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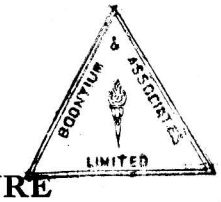

ENTRY OF FUNGI AND CHEMICAL SUBSTANCES IN SOLUTION INTO MATURE CEREAL GRAINS

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The literature on the subject is reviewed.

The layers of tissue enveloping mature cereal grains are considered in respect of their influence on the selective permeability of the grain. The nature of the semi-permeable system is discussed, as are its relation to grain viability, response to treatment with fungicides, and the possibility of acceleration of germination by such treatment.

THIS paper is a review and discussion of the literature and of the author's own work on the influence of the semi-permeability of mature cereal grains on the entry of fungi and chemical compounds in solution.

I. Functional significance of the outer layers of mature cereal grains: resistance to the entry of fungi

The husk and pericarp of the stored mature grain are composed of dead tissues, most of which have been much distorted and compressed by the pressure exerted by the swelling of the grain contents. Sub-epidermal fungi (many of which have been identified) have been found to be present in the outer layers of wheat.[1–3] Such sub-epidermal mycelia were most abundant in grain from a wet season, a layer of loosely packed hyphae lying between the exocarp and the endocarp in the space left by the disintegration of the mesocarp. None of the mycelium was found, however, to have penetrated into or through the living aleurone layer of undamaged wheat grain. When grains have been mechanically injured and the interior exposed, under damp conditions fungal mycelia, whether of sub-epidermal or other origin, develop on the injured area; Caldwell & Davies[4] photographed examples of this.

When the husk and pericarp of barley are removed by steeping the grains in sulphuric acid, increased attack by fungi in subsequent germination tests has been reported by Caldwell & Hampson,[5] while Meredith[6] has shown that the increased susceptibility of such grains to mould development is an important factor as regards the malting quality of peeled barley. One may conclude that these tissues when intact serve as a major factor in resistance to attack by fungi.

Myers[7] came to the same conclusion as to the value of undamaged pericarp of dent maize; he found that, where soil conditions favoured the growth of fungal spores, injuries to such maize seed reduced the stand of the crop. By use of a cold-germination technique to check the relation between percentage of field corn kernels with damaged pericarp over the embryo and the percentage germination of samples Tatum & Zuber[8] obtained a significant correlation.

The pericarp can only function effectively as a defence mechanism against fungal infection of the interior of the grain when it is intact. Hence the importance of the observation made by W. N. Brown[9] that with maize grains blistering of the pericarp predisposes to injury during harvesting and processing. He found that grains blistered on both germinal and abgerminal surfaces were usually large and plump, those blistered only over the scutellum were smaller, while grains with no blisters were smaller yet. Wortman & Rinke,[10] in comparing susceptibility of two dent maize hybrids to pericarp injury during harvesting and processing, showed that one hybrid was much more susceptible to such injury.

Another function of the pericarp is suggested by the results obtained by Gopalkrishna[11] working with sweet corn seed. After the seeds had been injured either physiologically by heat or physically by puncturing of the pericarp, the amount of water-soluble reducing substances was closely related to the vigour of seedlings subjected to a cold test. In the light of this it is possible that not only does breakage of the pericarp remove a physical impediment to fungal attack, but also it may, if sufficient moisture is available, yield extra nutriment to the fungi at a critical period in their establishment.

Between the pericarp and the tissues of the grain interior lies the testa, which if we take the inner integument and the chalaza together forms the innermost of the tissues surrounding the entire grain, for both the nucellar and aleurone layers when they reach the germ reduce in size until they become untraceable. R. Brown[12] demonstrated that the single layer of the testa and the cuticle-like membranes on its inner and outer surfaces form a semi-permeable system around the wheat grain. Similarly, the selective permeability of the barley grain was found by Tharp[13] to be due to the persistent crushed and cutinised inner integument along with the suberised, resistant tissue of the chalaza. The relation of the testa to resistance to infection by the fungus *Gibberella saubinetti* was studied in wheat by Pugh *et al.*[14] Resistance to the infection increased with the age of the grain and appeared to be proportional to the thickness of the membranes; the outer membrane of the testa was the most resistant layer of the kernel. The influence of grain moisture content and storage temperature on mould development in stored grain is well known;[15] these factors affect both permeability and resistance to fungi.

