration to tomato shoots. Increasing additions of the (non-toxic and weakly complexing) Mg^{++} ion to solutions of **I** or **II** have

no effect on the injury caused to the shoots. The Mn^{++} ion (in the non-toxic concn. used here) reduces (by $>61\%$) the toxicity of **I**, but not that of the weaker complexing agent **II**. The strongly complexing Co^{++}, Ni^{++} and Cu^{++} ions cause, due to chelating effects, in $>$ equimol. concn. with respect to **I** or **II**, considerable reductions in toxicity, but in higher concn. exert marked toxicity on their own account. The complexing capacity of the Fe^{+++} ion is weak, but its toxicity is of a high order. Symptoms of injury appear suddenly within a narrow range of low conc. of Fe^{+++} added to solutions of **II**. This critical effect is probably due to the gathering, from the tissues, of Fe^{+++} during passage through the stem, followed by decomplexing and consequent flooding injury in the leaves, a process which is probably assisted by the photosensitivity of the Fe^{+++}–**II** complex. P. S. ARUP.

Validity of the insecticide check method as a measure of the effectiveness of natural enemies of diaspine scale insects. P. DeBach (*J. econ. Ent.*, 1955, **48**, 584—588).—Repeated light applications of low-dosage DDT sprays did not increase populations of Californian red scale, *Aonidiella aurantii*, or of yellow scale, *A. citrina*. Relative increases occurred only when the effective natural enemies were selectively eliminated by DDT residues. Probably DDT caused some scale mortality and did not completely eliminate natural enemies. The insecticidal check method tends to give a conservative rather than an exaggerated measure of the effectiveness of natural enemies of these scales. A. A. MARSDEN.

Laboratory tests of the effect of insecticides on some beneficial insects. F. H. Harries and A. C. Valcarce (*J. econ. Ent.*, 1955, **48**, 614).—Of eleven insecticides tested against the convergent lady beetle (*Hippodamia convergens*), the striped collops (*Collops vittatus*) and the spotted lady beetle (*Coleomegilla maculata*), parathion, Malathion, Chlorthion and Diazinon were significantly more toxic than were the other materials tested, especially to the two lady beetles. Perthane, Strobane and Endrin had little toxicity. The striped collops was considerably more resistant to all insecticides tested than were the two lady beetles. A. A. MARSDEN.

Toxicity of five organic insecticides to resistant and non-resistant strains of *Blattella germanica* (L.). W. L. Butts and R. H. Davidson (*J. econ. Ent.*, 1955, **48**, 572—574).—Five insecticides were introduced into the hæmocœle by a micro-injection apparatus, using cockroaches of normal and chlordane-resistant strains. Results with normal cockroaches showed the following order of descending toxicity: lindane, Dieldrin, heptachlor, Aldrin, chlordane. For resistant roaches the order was: lindane, Dieldrin, Aldrin, heptachlor, chlordane. The resistant cockroaches showed some resistance to all the compounds, and especially towards heptachlor. A. A. MARSDEN.

Comparison of certain organic insecticides as sprays or baits against *Blattella germanica* (L.). S. Husain and F. W. Fisk (*J. econ. Ent.*, 1955, **48**, 576—578).—Aldrin, Dieldrin, Endrin, Isodrin, heptachlor, Diazinon and Bayer L13/59, were tested in the laboratory as sprays or baits against adult females of *Blattella germanica*. Aldrin, both as a bait and as a spray, was the most toxic; heptachlor was more effective in a bait than when sprayed, whilst Isodrin was much less effective in a bait. A. A. MARSDEN.

Agricultural emulsifiers. G. L. Brown and G. C. Riley (*Agric. Chemicals*, 1955, **10**, 34—36, 89—91).—Methods of testing emulsifiers to indicate their suitability for admixing with pesticides are described. A. H. CORNFIELD.

Effect of repeated spraying of insects on their resistance to insecticides. III. Conditioning by the administration of sublethal concentrations. F. Tattersfield and J. R. Kerridge (*Ann. appl. Biol.*, 1955, **43**, 630—644).—Treatment of *Drosophila melanogaster* with CO_2 in sublethal concn. or for sublethal periods of time increased the resistance of the organism to the toxic effects of the gas, although the effect was not permanent. Successive spraying with sublethal concn. of $C_6H_6Cl_6$ did not increase the resistance of *D. melanogaster* to the effects of this chemical. There was no evidence that an increased resistance to DDT arose from repeated conditioning treatments with DDT, either in the adults or their progeny, providing the conditioning concn. were kept at a level sufficiently low not to give rise to selection of less susceptible strains or mutants within the stock. Application of DDT in sublethal doses to adult insects reduced the amount and/or rate of egg laying. A. H. CORNFIELD.

Codling moth resistance to DDT in New York. E. H. Glass and B. Fiori (*J. econ. Ent.*, 1955, **48**, 598—599).—A strain of codling moths in one New York orchard developed resistance to several insecticides, and particularly to DDT. Codling moths from two other New York orchards showed a marked difference from this strain in their response to insecticides. A. A. MARSDEN.

DDT residues in fat from steers pastured on maize stover in DDT-treated fields. J. E. Fahey, T. A. Brindley and M. L. Spear (*J. econ. Ent.*, 1955, **48**, 606—607).—Maize fields receiving DDT sprays (1·5 lb. per acre) were harvested and steers were subsequently allowed to graze on the maize stubble for two months. Fat from these animals contained DDT (1·0 to 2·8 p.p.m. after the first month increasing to ~5 p.p.m. after two months). DDT residues in fat rapidly diminished when the animals were removed from the treated fields and fed a DDT-free diet, until $>0{\cdot}1$ p.p.m. of DDT was present. A. A. MARSDEN.

Residue studies of toxaphene, parathion and Malathion on some Florida vegetables. R. E. Waites and C. H. van Middelem (*J. econ. Ent.*, 1955, **48**, 590—593).—The amounts of residue from toxaphene, parathion and Malathion treatments on tomatoes, southern peas and okra are determined. Malathion and parathion residues on cabbage and turnip greens were quickly decomposed. Factors influencing the amounts of residue on these crops are discussed. A. A. MARSDEN.

Evaluation of dust deposits by polarography. N. T. Ban and W. M. Carleton (*Agric. Engng*, 1955, **36**, 803—805).—Details of the method are described, with particular reference to the determination of Cu residues on plant materials. A. H. CORNFIELD.

Some effects of insecticide seed treatment on dent maize. K. J. Starks and J. H. Lilly (*J. econ. Ent.*, 1955, **48**, 549—555).—Acetone as a solvent for the application of lindane to seed maize caused reduction in both stand and yields. Methyl cellulose as a sticker caused no injury under field conditions and was superior to dry applications of lindane. Captan was compatible with lindane, heptachlor and Diazinon at various dosages and also significantly increased the stands in treatments which included this fungicide. Heptachlor, Dieldrin and lindane protected germinating seed maize against wireworms at 1 oz. of toxicant per bushel of seed, and were effective against seed-attacking beetles at 0·5 oz. per bushel. Diazinon killed about as many wireworms but failed to prevent damage to the seed, whilst toxaphene was only slightly effective against wireworms. A. A. MARSDEN.

Certain species of *Chaetocnema*. F. W. Poos (*J. econ. Ent.*, 1955, **48**, 555—563).—*C. pulicaria* was the most efficient vector of bacterial wilt of maize caused by *Aplanobacter stewarti*. A DDT emulsion spray or dust gave the most effective control of this pest: several applications, beginning when the maize is coming through the ground, are recommended. A. A. MARSDEN.

Germination of seed maize treated to control soil insects. G. E. Gould (*Indiana agric. Exp. Sta.*, 1955, *Bull.* 624, 12 pp.).—Seed treatments protected the germinating seed, but were of little value in preventing wireworm attacking the young plants. $C_6H_6Cl_6$ (**I**) and Dieldrin were the most effective of the materials tested for protecting germinating seed from soil insects. When **I** was mixed with seed (<2 oz. per 100 lb. of seed) there was no noticeable injury to seedlings in field trials. Storage of treated seed had no effect on germination or growth of the plants. A. H. CORNFIELD.

Penetration of the foliage canopy of maize and potatoes by aerial spray. C. E. Deonier (*J. econ. Ent.*, 1955, **48**, 629).—Sprays of water containing a tracer dye were used to determine how much of a spray applied by an aeroplane penetrated downwards through the foliage canopy. On maize 42% as much spray penetrated to the silk and 11% as much to the ground as to the tassel level. With potatoes 37% as much spray penetrated to the hill level and 31% as much to the furrow level as to the vine top. The effective insecticide coverage, particularly for aphids on potatoes, was much less than these figures suggest, since most of the spray reaching the lower leaves is deposited on the upper surfaces whereas the aphids are on the undersides of the leaves. A. A. MARSDEN.

Effect of sucrose spraying on symptoms caused by beet yellows virus in sugar beet. M. A. Watson (*Ann. appl. Biol.*, 1955, **43**, 672—685).—The yellowing symptoms of sugar-beet plants infected with beet yellows virus were increased by spraying the plants daily with 10% aq. sucrose. Shading the plants depressed the extent of yellowing and this depression was reduced by the sucrose treatment. Spraying with sucrose increased the carbohydrate content of the leaves of both shaded and unshaded plants. Spraying increased the yield of roots of healthy and infected plants, most of this increase being accounted for by increasing sucrose content of the roots. A. H. CORNFIELD.

DDT tolerance by lygus bugs on seed lucerne. L. A. Andres, V. E. Burton, R. F. Smith and J. E. Swift (*J. econ. Ent.*, 1955, **48**, 509—513).—At harvest time the lygus bugs in lucerne seed fields were 3—4 times as tolerant of DDT as were those from untreated lucerne fields or those at the beginning of the season. Although some indications of increased tolerance to toxaphene were noted, this insecticide is recommended instead of DDT in resistant areas especially from mid to late season and in the desert regions. A. A. MARSDEN.

Control of the lucerne weevil with heptachlor. Anon. (*Agric. Chemicals*, 1955, **10**, 49).—Application of heptachlor sprays (4 oz. active ingredient per acre) in mid-April gave very good control of the lucerne weevil. A. H. CORNFIELD.

Meadow spittlebug control by pre-emergence treatment. J. T. Medler (*J. econ. Ent.*, 1955, **48**, 593—595).—Granulated $C_6H_6Cl_6$ (1·2% γ-isomer) applied to lucerne fields before hatching of the eggs effectively controlled nymphs of spittlebugs. Granulated Dieldrin and heptachlor at the same dosage were rather less effective. Pre-emergence applications of granulated insecticides should greatly reduce residue hazards. A. A. MARSDEN.

Control of insects on hairy vetch. N. Weaver and C. F. Garner (*J. econ. Ent.*, 1955, **48**, 625—626).—A single treatment of toxaphene (2 lb.)–Demeton (0·125—0·25 lb.) per acre applied in early May to vetch gave very promising control of lygus bugs, chiefly *Lygus lineolaris*, and pea aphids, *Macrosiphum pisi*, with min. damage to pollinating insects. A. A. MARSDEN.

Codling moth control. D. W. Hamilton (*Agric. Chemicals*, 1955, **10**, 41—42, 111).—A review of recent developments in codling moth control. A. H. CORNFIELD.

Insecticides for cherry fruit fly control. J. A. Cox (*J. econ. Ent.*, **48**, 575—576).—Three applications of parathion or EPN, or a split schedule of Pb arsenate followed by two applications of either parathion, Diazinon or EPN at weekly intervals gave excellent control of cherry fruit flies, *Rhagoletis fausta* and *R. cingulata*. Sprays of parathion, EPN, Diazinon and Malathion were quite effective against immature stages in the fruit. Applications of chlordane or Dieldrin to soil were of little value. A. A. MARSDEN.

Fumigation tests with ethylene dibromide for the control of cherry fruit fly eggs, larvæ and puparia. S. C. Jones (*J. econ. Ent.*, 1955, **48**, 617—618).—Fumigation with $C_2H_4Br_2$ (0·5 lb. per 1000 cu. ft.) for 2 hr. at 22° killed all eggs, larvæ and puparia of the cherry fruit fly, *Rhagoletis cinqulata*. Taste tests showed a slight change in flavour of the cherries four days after fumigation at this dosage. Definite off-flavours occurred with fumigation dosages of >1 lb. of $C_2H_4Br_2$ per 1000 cu. ft. A. A. MARSDEN.

Dormant *versus* summer control of the grape mealybug in the Yakima valley. K. E. Frick and R. E. Bry (*J. econ. Ent.*, 1955, **48**, 607—608).—A delayed dormant spray of parathion (0·5 lb. per 100 gal.) or a summer spray of 0·25 lb. per 100 gal. gave equally good control of grape mealybugs, *Pseudococcus maritimus*. More sooty mould, *Capnodium* sp. resulted if the summer spray was delayed until most of the bunches of grapes had honeydew on them. Vineyards needing pre-harvest control should be sprayed as the first honeydew is deposited on the fruit. A. A. MARDSEN.

Chemical control of spores of the mould causing white-rot of grapes (*Coniella diplodiella*, [Speg.] Pet. et Syd.) in soil. G. Turian and M. Staehelin (*Landw. Jahrb. Schweiz*, 1955, **69**, 799—808).—The min. efficient concn. of tetramethylthiuram disulphide for complete control of the spores of the fungus is 1 g. per kg. of soil. At this concn. the autotrophic soil bacteria (e.g., *Azotobacter* spp.) are unaffected. In practice, an application of a mixture containing 25% of the fungicide and 25% of S + $CaCO_3$ at 4 kg. per acre (120 sq. yards) destroys all the spores in the top-soil. P. S. ARUP.

Control of the strawberry aphid in Southern California. J. Wilcox and A. F. Howland (*J. econ. Ent.*, 1955, **48**, 581—583).—Many insecticides when used as dusts or sprays gave excellent control of the strawberry aphid, *Capitophorus fragæfolii*. In practice, however, parathion, Malathion and Metacide cannot be used on strawberries since cyclamen mites tend to build up after their use. Endrin, Demeton, lindane, schradan, Isolan and Pyrolan may present a residue hazard, whilst DN-289, lime–S and Sulphotepp caused burning of the plants. Two applications of a nicotine (4%) dust (about 50 lb. per acre) gave effective control. A. A. MARSDEN.

Controlling the Japanese beetle. W. E. Fleming (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 2004, 13 pp.).—Appearance, seasonal history and habits of *Popillia japonica* are described and control by spraying or dusting, hand collection, trapping, and by cultural means is discussed. E. G. BRICKELL.

Gastight fabrics for use in fumigating with hydrocyanic acid gas. A. W. Cressman, H. R. Yust and D. Eichorn (*U.S. Dep. Agric.*, 1955, *Circ.* 978, 14 pp.).—Investigations into the development of plastic-coated gas-tight fabrics for the fumigation of trees, are summarised, the important physical properties being strength, wt., base cloth weave, flexibility, abrasion resistance and adhesion of coating, permeability and stability to ageing and weathering. E. G. BRICKELL.

Control of cabbage worm. Anon. (*Agric. Chemicals*, 1955, **10**, 49).—Although the resistance of cabbage worm to DDT had increased greatly over 10 years, the application of 32 lb. of DDT per acre still gave satisfactory control of the pest. Dilan (32 lb. per acre) gave more effective control, but resulted in aphid damage. A. H. CORNFIELD.

Control of cabbage and carrot loopers. G. P. Wene and G. W. Otey (*Proc. 9th annu. Mtg Rio Grande Valley hort. Inst.*, 1955, 33—36).—The cabbage looper, *Trichoplusia ni*, was controlled by dusting with 2% Endrin, 2·5% Dieldrin, 20% toxaphene, ·12% toxaphene + 8% DDT or 5% Perthane. The carrot looper, *Rachiplusia ou*, was controlled by spraying with Endrin 0·3 or taxaphene 3 lb. per acre. HORT. ABSTR. (A. G. P.).

Control of the pea leaf miner in Southern California. J. Wilcox and A. F. Howland (*J. econ. Ent.*, 1955, **48**, 579—581).—Parathion (1—2%) markedly lowered the no. of leaf miners on spinach, romaine and beetroots; EPN emulsion sprays also gave very good results. Toxaphene (20%) and methyl parathion (2·5%) dusts severely burned spinach leaves in the absence of smog conditions, whilst parathion (1%) and EPN (2%) dusts in the presence of smog intensified the burn on the leaves. EPN alone or with DDT, parathion alone or with DDT, NPD, Demeton and DDT alone all gave good to excellent control of the spinach leaf miner, *Pegomya hyoscyami*, attacking beets and spinach. A. A. MARSDEN.

***Fusarium* rot of beans.** P. R. Miller (*Agric. Chemicals*, 1955, **10**, 91).—Treatment of bean seeds with Arasan, Ceresan, Spergon, Dow 9B, Dithane Z78 and Arasan + $C_6H_6Cl_6$ did not control *Fusarium* root rot when the beans were planted in infected soil. A. H. CORNFIELD.

Control of bean leaf-hopper. G. P. Wene and G. W. Otey (*Proc. 9th annu. Mtg Rio Grande Valley hort. Inst.*, 1955, 30—32).—The hopper (*Empoasca solana*) on peas was controlled by dusting with DDT (5) + S (82%). DDT 5, methoxychlor 5, TDE 5, parathion 1, EPN 1 and toxaphene 10% also gave good results. S increased the efficiency of toxaphene. HORT. ABSTR. (A. G. P.).

Control of the tomato fruitworm in South Carolina. W. F. Chamberlain and J. H. Cochran (*J. econ. Ent.*, 1955, **48**, 518—521).—DDT, DDD, Dilan and Dieldrin all gave good control of the tomato fruitworm, *Heliothis armigera*. Toxaphene, methoxychlor, Aldrin and parathion gave erratic or poor control. At the concn. used, dusts and sprays gave equally good control. Addition of sticking agents greatly increased control of this pest but their use in commercial control programmes was seldom necessary. A. A. MARSDEN.

Control of grey mould and ghost spot of tomatoes. P. R. Miller (*Agric. Chemicals*, 1955, **10**, 61, 115—119).—Of a no. of materials tested the best results over three years were obtained with Dichlone (0·75 lb. of 50% wettable powder in 100 gal. per acre). The treatment was ineffective against grey leaf spot, but this was controlled by alternating the Dichlone sprays with zineb, nabam–$ZnSO_4$ or maneb sprays. A. H. CORNFIELD.

Antagonism between cucumber mosaic and cucumber mildew. S. Blumer, L. Stalder and A. Harden (*Phytopath. Z.*, 1955, **25**, 39—54).—Antagonistic reactions between the mosaic virus and powdery mildew (*Erysiphe polyphaga*) are demonstrated. Probably the virus is inactivated by metabolic products of the mildew. A. G. POLLARD.

Insecticide tests on the squash bug. F. H. Harries and H. Matsumori (*J. econ. Ent.*, 1955, **48**, 613—614).—Laboratory and field tests showed that parathion, Malathion, EPN and Dieldrin were all very effective against *Anasa tristis*. Aldrin and chlordane (2 lb. per 100 gal.) sprays were quite effective in the field, whilst arsenomethane–As disulphide gave a good reduction of bugs mainly due to its repellent effect. A. A. MARSDEN.

***Aceria tulipæ*, (K.) damaging garlic in California.** W. H. Lange, jun. (*J. econ. Ent.*, 1955, **48**, 612—613).—Dusting stored garlic bulbs with S gave good protection against this pest. Fumigation with MeBr (2·5 lb. per 1000 cu. ft.) for 2 hr. at 26·5° gave a complete reduction of mites with no damage to the bulbs. A combination of fumigation and dusting S to prevent reinfestation is suggested for practical control. A. A. MARSDEN.

Antigenic relationship between Puerto Rican pepper mosaic virus and a strain of potato virus Y. J. E. Pérez and J. Adsuar (*J. Agric. Puerto Rico*, 1955, **39**, 165—167).—Antisera were prepared against the Puerto Rican pepper-mosaic virus (P.R.P.M.) and a typical strain of potato virus Y by inoculating rabbits with clarified sap from tobacco plants infected separately with each virus. All the four sera thus prepared reacted with both antigens. Cross-absorption tests showed that the P.R.P.M. virus possessed a minor antigenic component not found in the Y virus. A. H. CORNFIELD.

Toxicity of certain organic insecticides to the sweet potato weevil. W. J. Mistric, jun. (*J. econ. Ent.*, 1955, **48**, 615—616).—Of 11 insecticides tested in the laboratory against *Cylas formicarius*, Endrin,

methyl parathion and EPN were all highly effective. Malathion, Dieldrin, lindane, Aldrin, heptachlor, DDT, chlordane and toxaphene were all less toxic. A. A. MARSDEN.

Inactivation of tobacco mosaic virus by extracts and secretions of higher plants and of some micro-organisms. W. Bartels (*Phytopath. Z.*, 1955, **25**, 72—98).—The virus is inactivated to varying extents by culture filtrates of a no. of species of bacteria and fungi and by extracts of green leaves, of soils and of composts. A. G. POLLARD.

Reaction of some *Nicotiana* species to the pepper and common tobacco mosaic viruses. J. Adsuar and L. L. Matos (*J. Agric. Puerto Rico*, 1955, **39**, 168—171).—Of 24 species of *Nicotiana* tested, *N. glauca*, *N. tomentosiformis* and *N. otophora* developed no symptoms when inoculated with the pepper mosaic virus. *N. glauca* was the only species which did not develop symptoms when inoculated with the tobacco mosaic virus. A. H. CORNFIELD.

Control of the tobacco wireworm. Anon. (*Agric. Chemicals*, 1955, **10**, 46).—Effective control of the tobacco wireworm in tobacco transplants was obtained by adding to the water used for watering-in after transplanting 8 oz. of 40—50% chlordane wettable powder or 0·25 oz. of 25% $C_6H_6Cl_6$ wettable powder per 50 gal. A. H. CORNFIELD.

Insecticides for control of green June beetle larvæ. C. B. Dominick (*J. econ. Ent.*, 1955, **48**, 621—622).—Of seven org. insecticides tested for the control of *Cotinis nitida* in tobacco plantbeds, parathion was the most satisfactory material. When applied in early April one application of parathion as a dust, a granulated formulation, or a drench gave excellent control. Lindane and chlordane injured the seedlings. Dilan, Malathion, Potasan and Demeton gave variable results or were not very effective. A. A. MARSDEN.

Control of the brown cotton leafworm, *Acantia dacia*. D. F. Martin and W. J. Mistric, jun. (*Agric. Chemicals*, 1955, **10**, 43, 88).—Effective control of the pest in the field was obtained with 0·25—0·50% parathion and 0·33% Endrin sprays. 0·4% γ-$C_6H_6Cl_6$ was only partially effective. A. H. CORNFIELD.

Effect of Bayer 17147 on boll weevil. R. L. Robertson and F. S. Arant (*J. econ. Ent.*, 1955, **48**, 604—605).—Adults of the boll weevil, *Authonomous grandis*, were exposed to cotton leaves from plots treated with 5% Bayer 17147 (a benztriazine derivative of a dithio phosphoric acid methyl ester) and with 20% toxaphene applied as dusts at 10 and 20 lb. per acre. The initial kill was more rapid and lethal residues persisted longer on plants treated with Bayer 17147 than on those treated with toxaphene. A. A. MARSDEN.

Comparative effect of Systox and schradan on some predators of aphids in Egypt. M. K. Ahmed (*J. econ. Ent.*, 1955, **48**, 530—532).—Larvæ of the two predators *Sphæropharia flavicauda* and *Leucopis puncticornis* were susceptible to all cotton aphids poisoned with or without external contact by Systox and schradan. Adult and larval coccinellids and larvæ of *Chrysopa vulgaris* were not affected by any aphids killed with schradan. Aphids killed externally with Systox were highly poisonous to coccinellids, but aphids poisoned without external contact with Systox had little effect on coccinellid larvæ and almost none on the adults. *C. vulgaris* larvæ were almost immune to Systox when fed poisoned aphids of either group. A. A. MARSDEN.

Control of sugar-cane termites (1946—1953). S. B. D. Agarwala (*J. econ. Ent.*, 1955, **48**, 533—537).—Aldrin and Dieldrin were successfully applied to all stages of the sugar-cane crop against the termites *Microtermes obesi*. Almost complete protection of the sugar-cane crop was obtained by dipping the seed pieces before planting, applying to the cut ends of seed pieces or sprinkling the insecticide over the seed pieces in the furrows before covering. DDT, parathion, toxaphene and $C_6H_6Cl_6$ were unsatisfactory. A. A. MARSDEN.