The testa of wheat has been described by R. Brown[12] as mucilaginous in nature and the membranes as containing much true fat, while Pugh *et al.*[14] refer to the mature tissue as a narrow, brown, oily layer bordered by membranes that stain with Sudan III and which is relatively resistant to sulphuric acid. Tharp[13] found that the inner integument of barley has a thin inner cutin membrane and a much thicker outer layer of cutin of variable thickness, the thickness of these membranes varying with the variety of barley studied. It is of interest that one of the methods used to determine grain quality is the measurement of fat acidity[16, 17] and that such acidity has been attributed to the activity of fungal lipase by Dirks *et al.*[18] who also reported a non-dialysable heat-labile inhibitor of mould lipases in a water extract of wheat germ.

II. Functional significance of the outer layers of mature cereal grains: semi-permeability to chemical substances in solution

Adrian J. Brown[19] demonstrated that the grains of barley possess the property of selective permeability and that this also occurs in those of wheat, oats and rye.

In an investigation of the absorption of solute from aqueous solution by wheat grains R. Brown[12] found that the semi-permeable layer consists of a single layer of the testa and the cuticle-like membranes on its inner and outer surfaces, the membranes containing more true fat than would occur in the cuticle of a normal foliage leaf. He concluded that the factors in the seed regulating absorption of solute would seem to be electrical adsorption (cuticle-like membranes), mechanical adsorption (testa, endosperm, embryo), imbibitional pressure developed in the endosperm, and the size of the intermolecular spaces of the semi-permeable membranes, each solution reacting to each of these factors according to its own properties.

Of the testa, hyaline layer and aleurone layer surrounding the interior of the wheat grain Hinton[20] found that it is the testa that offers the greatest resistance to intake of water. He was unable to establish any relation between the permeability of the testa to water and the variables of grain colour, size, grade, thickness of skin or exposure to adverse weather, but he noted that fully mealy endosperm is twice as permeable to water as is fully vitreous.

A series of investigations into the selective permeability of the covering layers of barley grains was carried out by Adrian J. Brown and his co-workers.[19, 21] It was concluded that the solutes most strongly adsorbed by barley grains, viz., aniline, phenol and organic acids, are those yielding solutions with low surface tensions, while those solutes not adsorbed, such as sugars and polyhydric alcohols, give solutions with relatively high surface tensions. Thus it would appear that the selective action of the membrane is due to its property of selective adsorption.

The structural basis of the semi-permeability of barley grains was thoroughly investigated by Tharp[13] (see also above). The inner and outer cutin layers of the testa were examined, the outer being found to be much the thicker, though of variable thickness and thin over the embryo, the thinnest portion being the area immediately across the grouped cells at the micropyle. Thickness of these membranes was found to vary with the variety of barley. With the experimental techniques employed it was found that varieties exhibited wide differences in degree of permeability, a wet environment during maturation inducing decreased resistance to permeation, as also did premature harvesting but only slightly. Differences in relative per-

meability were not correlated definitely with any one variation in anatomical structure of the semi-permeable seed coat envelopes of the caryopsis, but in some varieties the heavier layer of cutin on the integument and heavier deposits of fat substance in the integument cells seemed to be connected with increased resistance to permeation. Hull-less varieties taken as a whole were more permeable than hulled varieties, but this difference was independent of the presence of the hulls.

Although this discussion is of the selective permeability evinced by mature cereal grains to chemical substances in solution, it is of interest that in an investigation concerned with the permeability to gases of the seed-coat membranes of *Cucurbita pepo* R. Brown[22] showed that the inner of the two membranes controls gaseous exchange. He noted, however, that with gaseous diffusion a living component of the seed coat was of great importance, the part of the inner membrane responsible appearing to be the layer identified by von Höhnel as the outermost, and only undigested, layer of the endosperm. ' This observation of von Höhnel's makes this layer morphologically homologous with the aleurone layer of cereal grains ' ; the two are very similar in appearance.