Insecticide seed treatment of soya-beans in relation to phytotoxicity and seed-maize maggot control. K. J. Starks and J. H. Lilly (*J. econ. Ent.*, 1955, **48**, 538—543).—Soya-beans of high viability were not injured by emulsions of Diazinon, Aldrin, heptachlor, Dieldrin or lindane: slight injury was caused by 2-oz. dosages of these insecticides when used in wettable powder formulations. Lower dosages of these compounds severely damaged seed which had been previously stored for 19 months. All insecticides tested were compatible with Captan. Heptachlor, toxaphene, lindane and Dieldrin were compatible with soya-bean bacterial inoculant, *Rhizobium leguminosarum*. The application of lindane to the seed gave soya-beans with oil showing an increased I value when this insecticide was used without inoculation. Diazinon, lindane and heptachlor seed treatments gave increases in stands where flies of the seed-maize maggot, *Hylemya cilicrura*, were attracted by application of moistened fish meal. A. A. MARSDEN.

Effect of some organic insecticides on the population levels of the serpentine leaf miner and its parasites. G. P. Wene (*J. econ. Ent.*, 1955, **48**, 596—597).—DDT, Aldrin and TDE dusts reduced the population of parasites of *Liriomyza subpusilla*. A toxaphene dust and a parathion spray reduced the no. of both the leaf miner and its parasites. Lindane, Endrin and Dieldrin reduced the no. of parasites less effectively than did parathion: they had little effect on the leaf miner. Under natural conditions, the parasites effectively controlled a leaf miner infestation. A. A. MARSDEN.

Methods of treating sorghum selfing bags for insect control. R. G. Dahms, J. B. Sieglinger and W. D. Guthrie (*J. econ. Ent.*, 1955, **48**, 568—572).—Several insecticides gave good control of either the maize earworm, *Heliothis armigera*, or the maize leaf aphid, *Rhopalosiphum maidis* when impregnated in or applied to selfing bags on sorghum heads. Aldrin and heptachlor gave satisfactory control of both insects and caused little sterility. When applied as a streak on the inside of the bags at 72 mg. per bag, Aldrin gave fair control of maize leaf aphids and excellent control of the earworms. A. A. MARSDEN.

Chemical control of the mountain pine beetle and Douglas-fir beetle. J. M. Kinghorn (*J. econ. Ent.*, 1955, **48**, 501—504).—Treatment of infested trees with concentrates of schradan and Systox failed to reduce brood survival. $C_2H_4Br_2$, Aldrin, heptachlor, lindane and Dieldrin when used as bark sprays were all effective against the mountain pine beetle, *Dendroctonus monticolæ*, in lodgepole pine blocks, although $C_2H_4Br_2$ had the most rapid effect. $C_2H_4Br_2$, Aldrin and heptachlor were effective against the Douglas-fir beetle, *Dendroctonus pseudotsugæ*, in relatively thin-barked Douglas fir. A. A. MARSDEN.

Control of the walnut aphid. A. E. Michelbacher (*J. econ. Ent.*, 1955, **48**, 504—509).—Systox and schradan gave better control of the walnut aphid, *Chromaphis juglandicola*, than did non-systemic aphicides; schradan being more effective than Systox. Good control with schradan depended on thorough coverage and application before the walnut foliage became too mature. Systox tends to cause leaf injury and applications should not be made until the trees come into full leaf. A. A. MARSDEN.

Notes on the biology and control of *Pseudocneorhinus bifasciatus*. F. F. Smith (*J. econ. Ent.*, 1955, **48**, 628—629).—Of 12 insecticidal dusts tested against Japanese weevils on caged azaleas, the min. concn. that killed all weevils was 2·5% of Aldrin, chlordane, heptachlor, Isodrin, Malathion and parathion, and 1% of Dieldrin. DDT, Endrin, lindane, methoxychlor and toxaphene were non-toxic at the concn. tested. A. A. MARSDEN.

***Fusarium* disease of gladioli.** C. Bruhn (*Phytopath. Z.*, 1955, **25**, 1—38).—The symptoms and means of transmission of the disease, due to *Fusarium oxysporum*, Schl. f. *gladioli* [Massey], are described. Promising results in controlling the disease were obtained by treating the corms with Hg prep., formaldehyde or an Fe carbamate prep. A. G. POLLARD.

Standard laboratory colonies of termites for evaluating the resistance of timber, timber preservatives and other materials to termite attack. F. J. Gay, T. Greaves, F. G. Holdaway and A. H. Wetherly (*Commonw. sci. industr. Res. Org. Aust.*, 1955, *Bull.* 277, 60 pp.).—A method is described for maintaining three species of Australian termites—*Nasutitermes exitiosus* (Hill), *Coptotermes lacteus* (Frogg) and *C. acinaciformis* (Frogg)—in laboratory colonies standardised with respect to the container, matrix, moisture content, ventilation and initial population and maintained at constant temp. and R.H. (16 references.) E. G. BRICKELL.

Organic termite repellents tested against *Cryptotermes brevis*, Walker. G. N. Wolcott (*J. Agric. Puerto Rico*, 1955, **39**, 115—149). A large no. of org. chemicals were tested for their effectiveness in preventing termite attack of almacigo and flamboyan woods impregnated with varying concn. of the chemicals. DDT at 2% concn. protected almacigo for 10·5 years whilst 1% pentachlorophenol was effective for 11 years. Impregnation with 48 other chemicals (0·1—1·0%) gave protection for a no. of years. Methoxychlor, Isodrin, Endrin and five other chemicals (0·05%) were effective for a no. of years. Pinosylvin monomethyl ether and two other chemicals were effective at 0·01% and 4 : 4′-dinitrostilbene and 2 : 4-dinitro-2′ : 4′-dichlorostilbene were effective at 0·005%. A. H. CORNFIELD.

Weedkiller for the control of grasses. Anon. (*Rubb. Res. Inst. Malaya, Plant. Bull.*, 1956, No. 23, 27).—Tests were made with Dalapon (Na 2 : 2-dichloropropionate), in the control of grasses on rubber estates. The substance is readily sol. in water and is not highly toxic to animals. It was specially effective against *Axonopus compressus*, *Paspalum conjugatum*, *Hemigymnia fusca* (*Panicum nodosum*) and *Panicum sarmentosum*, applications of 15 lb. per acre resulting in little or no recovery until ~7 months after treatment.

Two applications with dosages up to 50 lb. per acre had no detectable harmful effect on rubber trees seven years old. E. M. J.

Action of tauramide, 2-acetylamino-1 : 3 : 4-thiadiazole-5-sulphonamide and *N*-substituted sulphanilamides on duckweed, *Lemna minor*. F. Fromm and M. Lawrence O'Donnell (*Proc. Pa Acad. Sci.*, 1955, **29**, 135—140).—The sulphonamide group has a toxic action on duckweed and is phytotoxic in general; the aromatic sulphonamides are more toxic than are the aliphatic, but sulphonamides generally do not interfere with the *p*-aminobenzoic acid-metabolism of the plant. *L. minor* was killed by concn. of 8×10^{-2}M-tauramide, 10^{-2}M-N^1-acetylsulphanilamide, or 10^{-2}M-N^1N^4-diacetylsulphanilamide. Min. concn. which inhibited growth were 8×10^{-3}M-tauramide, 10^{-3}M-2-acetamido-1 : 3 : 4-thiadiazole-5-sulphonamide, 10^{-2}M-N^4-acetylsulphanilamide, 10^{-4}M-N^1-acetylsulphanilamide, 10^{-3}M-N^1N^4-diacetylsulphanilamide and 10^{-2}M-sulphaguanidine. Introduction of a free amino-group in the substituent increases its toxicity. E. M. J.

Substituted ureas and industrial weed and brush control. J. E. Naylor (*Farm. Chem.*, 1956, **119**, No. 3, 57—59).—A short report of a lecture mainly concerned with the application and formulation of phenyldimethylurea and some chlorinated derivatives. A. G. Pollard.

Destruction of brushwood in mountain pastures. W. Wurgler (*Landw. Jahrb. Schweiz*, 1955, **69**, 771—782).—Invading shrubs can best be destroyed by summer sprayings with aq. 0·2—0·25% emulsions of 2 : 4 : 5-T or 2 : 4-D, or by applications of the oily solutions of the chemicals to the trunks during winter. Details are given for the control of a no. of different shrubs. P. S. Arup.

Effect of rotary cultivation on rhizomatous weeds. H. Fail (*J. agric. Engng Res.*, 1956, **1**, 68—80).—Couch grass (*Agropyron repens*) and twitch (*Agrostis* spp.) were controlled by means of a rotary cultivator having L-shaped blades and operating about 6 in. below the soil surface. The treatment needed repetition 2—6 times according to soil type. For bracken, periodic rotary cultivations at increasing depths and finally to 10 in. gave satisfactory results if the first treatment was given in June when the fronds had expanded. A. G. Pollard.

Repellents to protect trees and shrubs from damage by rabbits. A. C. Hildreth and G. B. Brown (*U.S. Dep. Agric.*, 1955, *Tech. Bull.* 1134, 31 pp.).—Nicotine, nicotine sulphate, tetramethylthiuram disulphide and tetraethylthiuram monosulphide in water emulsions of asphalt or of certain synthetic plastics, proved satisfactory. An evaluation of 123 materials tested is appended. E. G. Brickell.

Animal Husbandry

Feeding of livestock. J. Duckworth (*J. roy. agric. Soc. England*, 1955, **116**, 126—137).—A review. Mineral nutrition (Ca, Mg, Cu), the effect of concentrates on butter-fat production, and the protein feeding of poultry are considered. A. G. Pollard.

Methods of assessing the energy values of foods for ruminan animals. K. L. Blaxter and N. McC. Graham (*Proc. Nutrit. Soc.*, 1955, **14**, 131—139).—A clear exposition of the theoretical basis of energy values and a critical review of the methods employed. This paper exposes the limited nature and inherent inaccuracies of the small amount of work on which modern food tables depend. (89 references.) W. F. J. Cuthbertson.

Inter-relations between passage of food through the digestive tract and its digestibility. K. L. Blaxter, N. McC. Graham and F. W. Wainman (*Proc. Nutrit. Soc.*, 1955, **14**, xv—xvi).—A sample of dried grass was fed to sheep as long material, coarsely ground or finely ground. The digestibility and retention time in the gut both decreased as the particle size diminished. Increasing the amount given decreased retention time and also the apparent digestibility, the latter effect being more marked with finely ground and negligible with coarse material. W. F. J. Cuthbertson.

Calorimetric measurements of the nutritive value for sheep of dried grass prepared in different ways. K. L. Blaxter and N. McC. Graham (*Proc. Nutrit. Soc.*, 1955, **14**, xv—xvi).—Dried grass was fed as chaff, as cubes made from coarsely ground or from finely ground material. When fed as cubes more energy was lost in the fæces and as methane than when fed as chaff but the net energy of the three products was identical. W. F. J. Cuthbertson.

Determination of the net utilisation of protein by a shortened method. D. S. Miller and A. E. Bender (*Brit. J. Nutrit.*, 1955, **9**, 382—388).—A 10-day rat test is described whereby net protein utilisation can be determined from N-consumption of the rats and the water content of their carcasses. The method gives results in good agreement with previously described values. A technique is described for the rapid detection of the limiting amino-acids in a protein. W. F. J. Cuthbertson.

Relation between protein efficiency and net protein utilisation. A. E. Bender (*Proc. Nutrit. Soc.*, 1955, **14**, xiii—xiv).—Protein efficiency (PE) has a very high correlation with net protein-utilisation values (NPU). From a series of tests on the same proteins in the same laboratory, it was shown that NPU = 30 + 14·5 PE with a correlation coeff. of 0·912 (P 0·001), whilst calculations on published work from different laboratories showed NPU = 37·2 + 14·05 PE (r = 0·838). In these tests the correlation coeff. between food intake and PE varied from 0·951 to 0·958. W. F. J. Cuthbertson.

Effect of grinding and pelleting on the digestibility of a ration by lambs. T. A. Long, A. B. Nelson and R. MacVicar (*J. Anim. Sci.*, 1955, **14**, 947—950).—A ration of prairie hay, lucerne hay, maize, cottonseed meal and molasses was fed to lambs, (i) without comminution, (ii) ground, (iii) ground and pelleted. The apparent digestibility of the org. matter of the ration was lowered by grinding but on pelleting rose again to the level of that of the natural ration. A. G. Pollard.

Digestibility and fodder-value of wheat and rye, and of their milling by-products. K. Hüni (*Landw. Jahrb. Schweiz*, 1955, **69**, 961—964).—No differences are found either between the digestibilities (by pigs or cows) of the two grains or their respective products, or between the digestive capacities (in relation to crude fibre contents) of the two animals. In practice, digestibility coeff. can be calculated for both classes of products fed to cows or pigs, by the formulæ given, which are based on the crude fibre contents alone. Starch values can also be calculated on similarly based formulæ. P. S. Arup.

Nutritive value of germinated wheat. W. Schneider (*Landw. Jahrb. Schweiz*, 1955, **69**, 965—970).—The nutritive value of sound germinated wheat is satisfactory, but the loss in wt. consequent on germination (due to consumption of sugars derived from the starch) should be borne by the producer. Mouldy germinated wheat is suitable only for feeding in limited proportions to mature animals, and is better tolerated by pigs or cattle than by horses. P. S. Arup.

Feeding value of stored maize. C. A. Cabell and N. R. Ellis (*J. Anim. Sci.*, 1955, **14**, 1167—1173).—Maize stored for 1—6 yr. showed a diminution in protein efficiency (for rats) from 2·72 to 1·81 g. of gain in wt. per g. of protein. When tested as an energy source no appreciable loss of feeding value resulted from storage for 6 yr. A. G. Pollard.

Fermentation processes in silage. I. Rôle of temperature. R. Nilsson, L. Tóth and C. Rydin. **II. Effect of various carbohydrates as supplements.** C. Rydin, R. Nilsson and L. Tóth (*Arch. Mikrobiol.*, 1956, **23**, 366—375, 376—384).—I. The quality of the silage produced in small-scale silos kept at various constant temp. (2—37°), and the course of the fermentation are followed by periodic analyses of the effluent and the press-juice of the silage. With forage rich in proteins, but poor in carbohydrates, low ratios of NH_3-N : total N, and low butyric acid contents are obtained by fermentation at temp. <20°, with satisfactory results. High pH and low lactic acid contents are obtained at higher temp., with unsatisfactory results.

II. In continuation of the above experiments, the addition of 3% of glucose to the forage promotes lactic acid fermentation sufficiently to allow of satisfactory silage production at 24° or 37°. Starch affects the fermentation unfavourably, whilst cellulose and straw meal are ineffective. Malt has a better effect than barley-meal, due to its higher sugar content and to its diastatic action. Additions of straw, cellulose, barley-meal and (to a smaller extent) of glucose reduce the amount of effluent. P. S. Arup.

Utilisation of urea and biuret as sources of nitrogen for growing-fattening lambs. J. C. Meiske, W. J. van Arsdell, R. W. Luecke and J. A. Holfer (*J. Anim. Sci.*, 1955, **14**, 941—946).—Urea, biuret or crude biuret (biuret 41, urea 46, triuret 6·5, cyanuric acid 6·5%) used to supplement a basal ration of molasses, maize and lucerne meal (crude protein 7·1%) increased the growth rates and the feed efficiency. The various N supplements were equally effective; biuret was somewhat less toxic than urea. A. G. Pollard.

Metabolism of carotene and vitamin A given by mouth or vein in oily solution or aqueous dispersion to calves, rabbits and rats. S. K. Kon, W. A. McGillivray and S. Y. Thompson (*Brit. J. Nutrit.*, 1955, **9**, 244—267).—In all species carotene given by mouth as a "Tween" dispersion was better absorbed and more efficiently converted to vitamin A than when given as an oily solution. Injected carotene, no matter how dispersed, formed little, if any, vitamin A in the calf. About one-tenth of an injected dose of vitamin A could be detected in the liver. W. F. J. Cuthbertson.

Rate of loss of carotenoids from artificially dehydrated lucerne under farm storage. K. L. Dolge, C. M. Dembiczak, J. E. Rousseau,

jun., H. D. Eaton, G. Beall and L. A. Moore (*Conn. agric. Exp. Sta.*, 1955, *Bull.* 314, 12 pp.).—The loss of carotenoids from artificially dehydrated lucerne hay as well as from calf starter rations containing 7—75% of lucerne took place essentially at a linear rate. The rate of decrease per four-week period ranged from 0·00017 to 0·00680 g. per lb. of dry matter. The rate of loss was greater from those materials having the higher initial carotenoid content. A nomograph is presented for estimating the carotenoid concn. of hays in storage when initial concn. and length of time in storage are known.
A. H. CORNFIELD.

Antibiotics and endocrine stimulants as promoters of growth in fattening pigs. R. Braude, R. C. Campbell, I. A. M. Lucas, J. R. Luscombe, K. L. Robinson and J. H. Taylor (*Brit. J. Nutr.*, 1955, **9,** 191—196).—The effects of thyroxine, stilbœstrol, penicillin and aureomycin were investigated at five experimental stations. In two out of three centres, L-thyroxine (0·3 mg./lb. of diet) increased the growth obtained on a diet containing an antibiotic (chlorotetracycline or penicillin). Marked variations in response to a given treatment were noted at the different stations at which the tests were carried out. In one instance, toxic signs were associated with stilbœstrol at a level which had improved growth when given together with antibiotic and thyroxine at two out of four centres.
W. F. J. CUTHBERTSON.

Thyroxine, stilbœstrol and antibiotics in rations for castrated male pigs. I. A. M. Lucas and A. F. C. Calder (*Brit. J. Nutrit.*, 1955, **9,** 267—279).—From weaning to 100 lb. wt., pigs given Aurofac or penicillin grew about 20% faster and used food about 15% more efficiently, whilst from 100 lb. wt. to slaughter improvements of 7% in growth rate and about 5% in feed efficiency were noted. Addition of thyroxine (30 mg./lb. of diet) and stilbœstrol (6 mg./lb. of diet) to the ration did not significantly improve growth up to 100 lb. wt. but caused an 8% increment in growth and feed efficiency after this stage of development of the animals. Addition of the hormones to rations containing either antibiotic reduced food conversion and growth to 100 lb. wt. by 8%. Thereafter the Aurofac-fed pigs were unaffected, but the growth and feed conversion of the penicillin-fed animals were improved by 6% and 8% respectively by addition of the hormones. Compared with the controls the hormones alone produced a 6% increase in growth and 4% in efficiency; antibiotics alone gave an increase of 15% in growth and 8% in feed efficiency over the whole period. During this time the hormones caused a 5% improvement in the feed efficiency of the penicillin-fed animals, but a 5% depression in feed conversion of those given Aurofac.
W. F. J. CUTHBERTSON.

Influence of oral administration of diethylstilbœstrol to beef cattle. W. Burroughs, C. C. Culbertson, E. Cheng, W. H. Hale and P. Homeyer (*J. Anim. Sci.*, 1955, **14,** 1015—1024).—Addition of diethylstilbœstrol (5—10 mg. per head, daily) to the ration of yearling steers or heifers increased the growth rate and average food consumption but lowered the food consumption per unit gain in wt. No undesirable side-effects resulted.
A. G. POLLARD.

Subcutaneous implantation of stilbœstrol in fattening bulls and steers. E. W. Klosterman, V. R. Cahill, L. E. Kunkle and A. L. Moxon (*J. Anim. Sci.*, 1955, **14,** 1050—1058).—The increased rate of gain in wt. of fattening bulls, effected by implantation of stilbœstrol, was smaller than that in steers. Carcass grades were higher for treated bulls than for treated steers. In untreated animals given the same rations bulls fattened faster than steers.
A. G. POLLARD.

Œstrogenic activity of legume, grass and maize silage. P. J. S. Pieterse and F. N. Andrews (*J. Dairy Sci.*, 1956, **39,** 81—89).—The œstrogenic activity of lucerne (*L*) silage was significantly greater than that of freshly cut *L*; that of maize (*M*) or broome grass (*B*) silage was insignificant. The activity of a mixed *L*, *B* and ladino clover silage, preserved with either $Na_2S_2O_5$ or molasses, increased during fermentation in steel upright silos. The activity of *L* silage at the top of an upright silo was less than at the bottom; silage prepared in 5-gal. tins had most activity when preserved with molasses. The activity of silage without preservative differed little from that in which ground *M* was used. All silages contained significantly more activity than did fresh *L*. The œstrogenic activity of the isoflavone, genistein, was significantly increased by treating with 1·5% alcoholic KOH solution.
S. C. JOLLY.

Antibiotic feeding for prophylaxis of experimentally-produced swine erysipelas. B. H. Schneider, G. R. Spencer and M. E. Ensminger (*J. Anim. Sci.*, 1955, **14,** 1140—1145).—Inclusion of penicillin or oxytetracycline in pig rations (15 and 40 mg. per lb. of feed) had no effect on experimentally induced (intracutaneously infected) erysipelas.
A. G. POLLARD.

Antibiotic and copper supplements for pigs. R. S. Barber, R. Braude and K. G. Mitchell (*Brit. J. Nutrit.*, 1955, **9,** 378—381).—$CuSO_4$, a high-Cu mineral mix and aureomycin were equally effective in significantly increasing the weight gains of pigs from 10—11 weeks of age to 25—26 weeks of age. The food consumptions of the groups given aureomycin or the mineral mix were increased but feed efficiencies were unchanged. The food consumption was unchanged by giving $CuSO_4$, but feed efficiency was improved by this treatment. The treatment had no effect on commercial carcass gradings.
W. F. J. CUTHBERTSON.

Influence of chlortetracycline on the growth and carcass characteristics of swine fed restricted rations. H. D. Wallace, J. I. McKigney, A. M. Pearson and T. J. Cunha (*J. Anim. Sci.*, 1955, **14,** 1095—1102).—In dry-feeding trials the food intake of pigs receiving chlortetracycline (**I**) was restricted to that of control animals. Under these conditions the antibiotic had no beneficial effect on growth or carcass quality. With pigs at pasture and receiving a ration of concentrates, **I** increased the consumption of pasture and thereby improved the feed efficiency.
A. G. POLLARD.

Effect of chlortetracycline supplementation on growth and feed utilisation of unsuckled baby pigs obtained by hysterectomy. E. G. Hill and N. L. Larson (*J. Anim. Sci.*, 1955, **14,** 1116—1121).—The piglings showed increased growth rates after receiving supplements of chlortetracycline (5 and 10 p.p.m. of the dry matter of the ration) at various ages (0—42 days). In other trials pigs receiving the antibiotic at a level of 10 p.p.m. up to 3 weeks of age and 200 p.p.m. from 3 to 8 weeks also showed increased gains and improved feed efficiency.
A. G. POLLARD.

Effects of chlortetracycline feeding on bovine rumen micro-organisms. R. E. Hungate, D. W. Fletcher and I. A. Dyer (*J. Anim. Sci.*, 1955, **14,** 997—1002).—The activity of rumen organisms was determined manometrically on samples collected by stomach tube. Depression of the activity caused by addition of chlortetracycline (**I**) to the diet was less in animals previously fed **I** than in previously untreated controls. Measurement of CH_4 production provided further evidence that ingestion of **I** modifies the microbial population of the rumen. The effect of **I** on the micro-organisms exceeded that of streptomycin.
A. G. POLLARD.

Some effects of feeding chlortetracycline on carcass characteristics and body composition of swine; a scheme for the resolution of the body composition. A. J. Clawson, B. E. Sheffy and J. T. Reid (*J. Anim. Sci.*, 1955, **14,** 1122—1132).—Chlortetracycline (Aurofac, 0·5% in the ration) slightly increased the growth rates of pigs without affecting, significantly, the dressing % or the proportion of wholesale cuts in the carcass or the composition of the meat. Close correlation was established between the % of water in, and the sp. gr. of the carcass and between the water and fat contents of the carcass. The direct and the antipyrine dilution methods for determining the water contents of carcasses gave results in close agreement.
A. G. POLLARD.

Survey of the beef-cattle industry of Australia. W. A. Beattie (*Bull. Commonw. sci. industr. Res. Org., Aust.*, 1956, No. 278, 135 pp.).
J. S. C.

The concept of fructose utilisation by bull spermatozoa and its relation to fertility. M. L. Hopwood, E. R. Rutherford and F. X. Gassner (*J. Dairy Sci.*, 1956, **39,** 51—59).—At 37° utilisation of fructose by semen diluted 2 : 1 with 0·125M-PO_4''' buffer (pH 7·4) fitted a first-order reaction. Calc. fructolysis rates were highly significantly correlated with non-return rates (0·604 ± 0·084), which compares favourably with the fructolysis index as an estimate of the probable fertility of bulls. The rate determination, adjusted for spermatozoa concn., is a more justifiable measure of the metabolic behaviour of individual semen samples as it represents more closely the manner by which spermatozoa remove fructose from seminal plasma.
S. C. JOLLY.