III. Absorption of solutes by the grain

Factors influencing the functioning of the semi-permeable system enveloping the grain may be grouped under three headings : (1) the condition of the surface and the composition of the grain ; (2) the nature of particular solutes ; (3) ambient conditions, particularly temperature and the concentration of the solute in solution.

It is clear that to function properly the enveloping tissues must be physically intact. Further it is clear that their properties are profoundly affected by their moisture content ; to take an extreme example, Caldwell[23] demonstrated that sulphuric acid of a strength that was not harmful to the subsequent germination of intact barley grains that were of low moisture content at the commencement of treatment, was lethal to similar grains that were damp at that stage. As has been noted, the testa of wheat is mucilaginous between its cuticularised membranes ; on the hypothesis that this is true of other cereals also, the effect of moisture variation on its permeability is understandable.

Another factor that will influence the rate of absorption of a compound by a grain is the adsorptive capacity of starch for that compound,[12] while fully mealy endosperm is more permeable to water than is fully vitreous,[20] both these facts having been determined with wheat grains as experimental material.

The functioning of the semi-permeable system may also be affected by the age of the grain. Caldwell & Hampson[5] showed that the resistance of dried uninjured barley to permeation by sulphuric acid during one year from time of collection was unimpaired as assessed by subsequent germination, but it was noted that not only did germination of harvested samples reflect the severity of harvest treatment when treated with acid prior to germination, but that this correlation became stronger the longer the barley had been stored. This may indicate that in grains where the tissues were becoming less viable with the passage of time due to some form of injury other than actual breakage during harvesting the semi-permeable system was affected. The importance attached by R. Brown[22] to the living tissue in controlling gaseous diffusion in *Cucurbita pepo* seeds would also lead one to expect a connexion between selective permeability and viability and in consequence between the former and age. Any such change in permeability as the grain aged might well of course not be in the same direction for all compounds.

Having touched upon some of the important aspects of the condition of the grain, and bearing in mind that differences in the tissues may arise from variation in weather during ripening or may be related to variety, permeability to specific solutes may now be considered.

A distinction is made by R. Brown[12] between two types of absorption by the grain of wheat : (1) that which occurs with a solute to which the membranes are freely permeable, as for instance iodine, where absorption is similar to that of water and in fact the presence of some water is necessary ; (2) that for a solute for which the membrane has only slight permeability, for example, silver nitrate. Brown found that in this case absorption takes place in three successive stages : absorption through the micropyle to the outer cuticle of the testa, then slow diffusion across the testa, and finally, after partial breakdown of the semi-permeability system, rapid

entry into the endosperm. He differentiated between the absorption of electrolytes and non-electrolytes and also pointed out the importance of adsorption and the effect of the osmotic pressure of the solution. He suggested that molecular volume may affect the rate of absorption of very complex molecules such as soluble carbohydrates. In earlier work Adrian J. Brown & Tinker[21c] had emphasised the importance of the surface tension of the solution of a solute to which barley grain is permeable as the determining factor of the rate of diffusion into the grain.

In the development of herbicides, differential or selective absorption is important since for most of these to be effective they have to enter the plant. Absorption is thought to be closely related to the polarity of the herbicidal molecule and consideration of this view in connexion with the selective permeability of grain harmonises the observations referred to above.

The term ' polarity ' in this context is used to describe the electrical nature of molecules and ions. Polar ions and molecules, such as those of water, amino-acids, carbohydrates and electrolytes, have some regions that are electrically positive and others electrically negative ; they are soluble in polar but not in non-polar solvents. When dissolved in water they tend to increase surface tension, although this effect may be only slight. Non-polar molecules on the other hand do not have strongly positive and negative areas within them ; they tend to be soluble in non-polar but not in polar solvents (e.g., in oil but not in water), but when they are soluble in water they usually decrease the surface tension. Most organic substances are non-polar, as are for instance most organic solvents, oils and waxes.

The waxy cuticle of plant leaves and stems is non-polar, hence non-polar compounds tend to be absorbed more speedily than do polar ones. This effect is particularly marked where the cuticle is heavy. The rate of penetration of plant cuticle by oil is related to the viscosity and surface tension of the oil ; light oils, having lower viscosity and lower surface tension, enter plant tissue more readily than do heavy oils.

The importance of the cuticularised membranes of the testa, which along with the suberised chalazal tissue envelop the grain, is clear from the work cited. It has been found by the several investigators that dry grains are relatively impermeable to some substances, somewhat more permeable to others and readily absorb yet others. Amongst substances to which dry grains are relatively impermeable are sulphuric acid, nitric acid, sodium chloride, sugars and polyhydric alcohols. They are somewhat permeable to mercuric chloride, acetic acid and trichloroacetic acid, and they readily absorb water, phenol, iodine, aniline and organic acids generally. These findings support the polar hypothesis which accommodates within itself the rôle of surface tension and of electrolytes in relation to grain permeability.

Temperature and concentration of the solute in solution have been indicated as factors influencing the relative permeability of the grain. In general the greater the concentration of solute and the higher the temperature the more rapid is the penetration by the solute of the tissues covering the grain.

IV. Effects of selective permeability on viability and response to treatment with fungicides

The use of fungicidal substances on grain prior to sowing is now so general and so much a matter of routine that one tends to overlook the fact that it is remarkable that we can apply toxic chemicals to a living organism for its protection. The semi-permeability of the grain is crucial in this matter.

Naturally-occurring defence mechanisms are present to resist infection, but those fungi which attack the grains successfully are by definition those against which the natural defences, whether physical or chemical, have proved to give insufficient protection. Thus measures against such fungi must take the form either of increasing the natural resistance of the grain, by plant breeding or possibly by stimulation of antifungal activity by chemotherapeutic means, or by adding extraneous substances. In the latter category one can envisage the possible use of antibiotics with toxic or fungistatic properties[24, 25] or the application of substances to increase the physical resistance of the grain surface to attack, but the present practical approach is the application of fungicidal compounds to the surface.

The effectiveness of fungicidal seed treatments in destroying specific fungi and in benefiting crops planted in heavily infected conditions favourable to fungal development has been much

studied. Less attention appears to have been given to the effect of fungicides on the seeds of different species or of different varieties of the same species, and under conditions of little or no infection. There is evidence that such studies would be valuable.

The fungicidal action of copper appears to be linked with the precipitation of protein and with the presence of enzymes with sulphydryl groups; its fungicidal action is inhibited by the presence of glycine. Foster[26] investigated the acceleration and retardation of germination of some vegetable seeds resulting from treatment with copper fungicides. He noted that previous investigators had reported injury by such fungicides to seeds of peas, cucumbers and crucifiers, while consistently beneficial effects had been obtained with seeds of beets, spinach, eggplant, pepper and tomato. The beneficial effects had been attributed entirely to the protection from fungal attack provided by the fungicide. Foster demonstrated that copper compounds can in fact exert a positive influence, the nature of which depends on the respiration-controlling enzyme systems of seeds. One enzyme system, which is present in barley, oats, cucumber and peas, needs sulphydryl groups in one or more of its constituent enzymes; such enzymes are inhibited by copper. Conversely, a different type of enzyme system which occurs in potatoes, beets, spinach and mushrooms contains the enzyme tyrosinase, which requires the presence of copper ions for its action.

Foster confirmed experimentally that seeds with the sulphydryl groups (pea, cucumber, cabbage) have reduced or retarded germination after treatment with concentrations of copper sulphate and cuprous oxide which increase or accelerate germination of beet, eggplant, pepper and spinach seeds, sulphydryl groups being absent from these seeds. Further, suspensions of cuprous oxide reduced the respiration of ground seeds and seedlings of peas, while increasing that of similar spinach material. Foster concluded that it is necessary when rating fungicides to specify the seeds used, citing as an example that chloranil is outstandingly superior to cuprous oxide for treatment of peas or beans but that the reverse is true when treating beet and spinach seeds.