Diluters for bovine semen. VII. Effects of time and temperature of heating skim milk on the livability of bovine spermatozoa. R. G. Saacke, J. O. Almquist and R. J. Flipse (*J. Dairy Sci.*, 1956, **39,** 90—96).—Fresh skim milk used for diluting bovine semen was heated under practical conditions to temp. between 77° and 97° for 1 or 10 min. Optimum livability of spermatozoa was obtained with heating conditions of 87—97° for 1 min. or 77—97° for 10 min. Significant decreases in livability occurred with milk heated for 1 min. at 77° or 82°.
S. C. JOLLY.

Absorption of rumen volatile fatty acids from the forestomachs of young dairy calves fed high-roughage rations. H. R. Conrad, J. W. Hibbs and W. D. Pounden (*J. Dairy Sci.*, 1956, **39,** 97—98).—Rumen volatile fatty acids are almost completely absorbed from the forestomachs of young dairy calves on a high-roughage ration. Cæcal fermentation is a secondary source of such acids.
S. C. JOLLY.

Effect of nursing calves on milk production of identical twin heifers. E. W. Swanson (*J. Dairy Sci.*, 1956, **39,** 73—80).—Using identical twin heifers for comparison, milk production in the first week after

vealing averaged 5·1 lb. per cow per day lower in the animals that nursed their calves until they were of vealing size than in those animals milked in the usual way. Lactation in the former group subsequently increased until both groups were producing equally six weeks after vealing. The total loss of milk due to nursing the calves amounted on average to 1496 lb. per cow over six lactations. S. C. JOLLY.

Nutritive value of colostrum for the calf. X. Relation between the period of time that a calf house has been occupied and the incidence of scouring and mortality in young calves. J. H. B. Roy, J. Palmer, K. W. G. Shillam, P. L. Ingram and P. C. Wood (*Brit. J. Nutrit.*, 1955, **9**, 11—20).—The rate of growth during the first three weeks of life decreases and the rate of scouring of calves in a calf house increases with the time during which the calf house has been in continuous use. These effects are independent of the quantity of colostrum given the calves and of the presence or absence of calves deprived of colostrum. Deaths amongst calves given colostrum occurred only when the incidence of scours reached a high level. A resting period of 15 days was insufficient to prevent scouring at the start of a subsequent test but a break of 47 days did eliminate scouring at the start of the following test. The build-up of infection was related to the occurrence of *Bact. coli* types pathological to the calf. W. F. J. CUTHBERTSON.

Nutritive value of colostrum for the calf. XI. Effect of aureomycin on the performance of colostrum-deprived calves. J. H. B. Roy, K. W. G. Shillam, J. Palmer and P. L. Ingram (*Brit. J. Nutrit.*, 1955, **9**, 94—103).—Dietary aureomycin given during the first 10 days of life reduced the incidence of scours and high rectal temp. and increased the live-wt. gains of colostrum-deprived calves. In this respect there was no difference between the colostrum-deprived calves given the antibiotic and the colostrum-fed calves. There was an indication that aureomycin depressed mortality of colostrum-deprived calves. W. F. J. CUTHBERTSON.

Energy and carbohydrate metabolism in magnesium-deficient calves. K. L. Blaxter and J. A. F. Rook (*Brit. J. Nutrit.*, 1955, **9**, 121—132).—Magnesium-deficiency in the calf leads to increased heat output and decreased energy retention associated with increased muscular activity. Exercise of normal calves increased their blood-pyruvate and -lactate levels to those of Mg-deficient calves in mild tetany. The abnormalities in metabolism and energy production during Mg-deficiency are due to the increased muscular work of tetany and not to effects on the enzyme systems employed in carbohydrate metabolism. W. F. J. CUTHBERTSON.

Effect of proteins of plant and animal origin on growth, reproductive development and semen production of young dairy bulls. R. J. Flipse, J. O. Almquist and P. E. Johnson (*J. Dairy Sci.*, 1956, **39**, 60—65).—Source of protein [fluid (*F*) or dried (*D*) skim milk or vegetable protein] had no significant effect on growth, hæmoglobin concn., total serum protein, urea- and non-protein-N in the blood, or the age or size at which viable spermatozoa first appeared in ejaculates of young dairy bulls. Volume of ejaculate was adversely affected by rations containing *D*, but spermatozoa concn. was increased by *F*. Other semen characteristics were not affected by the rations. S. C. JOLLY.

Effect of particle size on the utilisation of bone meal and limestone by beef cattle. J. Matsushima, T. W. Dowe, C. L. Comar, S. L. Hansard and W. J. Visek (*J. Anim. Sci.*, 1955, **14**, 1042—1049).—Finely ground (<28-mesh) and coarsely ground (>28-mesh) materials were equally effective as mineral supplements for steers. A. G. POLLARD.

Serum values in "wheat pasture poisoning" cases. H. R. Crookshank and F. H. Sims (*J. Anim. Sci.*, 1955, **14**, 964—969).—Changes in blood components are examined in cows suffering from poisoning after grazing on winter wheat, notably during the period of lush growth. The principal effects were diminution in inorg. PO_4''', Ca, Mg and albumin : globulin ratio and increased contents of total protein, globulin and K. A. G. POLLARD.

Influence of dietary calcium and phosphorus on the incidence of milk fever [in dairy cattle]. J. M. Boda (*J. Dairy Sci.*, 1956, **39**, 66—72).—The incidence of milk fever in susceptible Jersey cows was lowered by prepartum feeding of rations supplying ~8·5—11 g. of Ca and 74—102 g. of P daily ; some reduction also occurred apparently with a ration supplying 44 g. of P with 11 g. of Ca daily. All rations gave adequate wt. gains before parturition and had no adverse effects on parturition, subsequent lactation records or health of the calves. S. C. JOLLY.

The methionine–cystine need of the young pig. D. E. Becker, A. H. Jensen, S. W. Terrill and H. W. Norton (*J. Anim. Sci.*, 1955, **14**, 1086—1094).—In trials with weanling pigs a synthetic diet containing 12·6% of isolated soya-bean protein and providing methionine 0·15% and cystine (0·17%) failed to support normal growth. No improvement followed the addition of 0·1—0·2% of L-cystine. Satisfactory growth was obtained with a diet which included methionine 0·25 and cystine 0·1%. The total methionine–cystine requirement was 3·33% of the total protein. Of the total S-bearing amino-acids 40% may be contributed by cystine. Supplements of DL-methionine (0·3 or 0·4%) depressed growth. DL-α-Hydroxy-γ-methylmercaptobutyric acid could serve as part of the methionine requirement. Antibiotics (equal parts of oxytetracycline hydrochloride, procaine penicillin and streptomycin, at the rate of 36 mg. per lb. of ration) did not affect the methionine requirement of the pigs. A. G. POLLARD.

Sugar in pig starter rations. C. J. Lewis, D. V. Catron, G. E. Combs, jun., G. C. Ashton and C. C. Culbertson (*J. Anim. Sci.*, 1955, **14**, 1103—1115).—In free-choice experiments piglings showed a preference for starter rations containing sucrose and, among sugar-containing pellets, tended to select those having sugar inside the pellett rather than those with a sugar coating. Addition of sugar to the ration (meal or pellets) improved feed efficiency and when in pellets, increased early growth rates. A. G. POLLARD.

Saccharin and dried cane molasses in swine rations. R. A. Notzold, D. E. Becker, S. W. Terrill and A. H. Jensen (*J. Anim. Sci.*, 1955, **14**, 1068—1072).—Neither molasses (7·2%) nor saccharin (0·03—0·10%), added to pig rations, had any effect on rates of increase in live wt. or on food consumption or efficiency. With self-fed shelled maize pigs showed a temporarily greater preference for that treated with 0·05% of saccharin. A. G. POLLARD.

Comparison of phosphorus from different sources for growing and finishing swine. H. L. Chapman, jun., J. Kastelic, G. C. Ashton and D. V. Catron (*J. Anim. Sci.*, 1955, **14**, 1073—1085).—Steamed bone meal, $CaHPO_4$ and soft phosphate with colloidal clay (a by-product from rock-phosphate mines) are compared. Use of the soft phosphate + clay resulted in inferior gains in wt., feed efficiency and breaking strength of femurs ; the ash and F contents of the femurs increased. The P content of the bone ash and of blood serum were unaffected by either P source. Chlortetracycline may affect the P metabolism of pig bones. A. G. POLLARD.

High-copper mineral mixtures for pigs. R. J. Bowler, R. Braude, R. C. Campbell, J. N. Craddock-Turnbull, H. F. Fieldsend, E. K. Griffiths, I. A. M. Lucas, K. G. Mitchell, N. J. D. Nickalls and J. H. Taylor (*Brit. J. Nutrit.*, 1955, **9**, 358—362).—Experiments carried out at eight different centres showed that Cu (250 p.p.m. of diet) significantly increased the growth of pigs (from 1·40 to 1·48 lb. per day) from 10—12 weeks of age up to bacon wt. A slight but non-significant improvement in feed efficiency was noted. There was no difference between the carcass grading of the normal and Cu-fed pigs. W. F. J. CUTHBERTSON.

Feeding standards for poultry. W. Bolton (*Proc. Nutrit. Soc.*, 1955, **14**, i).—Neither crude fibre nor nitrogen-free extractive determinations give a good indication of digestible carbohydrate, but available carbohydrate (sum of sugars, dextrin and starch expressed as starch) accounts for 90% of the digestible carbohydrate. Thus it is suggested that this value rather than crude fibre would assist in the valuation of poultry diets. W. F. J. CUTHBERTSON.

Retention of vitamin B_{12} by growing cockerels. D. H. Shrimpton (*Proc. Nutrit. Soc.*, 1955, **14**, ii).—At 23 weeks of age, cockerels from vitamin B_{12}-depleted dams, reared on a vitamin B_{12}-deficient diet had body stores of vitamin B_{12} comparable with those in birds with a dietary source of vitamin B_{12}. It is suggested that after 4—6 weeks of age fowls can utilise significant amounts of endogenous vitamin B_{12} presumably derived from the gut flora. This amount of vitamin B_{12} is sufficient for normal growth after 4 weeks of age. Between the 3rd and 23rd week the birds stored about 20 μg. of vitamin B_{12} each. W. F. J. CUTHBERTSON.

Quantitative aspects of the nicotinic acid–tryptophan inter-relationship in the chick. Hans Fisher, H. M. Scott and B. Connor Johnson (*Brit. J. Nutrit.*, 1955, **9**, 340—349).—In the presence of nicotinic acid, 0·15% of L-tryptophan in the diet satisfies the chicks' requirement for this amino-acid, whereas 0·2% of L-tryptophan is needed to satisfy both tryptophan and nicotinic acid needs for growth. Addition of maize or of histidine, leucine and threonine to the diet decreased growth and increased the incidence of perosis. These effects were overcome by the addition of 10 mg. of nicotinic acid per 100 g. of diet but were not modified by alterations in tryptophan supply. It is suggested that the effects of maize diets are due to their amino-acid content and that nicotinic acid is directly involved in the metabolism of threonine, histidine and leucine. W. F. J. CUTHBERTSON.

Comparison of Hawaiian meat and bone meal, soya-bean oil meal, and herring meal in chick starter rations. A. L. Palafox and M. M. Rosenberg (*Hawaii agric. Exp. Sta.*, 1955, *Tech. Bull.* 28, 15 pp.).—

Chicks reared on an all-vegetable diet containing soya-bean oil meal showed wt. gains similar to those receiving 14—16% meat and bone meal and/or 5% herring meal in their diets. Feed efficiency was somewhat poorer with the all-vegetable than with the other diets. A. H. CORNFIELD.

Cattle disease of unknown origin in southeastern Utah. R. J. Raleigh, W. Binns, J. LeGrande Shupe, L. E. Harris and L. L. Madsen (*J. Anim. Sci.*, 1955, **14,** 951—963).—Of various dietary factors (including trace elements) examined, only lucerne hay had a curative effect on " brisket disease " (symptoms etc. described) in Utah. A. G. POLLARD.

Use of synergised pyrethrins to prevent oviposition by cattle grubs. E. S. Raun (*J. econ. Ent.*, 1955, **48,** 603—604).—When applied to cattle with an automatic treadle sprayer, a piperonyl butoxide (10%)–pyrethrins (1%) spray prevented oviposition by *Hypoderma lineatum* on cattle. Due to different oviposition habits, however, this treatment did not control the northern cattle grub, *Hypoderma bovis*, under the conditions of the experiment. A. A. MARSDEN.