The tenacity with which a fungicide is retained by seed will influence its effect on fungi and on the seed itself. Mercuric chloride and silver nitrate were found by Dadd & Jacobs[27] to be retained to some extent by treated sweet pea seeds even after repeated washing and soaking of the seeds in water, while chloramine-T was readily released from seed treated with it. Amongst the organic mercurial compounds, the anion present in each particular compound greatly modifies the solubility of such (usually volatile) compounds in water. Roane & Starling[28] have shown that mercurial fungicides are phytotoxic when applied in excessive amounts to wheat, or to wheat at high moisture levels, or when used on injured (particularly chipped) seed grain; likewise copper compounds have similar harmful effects under such circumstances. Such harmful effects arising from the treatment of injured grains may well be due not only to the physical breaking of the selective permeability system but also to the retention of relatively large amounts of fungicide in the breaks and cracks in the grain surface. The need to avoid on the one hand excessive concentration of fungicide and on the other excessive grain moisture contents reflects the limitations of the power of the grain to prevent or minimise the entry of harmful substances.

Conclusion

The functional significance of the outer layers of mature cereal grains has been considered in relation to resistance to entry of fungi and to the selective permeability of the grains to chemical substances in solution. After examination of the functioning of the semi-permeable system, the viability and response of the grain to treatment with fungicides have been briefly considered. With the passage of time the emergence of strains of fungi increasingly resistant to fungicides in use at present is probable. Since the extent to which dosage with a given fungicide can be increased without harm to the seed and plant is limited, not only would it then become even more important that the selective permeability of the grain should not be marred, but the use of seed treatments that would act both to injure the fungi and also to stimulate the plant might well become of importance. The action of copper fungicides in stimulating the seeds of some species appears promising in this connexion and points to the need for more information on the

effects, whether harmful or beneficial, of fungicides on the germination of seeds in conditions where such influences are not masked by fungicidal action.

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FATTY ACID COMPOSITION OF THE DEPOT FATS OF THE KIWI *(APTERYX AUSTRALIS MANTELLI)*

By F. B. SHORLAND and JOAN P. GASS

The depot fat of a flightless bird, the New Zealand kiwi (*Apteryx australis mantelli*) has been shown to be similar in fatty acid composition to that of other land birds apart from its exceptionally high content (~10%) of C_{20} highly unsaturated acids the origin of which has not been established.

Introduction

Information on the fatty acid composition of bird depot fats is rather scanty and in particular quantitative data are lacking. Of the land birds fats the composition of those of the hen,[1] grey goose,[2] emu,[2] ostrich[3] and the flamingo[4] have been determined. Cruickshank[5] has shown that the nature of the ingested fat markedly influences the composition of hen depot fats. The importance of the dietary fat is also suggested by the similarity in fatty acid composition between the fats of marine birds and fishes[6] which contrasts sharply with the simpler pattern of fatty acid distribution found in the fats of land birds.

In the present investigation is reported the fatty acid composition of the depot fat of the kiwi (*Apteryx australis mantelli*), a New Zealand species of flightless bird.

Experimental and results

The specimen used in this investigation was an adult female bird kindly supplied by Dr. R. A. Falla, Director of the Dominion Museum, Wellington. It was forwarded to the museum by Mr. T. Short of Raetihi, who reported that it had been killed by a dog on 24/11/56.

As received in the Laboratory the bird weighed 2450 g. It was dissected by Mr. P. C. Bull of the D.S.I.R. Animal Ecology Section, who has reported elsewhere on the stomach contents.[7] Essentially they consisted of insect remains together with those of two large native slugs, earthworms, leaves and fruits. These findings resemble those earlier reported by Gurr.[8] After plucking, the skin was removed and the muscle tissue separated from the bone. The bones, including traces of adhering muscle tissue, viscera and alimentary tract freed from contents were bulked and designated residue. The relative proportions of skin, bone and residue together with their lipid contents determined as described by Hartman *et al.*[9] are shown in Table I.

The amounts of polyunsaturated acids were determined as described by Herb & Riemenschneider[10] and are given in Table II together with the general chracteristics.