Weight gains in feeder calves treated with low pressure rotenone sprays to control cattle grubs. E. S. Raun (*J. econ. Ent.*, 1955, **48,** 604).—Although the differences in weight gains were not significant, calves treated with rotenone sprays for the control of *Hypoderma lineatum* and *H. bovis* gained an average of 4·7 and 11·6 lb. more than did untreated animals during the 3-months test period. A. A. MARSDEN.

Hog lice and hog mange. H. E. Kemper and H. O. Peterson (*U.S. Dep. Agric.*, 1955, *Farm. Bull.* 1085, 21 pp.).—The nature and habits of *Hæmatopinus adventicius* var. *chinensis* (*H. suis*), *Sarcoptes scabiei* var. *suis* and *Demodex phylloides* (Csokor), symptoms caused by each species, and methods of control and eradication are discussed. Plans for hog wallows and dipping plants are given. E. G. BRICKELL.

2.—FOODS

Sorbic acid as a fungistatic agent for foods. XI. Effectiveness of sorbic acid in protecting cakes. D. Melnick, H. W. Vahlteich and A. Hackett (*Food Res.*, 1956, **21,** 133—146; cf. J.S.F.A. Abstr., 1956, i, 235).—The literature covering the use of sorbic acid as a fungistatic agent for protecting various foods is reviewed. In protecting cakes, propionates can be used only at low concentrations without imparting flavour to the cakes. With sorbic acid at 0·1% level (batter wt. basis) a significantly greater degree of protection against mould spoilage is obtained without alteration of cake flavour. The U.S. Food and Drug Administration allows the use of sorbic acid in concentrations up to 0·30% in cakes. (24 references.) E. M. J.

Theory of the diffusion process in sugar manufacture. VIII. Water economy problems in juice production. G. Oplatka (*Cukoripari Kutatóintézet Közleményei*, 1955, **2,** 72—79; cf. S.I.A., 1955, 767).—Diffusion waste water recovery in the Robert battery is not economic in regard to the sugar content. The waste of water can be obviated by adding fresh water only during draw-off, flow being subsequently maintained by pumps between the vessels. The return of pulp-press water is similarly valuable only in regard to the water recovery, indicated by calculations of the sugar which can be recovered; this is only about half that present in the pulp-press water. The optimum point of return of the water to the battery is given by an equation. If the returned water is just mixed with the entering fresh water sugar losses are slightly higher. SUG. IND. ABSTR. (E. M. J.).

Discontinuous [beet] juice purification process. S. Vajna and B. Gabos (*Cukoripari Kutatóintézet Közleményei*, 1955, **2,** 80—83).—The optimum predefecation pH is 10·8 to 11·0, but there is another optimum colloid coagulation point at pH 9·3—9·8. If the raw juice is treated with 0·075% of lime, boiled, and then further treated with $CaCO_3$, equiv. to 0·8% of CaO and more lime corresponding to the juice solids (e.g., 0·045% of CaO at 11° Brix, plus 0·01% CaO for each degree Brix higher), followed by boiling for 5 min. (at pH 9·2—9·4) filtration is 2—3 times better than usual. The process is continued by addition of 0·8% of lime, saturation at 80°, boiling and filtering. The unwashed muds can be returned to the pre-defectation process. Juice quality is as good as by Silin's process. The lime salt content is higher than when predefecation is carried out at the higher pH. SUG. IND. ABSTR. (E. M. J.).

Crystallisation of low grade products with continuous addition of mixing water. J. Hrubíšek (*Listy Cukr.*, 1955, 297—300).—The exhaustion of low-grade beet sugars, completed largely in the crystallisers, reaches optimum processing if the syrup supersaturation is brought to 1·4 by water addition on dropping, and is maintained at that figure; the results depend on the amount of water added and the time of addition. Improved results were obtained if the calculated amount of water was added gradually during dropping and crystallising to keep the supersaturation steady. The water was added from jets over the whole length of the cooler. Compared with the usual method, molasses purities were reduced from 64·0 to 63·4 or from 63·9 to 62·9. An equation to determine the coeff. of the efficiency of exhaustion is given. SUG. IND. ABSTR. (E. M. J.).

Buffered paper chromatography of sugar and related substances. II. S. N. Parikh and A. N. Godbole (*J. Maharaja Sayajirao, Univ. Baroda*, 1955, **4,** 7—13; cf. J.S.F.A. Abstr., 1955, i, 314).—R_F values of 13 sugars and related substances are listed: (*a*) obtained on plain paper with phenol–water and ethyl acetate–pyridine–water systems; (*b*) phenol–water, paper buffered with Na acetate–HCl to give pH in the range of 0·65—4·95; (*c*) paper buffered with borate–KCl–NaOH to give pH 8, 9, 10; (*d*) paper impregnated with universal buffers to give pH ranging from 1·99 to 11·91; (*e*) ethyl acetate–pyridine–water, paper impregnated with borax–succinic acid to give pH 3, 4, 5, 5·8; (*f*) buffered with phosphate to pH 5·91, 6·98, 8·04; (*g*) phenol–water, buffered in seven different ways. These findings are discussed. The effect of the buffers on R_F values appears to be caused by complex formation. E. M. J.

Low oxygen gas storage trials of apples in Tasmania. D. Martin and J. Cerny (*Div. of Plant Ind., Commonw. sci. industr. Res. Org. Aust., Tech. Pap.* 1956, No. 6, 19 pp.).—In many varieties of Tasmanian apples susceptibility to scald and breakdown is increased when stored in gas mixtures containing 5% of CO_2 and 16% of O_2. Low O_2 concentrations (3%) in the absence of CO_2 have allowed the use of low storage temp. without increasing the susceptibility to low-temp. breakdown; and have given good control of softening, superior texture and reduced wastage from disorders and rots, and have reduced markedly the proportion of soluble pectin. The method was suitable for many varieties including Cox, Cleopatra, Granny Smith, Delicious, etc.; gave better results than storage in gas mixtures of 5% CO_2 and 16% of O_2 with varieties susceptible to scald and breakdown, and as good for varieties resistant to these disorders. With deep scald of Jonathan, incidence was less in low O_2 treatment, than in CO_2 treatment. (17 references.) E. M. J.

Damage to Colorado Elberta peaches during harvesting. C. J. C. Jorgensen (*Colorado agric. Exp. Sta., Tech. Bull.* 56, 32 pp.).—The extent of damage caused to peaches at various stages (picking, sorting, etc.) in the harvesting of peaches packed in both bushels and in boxes is reported. The extent of damage varied considerably from year to year. A. H. CORNFIELD.

Mango varieties grown in Hawaii and their suitability for freezing. K. J. Orr and C. D. Miller (*Hawaii agric. Exp. Sta.*, 1955, *Tech. Bull.* 26, 24 pp.).—Physical and chemical characteristics (including ascorbic acid content) of the fruit of a no. of varieties of mango are described. The effect of various methods of freezing on the organoleptic properties of the fruit are reported. A. H. CORNFIELD.

Determination of fruit juice in fruit beverages and turbid lemonades. E. Benk (*Brauwelt*, 1956, **96, B,** 119—120).—Adulteration of the beverages or the use of juices which have deteriorated through fermentation can readily be detected by means of the formol titration. The beverage or diluted (1 : 9) fruit juice is treated with active C and filtered before titration. Elimination of H_2SO_3 or its salts (which interfere with the results) can be achieved either by evaporating 100 ml. of the filtrate (made faintly acid to phenolphthalein) down to 10 ml., or by the addition of H_2O_2. A third method (due to Sciacca, and used in Switzerland and Italy) is also described. Formol titration values (min. and max.) obtained by the author and by Swiss and Italian investigators are given for the juices of lemons, oranges (several varieties), grapefruit and other fruits. The manufacture of the beverages should be controlled by the analysis of the juices used as raw materials and of the bottled products. P. S. ARUP.

Use of preparations containing silver for sterilisation of sugar-containing liquids. [A] —. Walter. [B] U. Kutscher (*Mschr. Brauerei, wissen. Beil.*, 1955, **8,** 161—162, 162—163).—[A] A criticism of Kutscher's paper (cf. *ibid.*, 1955, No. 8) in which the efficacy of prep. of Ag for sterilising lemonades, etc. is denied. [B] A reply to the above criticism. P. S. ARUP.

Analysis of scores for bitterness of orange juice. G. G. Coote (*Food Res.*, 1956, **21,** 1—10).—The development of a bitter taste resulting from the presence of limonin and precision of scoring by taste panel are discussed in connexion with orange juice canning. (12 references.) E. M. J.

Texture changes during the dark-ripe processing of olives. C. Sterling (*Food Res.*, 1956, **21,** 93—102).—The effects of the brining operation, the treatment of the olives with lye solutions, the leaching of the lye from the olives, salting in 2·5% NaCl filling solution and

heating in retorts at 240—250°F. are discussed. Increase in firmness of the cooked or canned fruit tissues may be related to increasing de-esterification (plus Ca neutralisation of the newly-formed free carboxyl groups) of the pectic material of the cell walls, and to the dehydration rôle of the solutes in the holding brine. The lye treatment during the dark-ripe process may result in further de-esterification and depolymerisation of pectic substances. (15 references.) E. M. J.

Acid-forming bacteria from cucumber fermentations in Michigan. R. N. Costilow, F. M. Coughlin, D. L. Robach and H. S. Ragheb (*Food Res.*, 1956, **21**, 27—28).—From 347 brine samples of 84 cucumber fermentations, 848 cultures of acid-forming bacteria were obtained; 284 were classified as *Pediococcus cerevisiæ*, 333 as *Lactobacillus plantarum*, 188 as *L. brevis* and the remaining 43 comprised a miscellaneous group one of which was *Leuconostoc mesenteroides*. (13 references.) E. M. J.

Direct microscopical enumeration of bacteria in the washings of green beans. J. O. Mundt (*Food Res.*, 1956, **21**, 21—26).—A study of stains and procedures adaptable to the staining of bacteria in suspensions prepared from green beans was made, the stain being used to evaluate quality. Hucker's modification of crystal violet stain gave the best results. (13 references.) E. M. J.

Frozen pea reference standard for taste tests involving storage. A. C. Ward and M. M. Boggs (*Food Technol.*, 1956, **10**, 117—119).—When peas were handled and processed under excellent conditions, judges could not distinguish between pairs of samples: (*a*) packs stored in air and in N_2 for 13 months at −60°F; (*b*) packs stored in air at −30°F. and at −60°F. for 13 months; and (*c*) packs in air or N_2 stored 13 months at −60°F. and freshly prepared packs of comparable raw material. Air packs, hermetically sealed, of frozen peas stored at −30°F. or below are recommended for controls in taste panel work when storage time does not exceed 13 months. E. M. J.

[A] **Factors affecting growth of *Bacillus coagulans* in tomato juice.** [B] **Heat-activation of bacterial spores.** N. W. Desrosier and F. Heiligman. **Spore germination. I. Activators. II. Inhibitors.** F. Heiligman, N. W. Desrosier and H. Broumand (*Food Res.*, 1956, **21**, 47—53, 54—62, 63—69, 70—74).—[A] The spores of *B. coagulans* are capable of germinating and growing in tomato juice if they are inoculated immediately after heat treatment, or if the pH of the juice is increased. If the spores are heated and then stored at 2—4°, no spoilage occurs, but the same spores will spoil tomato juice if the pH is increased or if KH_2PO_4 is added.

[B] In tests on the effect of preheating on the germination of *Bacillus thermoacidurans* F.S. 787, *Bacillus globigii* and P.A. 3679 spores, there was a time–temp. relationship for obtaining a max. stimulation of germination activity. Results suggest that sub-lethal heating may supply the energy of activation or increase the rate of various reactions which precede or are necessary for the germination process. (18 references.)

I. Spores could germinate in a substrate containing only carbohydrate or its related compounds which act as a source of energy. Available phosphate played an important rôle in spore germination. Spores did not respond to fatty acids as a source of energy for germination. Out of amino-acids tested, only L-alanine had any stimulating activity on the germinating process. The highest rate of germination occurred when glucose, L-alanine and adenosine were combined in the substrate. (16 references.)

II. The effects of respiratory inhibitors on the germination of *B. thermoacidurans* F.S. 787 spores were studied. Malonic acid can inhibit the metabolism of citric and succinic acids, but has little effect on fumaric and malic acids in yielding energy for spore germination. The stimulatory action of either glucose or sodium pyruvate on spore germination can be inhibited by materials which inhibit the oxidation of glucose or pyruvate, although the patterns of inhibition differ. The presence of 2 : 4-dinitrophenol, Na arsenate, Na azide, Na arsenite, Na fluoride or iodoacetic acid reduced the availability of certain energy-yielding metabolic pathways, substantially retarding spore germination. (14 references.) E. M. J.

Effect of temperature and acidity on dry storage of sodium carrageenate. D. A. I. Goring (*Canad. J. Technol.*, 1956, **34**, 39—41).—The degradation and decreased η of dried carrageenin on storage for 1—1½ years has been confirmed on samples dialysed (with control of pH) to form Na carrageenate or free carrageenic acid. The degradation was followed by the change in η of freshly prepared solutions (in acetate buffer) of samples (pH 3, 5, 6·9 and 9·5, respectively) stored at 25°, 4° and −13°. Increase of storage temp. or of acidity accelerates the rate of degradation and the decrease in η. Solid extracts prepared from solutions of pH 9—10 can be stored at $<4°$ for long periods without change in η. The marked instability of carrageenic acid is also confirmed. W. J. BAKER.

Practical notes on the determination of aldehydes in rectified spirits by the official method. T. Caldemaisous (*Industr. aliment. agric.*, 1955, **72**, 797—800).—The purity requirements and methods of preparation of the reagents used in the official method, i.e., basic fuchsine, aldehydate of ammonia, 50% aldehyde-free alcohol, and $NaHSO_3$, are defined and details of manipulation, including the preparation of aldehyde standards and the detailed procedure of analysis, are described. J. S. C.

Influence of barley-husk in brewing technique. I. Small-scale malting experiments. K. Schuster. **II. Experimental brewings with grist of varying husk content.** G. Krauss (*Brauwelt*, 1956, **96, B,** 65—71, 93—100).—I. Clarification of the wort is prolonged by decreasing the husk content in the grist (15—5%) or by increasing the fineness of the husk. The contents and properties of the sol. constituents of husk, and their influence on the composition of the wort are fairly uniform for husks from different sources. A formula is given for predicting the extract yield from the % of husk and of the protein in the grist. Reducing the husk content and increasing its fineness offer no advantages. (23 references.)

II. The effects on wort composition and beer quality of variations in the husk content (7·5—~22%) of the grist are examined. The effects on the composition of the compounds of N are irregular. The introduction of 10% of husk has no effect on the formation of chill- or shaking-haze or on foaming capacity. The effect on the taste of the beer is slight, with an inclination to harshness which becomes somewhat more noticeable on increasing the husk to 22%. P. S. ARUP.

Evaluation of brewing qualities of barley and malt. H. Kieninger (*Brauwelt*, 1956, **96, B,** 53—58).—The experimental small-scale malting equipment at Weihenstephan is described. Methods for the physical and chemical examination of barley and malt are described and examples are given of varietal and seasonal variations in the results. P. S. ARUP.

Control of corn-beetles and other pests with regard to brewing and malting requirements. B. Mändl (*Brauwelt*, 1956, **96, B,** 25—30).—A review covering types of pests, types of insecticides, their suitability, mode of action, and application, and the development by pests of resistance to insecticides. The examination of insecticides for non-interference with malting and brewing when applied to barley or malt is dealt with in detail. P. S. ARUP.

Modern treatment of boiler water. L. Macher (*Brauwelt*, 1956, **96, B,** 1—7).—A review covering methods for softening and otherwise purifying water for use in boilers, and methods for the analytical control of impurities in water and steam. P. S. ARUP.

Determination of inorganic phosphoric acid in wort and beer. C. W. Naumann (*Brauwissenschaft*, 1956, **9**, 49—50).—In a laboratory fermentation of 10% wort, 15% (only) of the available inorg. P was absorbed by the yeast. The statement by Schönfeld that yeasts show normal contents of P even in 2—3% worts is thus confirmed. P. S. ARUP.

Nitrogenous constituents of brewing materials. V. Action of selected precipitants on brewers' worts. VI. Use of ion-exchange resins in fractionating the nitrogen compounds of brewers' worts. VII. Complex nitrogen compounds in unhopped wort. E. M. Shooter (*J. Inst. Brew.*, 1956, **62**, [New Series, **53**], 31—38, 38—51, 51—56).—V. The Lundin system of wort analysis (*Woch. Brau.*, 1931, **48**, 347, 357) was found to give reproducible results. It is based on pptn. of proteins by tannic acid and of proteins and peptides by phosphomolybdic acid. Results obtained were closely comparable with those obtained with $MgSO_4$ for proteins and uranyl acetate for proteins and polypeptides (Myrbäck, *ibid.*, 43). The method was used to study the influence of prolonged mashing and of fermentation on the composition of worts. Prolonged mashing appears to increase the nitrogenous constituents whereas, during fermentation, the nitrogenous fractions of worts diminish, chiefly by assimilation of simpler compounds. (35 references.)

VI. Amino-acids and some simple peptides were adsorbed on cation-exchange resins and fractionally eluted in an unchanged condition with aq. NH_3. The more complex nitrogenous compounds were, however, partially degraded by hydrolysis in the process. A no. of amino-acids and simple peptides, thus prepared, or prepared by absorption on C, were examined by paper chromatography: some were identified and a no. of new compounds, as yet unidentified, were revealed. (24 references.)

VII. Four fractions were prepared by pptn. of a standard unhopped wort with $(NH_4)_2SO_4$, their general composition established, and submitted to electrophoretic analysis. They contained more non-proteins than did the corresponding fractions from a saline extract of barley. Electrophoretic analysis showed the wort fractions to be less complex than the corresponding barley fractions. (10 references.) J. S. C.

Nitrogen metabolism of yeast. W. Hoppe (*Brauwissenschaft*, 1956, **9**, 34—41).—Separation, determination and analysis of the N given up as amino-acids to water or liquid media by brewing yeasts is effected by exchange-column and buffered filter-paper chromatographic techniques (described). Calculated on the total yeast-N, the N given up to water during six days is $<2\%$. The N yielded by fermenting yeast to aq. 10% sucrose is approx. three times that yielded to aq. 10% lactose, and approx. nine times that yielded to water. Brewing yeasts (three types) in an active state of growth yield approx. half the amount of N to the medium in comparison with the amount yielded by the same yeasts in a starved condition. (18 references.) P. S. ARUP.

Comparative investigations of cocci of various origins. S. Strese and S. Windisch (*Mschr. Brauerei, wissen. Beil.*, 1955, **8**, 151—160; cf. *ibid.*, 141).—Cocci isolated mainly from beer and from horse-urine are classified (largely according to Bergey's system) into 10 groups. Morphological characteristics vary considerably with cultural conditions, especially the pH of the medium. In addition to the usual cultural characteristics the ability to assimilate (as distinct from fermentative capacity) various sugars has proved a useful distinctive criterion. Optimum pH ranges are also distinctive, but the possibility of adaptation to the lower pH range of beer must be taken into account. Appreciable similarities are found between cocci isolated from horse-urine and those found in beer. Types isolated from air do not occur in beer or urine. (34 references.) P. S. ARUP.

Detection of wild yeasts by culture in wort containing tartaric acid. S. Windisch (*Mschr. Brauerei, wissen. Beil.*, 1956, **9**, 3—5).—Results of tests with 163 yeasts show that some wild yeasts tolerate much higher and some much lower concn. of tartaric acid than do culture yeasts. Morphological alterations under the influence of the acid rule out microscopical identification. P. S. ARUP.

Pure culture of micro-organisms. S. Windisch (*Mschr. Brauerei, wissen. Beil.*, 1956, **9**, 17—19).—The nature and methods of producing pure cultures of micro-organisms are discussed, a pure culture being one which contains one species only, or a culture obtained by selective growth of one species from a mixed culture. The variation of the length of life of individual cells in a mixed culture, the repression of a certain type so that it apparently disappears, but in reality is maintained in united cells, until change of culture conditions permits growth, are discussed. The view of Kleber and Hoffmann that cocci in beer originate from yeast cells is considered, but there is no proof by microscopical findings to support the view. Further study without reference to the origin of cocci with resemblance to "beer-sarcinæ" is recommended. (17 references.) E. M. J.

Beer-yeast in the light of results of recent researches. F. Kretschmer (*Brauwelt*, 1955, **95, B**, 1719—1726).—A review covering yeast genetics (with reference to the work of Winge and of Lindegren), the antibiotic and probiotic phases of the yeast fermentation, the relationship (according to Shimwell) of beer-sarcinæ to *Streptococcus damnosus*, and evidence against Schandler's theory of the transformation of yeasts into micrococci. (33 references.) P. S. ARUP.

Comparison of methylene-blue and acridine-orange methods with culture method as proof of survival or death of various yeasts after injury by various methods. II. H. Ketterer (*Brauwissenschaft*, 1956, **9**, 59—64; cf. J.S.F.A. Abstr., 1956, i, 278).—Agreement between the results of the two staining methods and the culture method is observed after treatment of the yeasts with aq. $HgCl_2$, but not after treatment with $AgNO_3$ or quaternary NH_4 compounds. The staining methods overestimate the no. of surviving cells and are not in mutual agreement. (18 references.) P. S. ARUP.

Comparative studies of hop analysis. I. Estimation of moisture content. II. Estimation of humulone and other resin constituents. L. R. Bishop (*Mitt. VersSta. Gärungsgew.*, 1955, **9**, 151—169).—I. cf. J.S.F.A. Abstr., 1956, i, 41; II. cf. *ibid.*, i, 167. E. M. J.

Charcoal method for estimation of the content of humulones in hops. W. H. Gough (*J. Inst. Brew.*, 1956, **62**, [New Series, **53**], 9—15).—The ether extract of hops is treated with charcoal, which has undergone preliminary treatment with SO_2, the colouring matter being readily removed and the resins left unabsorbed. After transfer to pentane, the humulones are determined by polarimetry. J. S. C.

Evaluation of hops. III. Convenient method for polarimetric determination of the α-soft resin of hops. R. D. Hall (*J. Inst. Brew.*, 1956, **62**, [New Series, **53**], 16—19).—The standard method of polarimetric estimation of total "humulones" in the α-soft resin of hops (Ford and Tait, *ibid.*, 1932, 351) is modified by decolorisation of the methanol extract with activated carbon, enabling concentrated solutions to be examined and improving stability. J. S. C.

Evaluation of hops: new approach to the detailed analysis of hop resins. G. A. Howard and A. R. Tatchell (*J. Inst. Brew.*, 1956, **62**, [New Series, **53**], 20—27).—Procedures of gas–liquid partition chromatography of the mixture of fatty acids derived from oxidation of the α-soft resin of hops enable cohumulone, humulone and adhumulone to be quickly and accurately estimated. Similar procedures can be used for determining colupulone, lupulone and adlupulone in the "β-acids" or, alternatively, liquid–liquid partition methods may be used. (14 references.) J. S. C.

Effect of some constituents, especially the colloids, of malt extract on course of hop-boiling. V. Salač, M. Kotrlá-Hapalová and M. Vančura (*Brauwelt*, 1956, **96, B**, 189—196).—The proteolytic prep. Collupulin is chosen (in preference to four other prep.) for experiments on the effects of modification of the wort proteins. The best results (with average hop additions) as regards utilisation of the hop-bitters and the general quality of the resulting beer are obtained with the use of 1 g. of the prep. per l. (the smallest amount tried) of first wort, which gives a ratio of the Lundin protein fractions A : B : C of 3 : 2 : 4—5 in the wort, and 1 : 1—2 : 6—7 in the beer. The proteolytic prep. appears also to have an amylolytic effect on the complex carbohydrates. Collupulin increases colloidal and biological stability, but excessive pptn. of the proteins of high and medium mol. wt. tends to spoil the taste of the beer. P. S. ARUP.

Solubility of carbon dioxide, oxygen and nitrogen in beer and water. ii. C. Enders, W. Kleber and E. Paukner (*Brauwissenschaft*, 1956, **9**, 50—58; cf. J.S.F.A. Abstr., 1956, i, 279).—The solubilities of the gases in beer are less than those in water. The gases are slightly more sol. in water containing 4% of EtOH than in pure water, but solubilities decrease with increasing amounts of dissolved extract. At temp. $<60°$, increases in solubility due to increased pressure are less than the values calculated according to Henry's law, but reversal of this relationship occurs in the range 60—80°. The solubility of CO_2 in beer is a purely physical relationship, independent of any binding effect of the colloids. The higher η and γ of the beer as compared with these values for water, however, reduce the tendency to form bubbles. Saturation-pressure equilibrium in bottled beer at rest is reached after several weeks. With increases in the phase-limiting surface, and agitation, the equilibrium is quickly established. (126 references.) P. S. ARUP.