Table I

Composition of the tissues used for fat extraction

Tissue	Wet weight, g.	Fat-free dry matter, %	Fat, %
Muscle	505	17·2	5·1
Skin	499	18·8	29·4
Residue	517	19·5	11·1

Table II

Characteristics of kiwi lipids

Tissue	Sap. equiv.	Iodine value (Wijs, 1 h.)	Free fatty acids, %	Unconjugated acids				Conjugated acids	
				Diene	Triene	Tetraene	Pentaene	Diene	Triene
Muscle	290·2	99·1	18·1	10·4	5·9	4·1	2·2	0·8	0·2
Skin	292·6	98·8	2·9	10·5	8·4	0·9	1·2	0·6	0·1
Residue	294·7	92·8	11·3	8·7	5·4	1·6	1·5	1·1	0·3

As the lipids from the various parts of the kiwi show similar characteristics (see Table II), the skin fat comprising the major fatty depot was selected for further study. The low phosphorus content (P 0·038%) indicated the absence of phospholipids. The total lipid was therefore used for ester fractionation analysis as described by Shorland *et al.*[11] The methyl esters of the skin fat were also analysed by gas–liquid chromatography[12] by means of the sensitive detector of Lovelock *et al.*[13] with argon carrier gas. For the resolution of the methyl esters into their individual saturated and unsaturated components a column with a polyester (LAC-3-R-728, Cambridge Industries, U.S.A.) supported on five times its weight of Celite 545 was used at a temperature of 150°. As no suitable reference standards for the C_{20} and C_{22} unsaturated acids were available, the amounts of these were determined from the corresponding saturated acids following hydrogenation of the methyl esters at 170° with Ni catalyst supported on kieselguhr. For the separation of the methyl esters of these higher saturated acids, Apiezon L was substituted for the polyester liquid phase and the temperature was raised to 225°.

The methyl esters of the C_{18} unsaturated acids isolated during ester fractionation were further analysed by gas-liquid chromatography to give the amounts of oleic, linoleic and linolenic acids. The results thus obtained are compared in Table III.

Discussion

It will be noted from Table IV that the fatty acid composition of kiwi depot fat is generally similar to that of other land birds apart from the occurrence of substantial amounts of C_{20} highly unsaturated acids. As a rule such acids are not present in quantity in the depot fats of higher land animals unless supplied in the diet. They occur, however, in the phospholipids of non-fatty tissues. The earlier unpublished results of Shorland & Jessop for kiwi breast

Table III

Comparison of results for fatty acid composition by gas–liquid chromatography and ester fractionation

Fatty acids (mol.-%)

Acids	Gas–liquid chromatography	Ester fractionation	Acids	Gas–liquid chromatography	Ester fractionation
Saturated			*Unsaturated*		
Lauric	0·1	0·4	C_{12}	—	0·2 (2·0 H)
Myristic	0·8	1·1	C_{14}	—	1·0 (2·0 H)
n-Pentadecanoic	0·6	—	C_{16}	2·4	3·5 (2·0 H)
Branched C_{15}	1·0	—	Oleic	44·9	45·2
Palmitic	20·8	19·6	Linoleic	10·5	10·6
n-Hepta-decanoic	0·4	—	Linolenic	5·3	5·3
Branched C_{17}	0·7	—	C_{20}	6·8	8·4 (5·3 H)
Stearic and above	5·6	4·7			

Table IV

Fatty acid composition of bird fats

Fatty acids (wt.-%)

	Flamingo[4]	Grey goose[2] (abdominal)	Emu[2] (sub-cutaneous)	Light Sussex hen[1] (abdominal)	Ostrich[3]	Kiwi (skin)	Kiwi* (breast)
Iodine value	67·9	57·1	65·8	78·5	80·5	98·8	92·9
Lauric	—	12·3	—	—	—	0·3	—
Myristic	—	8·2	0·9	1·2	0·9	0·9	1·6
Palmitic	24·8	20·3	17·5	24·0	24·8	18·1	17·6
Stearic	7·7	5·6	10·1	4·1	5·9	3·6	7·5
Arachidic	—	—	0·6	—	0·4	1·5	—
Dodecenoic	—	—	—	—	—	0·2	—
Tetradecenoic	—	0·6	0·9	—	0·9	0·8	0·5
Hexadecenoic	4·9	2·5	2·1	6·7	6·1	3·2	2·9
Octadecenoic	53·4	41·6	62·2	42·5	39·8	45·9	
Octadecadienoic	7·1	6·6	5·2	20·8	17·1	10·8	59·6
Octadecatrienoic	0·2	—	—	—	3·8	5·4	
Unsaturated C_{20}–C_{22}	1·9	2·3	0·5	0·7	0·3	9·3	10·3