[A] **Redox titration of beer with [added] ascorbic acid.** K. Raible. [B] **Provisional short reply to the above paper.** W. Kleber and W. Heyer (*Brauwissenschaft*, 1956, **9**, 42—49).—[A] The Kleber and Heyer method (cf. *Brauwelt*, 1953, **93**, 496) is critically examined. For reasons given, the values obtained do not represent true stability values.

[B] The validity of the above findings is briefly contested. P. S. ARUP.

Measurement of the surface tension of beer by the drop-weight method. A. D. Rudin (*J. Inst. Brew.*, 1956, **62**, [New Series, **53**], 27—30).—The surface tension of beer was measured by a modification of the drop-weight method, using a micrometer syringe consisting of a screw micrometer with 25-mm. movement, subdivided to give readings to 0·01 mm., for the purpose of determining the drop vol. Variations of surface tension with the time taken to form a drop, due to development of surface viscosity, were observed and are discussed. (13 references.) J. S. C.

Comparative tests of moisture ovens. Analysis Committee, Institute of Brewing (*J. Inst. Brew.*, 1956, **62**, [New Series, **53**], 7—9).—A series of tests with different types of drying ovens, in comparison with the boiling-water oven used in the Standard Method of the Institute of Brewing, was carried out. The tests were based on the dehydration of $CuSO_4,5H_2O$ under standard conditions. It was found that only the boiling-water oven gave consistent results and that the method of testing used can distinguish many serious errors in ovens but cannot distinguish those which arise from variations in the humidity of the air in the oven. J. S. C.

Detection and determination of dehydroacetic acid in beverages. J. A. Gautier, J. Renault and H. Ghadichah (*Ann. Falsif., Paris*, 1956, **49**, 7—21).—The determination of dehydroacetic acid (6-methyl-3-acetopyran-2-one) in pure solutions, its extraction, and its detection in wines and beers, are reviewed. The methods particularly considered are: formation of the dinitrophenylhydrazone, pptn. of the Cu^{++} salt, examination of crystals obtained by sublimation, and determination of m.p. by the Köfler microscopic apparatus. (17 references.) J. S. C.

Heating of milk at very high temperatures. J. R. Cuttel (*Industr. aliment. agric.*, 1955, **72**, 803—806; translated from *Dairy Industries*, 1954, **19**, 917—919).—The ultra-high-temp. pre-heating process for milk is reviewed and discussed (cf. Williams *et al.*, J.S.F.A. Abstr., 1956, i, 168). A detailed layout of a typical plant is given in diagrammatic form and described. J. S. C.

Chemistry of milk fat. E. L. Jack and L. M. Smith (*J. Dairy Sci.*, 1956, **39**, 1—25).—The chemistry of the triglycerides of milk fat and of their constituent fatty acids is reviewed and critically discussed. The amounts of non-glyceride components in milk fat are given. (180 references.) S. C. JOLLY.

Reduction of fat separation in evaporated milk. A. F. Tamsma and N. P. Tarassuk (*J. Dairy Sci.*, 1956, **39**, 26—35).—During the first 2 months of 6-months' storage at room temp., η of evaporated milk decreased to an approx. constant value if the cans were not periodically inverted; approx. 5% of the fat rose to the top portion per month. Commercially evaporated milk varied considerably in η and homogenisation effectiveness. Enzymic treatment before sterilisation increased average η to about 1·5 during storage and approx. halved the extent of creaming. With some enzymes the decrease in creaming was less than could be attributed to increase in η and was probably partly due to adsorbed material on the fat-globule surface. The use of enzymes was limited by the heat stability of the milk. Increased homogenisation effectiveness obtained either by rehomogenisation or by separation of the larger fat globules approx. halved the extent of creaming. S. C. JOLLY.

Colloidal proteins of skim milk. I. Effect of heat and certain salts on the centrifugal sedimentation of milk proteins. L. F. Edmondson and N. P. Tarassuk (*J. Dairy Sci.*, 1956, **39**, 36—45).—Heating skim milk at 88° for 15 min. decreased total-N sedimentation for centrifuging periods $\gtrsim$40 min. at 26,000 g., after which time sedimentation increased compared with unheated milk. These effects are probably due to a change in size distribution of the caseinate complex and sedimentation of part of the heat-denatured serum proteins in addition to the increased η caused by heating. Caseinate sedimentation rate in the raw milk was markedly decreased by 0·15% of Na_2HPO_4, due partly to increase in η and partly to decreased particle size or increase in degree of hydration; heating at 88° for 15 min. further decreased sedimentation rate with pronounced increase in η. Total-N sedimentation was increased by increasing Ca content of raw milk $\gtrsim$3-fold by addition of $CaCl_2$, but $\gtrsim$6-fold increase in Ca caused no further effect. Whey proteins of raw milk were unaffected by centrifugation; in heated milk part of the heat-denatured whey proteins was removed with the caseinate complex. S. C. JOLLY.

Rapid silica gel method for measuring total free fatty acids in milk. W. J. Harper, D. P. Schwartz and I. S. El-Hagarawy (*J. Dairy Sci.*, 1956, **39**, 46—50).—The chromatographic method of Harper and Armstrong (*ibid.*, 1954, **37**, 481) for the determination of butyric and higher fatty acids has been modified so that the total amount of free fatty acids in milk or cream is recovered in one fraction using a single mixed solvent in ~15 min. without prior extraction of the fat. The method is more quant. than are previously suggested methods for recovering free fatty acids in milk. S. C. JOLLY.

Heat stability of vitamin A in ghee and vanaspati. G. S. Hattiangdi and K. F. Kanga (*J. sci. industr. Res.*, 1956, **15, C**, 48—51).—The stability of vitamin A in ghee and vanaspati at approx. levels of 650 i.u. per oz. was examined in tests based on deep frying (200°) shallow frying (220°) and simmering (100°). The losses in potency were greatest in shallow frying and least in simmering. The losses were greater in ghee than in vanaspati. Normally "Vanitin" contains DL-α-tocopherol and lecithin as stabilisers. When butylated hydroxyanisole is incorporated as an additional antioxidant, the stability of the vitamin-containing vanaspati is improved. E. M. J.

Method for braising beef round steaks. P. Paul and M. Bean (*Food Res.*, 1956, **21**, 75—86).—On the basis of data obtained from many trials the following method for braising is suggested: the steak is browned at 475°F. for 1 min. on each side; 50 ml. of water are added, the pan is covered, placed on a rack with ½-in. legs in an oven preheated to 250°F. and the steak is cooked for ½ an hour longer than required to bring the internal temp. of the steak to 98°c. E. M. J.

Hydroxyproline content of animal tissues. J. N. Aronson and C. A. Elvehjem (*Food Res.*, 1956, **21**, 109—116).—A modification of the method of Troll and Cannan, based on the reaction of hydroxyproline and proline with ninhydrin in phosphate buffer (pH 7·0) to yield a red pigment which can be extracted with benzene, was used to determine the hydroxyproline content in animal tissues. A quant. estimate is made by comparison of the absorption at 570 mμ, the max. for hydroxyproline, and 550 mμ., the max. for proline. Muscle tissues from beef, veal, pork and lamb, contained approx. 0·9 g. of hydroxyproline per 16 g. of N. The relation of the hydroxyproline to tissue collagen is discussed. (13 references.) E. M. J.

Cathode ray irradiation of chicken meat for the extension of shelf life. B. E. Proctor, J. T. R. Nickerson and J. J. Licciardello (*Food Res.*, 1956, **21**, 11—20).—Tests were made to determine whether cut-up chicken meat could be treated with high-voltage rays to extend the storage life at refrigerator temp. above freezing and prevent the growth of spoilage bacteria during shelf storage. Treatment of samples at 2×10^6 rep gave results not significantly different in flavour from controls in four out of six trials, but at $2{\cdot}5 \times 10^6$ rep six irradiated samples were significantly different from controls in every case. In 1·5 g.-samples containing 260 spores of *Clostridium sporogenes* per g. sterility was achieved with $1{\cdot}5 \times 10^6$ rep. E. M. J.

Chemistry of cured meat pigment fading. H. N. Draudt and F. E. Deatherage (*Food Res.*, 1956, **21**, 122—132).—Several possible mechanisms of colour changes in cured meat pigments involving the nitric oxide derivative of either myoglobin or denatured myoglobin are discussed. In the presence of visible light and air or air alone, part of the nitric oxide of purified heat-denatured globin nitric oxide myohæmochrome is further oxidised to give nitrite and nitrate ions. Nitric oxide from the pigment of cured meat can be lost as gas, as nitric oxide or nitrogen dioxide. A gas (probably CO_2) adsorbed by KOH solutions, is produced in the oxidation of hæmochrome in air and intense light. O_2 adsorption is associated with loss of nitric acid from the pigment and oxidation of the resulting hæmochrome. Denatured globin hæmochrome shaken in the presence of free fatty acids undergoes darkening not observed in light. Oxidation of the hæmochrome may be of significance in cured meat colour deterioration. (16 references.) E. M. J.

Spoilage of fish in the vessels at sea. II. Treatment on the deck and in the hold. C. H. Castell, W. A. MacCallum and H. E. Power (*J. Fish Res. Bd. Canada*, 1956, **13**, 21—39).—The effects of various kinds of treatment and handling of haddocks in trawlers at sea on spoilage were examined using pH values, trimethylamine values, bacterial counts and organoleptic tests. It was found that two conditions were major causes of accelerated spoilage: (1) treatments which caused very heavy initial contamination such as storing fish in contact with slimy wooden pen boards, and (2) treatments that increased the temp. of the fish either by considerable rises for short periods or smaller increases for a long period, such as might result from inadequate or inefficient icing. J. S. C.

Frozen oysters. M. L. Morton and W. J. Dyer (*J. Fish. Res. Bd. Canada*, 1956, **13**, 47—51).—Shucked oysters were (1) slowly frozen and stored at −12°, or (2) quickly frozen and stored at −23° with desiccation held to a minimum. Organoleptic assessments indicated that the former deteriorated and the latter suffered little change in either texture or taste. When stewed or deep fried, the deterioration in (1) can be masked. J. S. C.

3.—SANITATION

Insecticides and repellents for the control of insects of medical importance to the armed forces. Anon. (*U.S. Dep. Agric.*, 1955, *Circ.* 977, 91 pp.).—Control measures for mosquitoes, flies, human lice and scabies, bed bugs, cockroaches, fleas, ants, sand-flies, black flies, ticks and chiggers are described together with details of repellents and the toxicology of both these and of insecticides. (15 references.) E. G. BRICKELL.

Poison bait for the control of house flies on military reservations. J. C. Keller (*J. econ. Ent.*, 1955, **48**, 528—529).—Daily applications of a Chlorthion (1 or 2%)-granulated sugar bait gave excellent control of flies on military installations. A. A. MARSDEN.

DDVP as a toxicant in poison baits for house-fly control. J. W. Kilpatrick and H. F. Schoof (*J. econ. Ent.*, 1955, **48**, 623—624).—When used in dairy barns and calf pens a bait of 0·1% DDVP (*OO*-dimethyl 2 : 2-dichlorovinyl phosphate) in 10% sugar solution was highly effective against house flies for approx. 48 hr. Frequent applications were necessary to insure continuous low fly populations. A. A. MARSDEN.

Poison baits for the control of blow flies and house flies. J. C. Keller, H. G. Wilson and C. N. Smith (*J. econ. Ent.*, 1955, **48**, 563—565).—Liquid baits containing sweetening materials (malt, sugar, or blackstrap molasses) and suitable concn. (1 and 2%) of insecticides were highly effective in controlling blow flies or combined populations of blow flies and house flies. No consistent difference in the effectiveness of the following four insecticides was observed against blow flies: Shell OS 2046 (1-carbomethoxy-1-propen-2-yl dimethyl phosphate), Shell OS 1808 (1 carbomethoxy-1-propen-2-yl diethyl phosphate), Bayer L13/59, and Chlorthion. Against house flies, Bayer L13/59 was consistently rather more effective than the other materials. Repeated daily applications are recommended for good control. A. A. MARSDEN.

Relative toxicity of six insecticides to two strains of the house fly. M. W. Marsh and W. G. Eden (*J. econ. Ent.*, 1955, **48**, 610—611).—When determined by topical action to house flies, one strain of house flies was 14 times more resistant to DDT than was the other. The LD_{50} to both strains of flies were determined for methoxychlor, chlordane, heptachlor, Chlorthion, Diazinon and Isochlorthion. With the exception of Diazinon there was no difference between the LD_{50} of the two strains. A. A. MARSDEN.

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