* Unpublished results (Shorland & Jessop)

fat (Table IV), supplied by Dr. W. H. Dawbin of Victoria University, Wellington, from a male bird taken near Taupo, confirm the high content of C_{20} unsaturated acids. The content (10%) of C_{20} unsaturated acids, however, is less than found in the depot fats of sea birds (20–27%) where in addition 16–22% of C_{22} unsaturated acids which appear to be absent from the kiwi fat also occur.

In keeping with the results for other land bird fats the kiwi fats contain similar amounts (4–8%) of stearic acid, but lesser amounts of palmitic acid (18%) than is typically found in land mammals. The abnormally high amounts of lauric acid in the fat of the grey goose is attributed by Hilditch *et al.*[2] to the coconut oil in the diet.

When the unsaturated fatty acids of the bird fats (Table IV) are compared the content of octadecadienoic (linoleic) acid is found to be variable. This, however, is probably a reflection of the diet, as Cruickshank[5] has shown that the iodine values of hen depot fats readily respond to changes in the iodine values of the dietary fats. In addition, the results for the octadecadienoic acid contents of the fats of the grey goose, hen and emu are subject to doubt as they have not been determined directly, but have been calculated empirically by attributing to linoleic acid the unsaturation in excess of that required for oleic acid. It is more likely that part of this unsaturation should be attributed to linolenic acid which has generally been revealed when the C_{18} unsaturated acids have been examined by spectroscopic techniques following alkali isomerisation, as in the ostrich and flamingo fats, or by gas–liquid chromatography as in the kiwi fats. The presence of considerable amounts of linolenic acid in the kiwi fat is probably, as in the case of linoleic acid, a reflection of the diet, both these constituents being found in quantity in insect fats, as well as in leaf lipids (cf. Hilditch[14]).

In Table III the results of analysis of the kiwi fat by ester fractionation and gas–liquid chromatography are compared and show general agreement. However, as may be expected, the results obtained by the more sensitive gas–liquid chromatographic technique are consistent with the presence of *n*-odd numbered C_{15} and C_{17} straight-chain and branched fatty acids. For the definite proof of the existence of these acids in bird fats it would be desirable to isolate these constituents in pure form. Their presence is rendered likely as such constituents have already been isolated and identified in animal fats,[15, 16] in shark liver oil[17, 18] and in tall oil.[19, 20]

Studies on bird depot fats[1, 5] show that the fats from different depots have a very similar composition as do the present results for the kiwi depot fats. This situation contrasts with that of sheep, cattle and pigs where the internal depot fats are much more saturated than the external.[14] It is therefore possible that the fats from different depots of the same bird generally tend to be more uniform in composition than are those from the different depots of the same mammal.

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TESTING OF PAPER AND OTHER SACK MATERIALS FOR PENETRATION BY INSECTS WHICH INFEST STORED PRODUCTS

By PAULINE M. DAVEY and T. G. AMOS

Seven types of materials used for storage of produce (maize, groundnuts, etc.) were tested for penetration by nine species of insects which infest such produce. The relative values of the materials for preventing damage to the produce are determined and recommendations made as to suitable materials in certain cases and the procedure which should be followed in the storage of produce.

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Introduction

In the tropics, large quantities of produce such as maize, groundnuts, cocoa beans, paddy-rice, etc., are stored in sacks mainly on small farms where each farmer handles and stores small quantities. Although certain territories have changed from sack to bulk storage, it is envisaged that sacks will continue to be used for many years to come, since adaptations to new methods of transport and storage involve large capital outlays and a great deal of reorganisation.

Jute sacks made from Calcutta and Dundee hessians are cheap and strong,* can be used many times, are easy to handle, are not excessively damaged by hooks and spear samplers and can be easily closed and repaired by sewing. Under dry climatic conditions produce in sacks may continue to dry out in store, which is often an advantage, but under high-humidity conditions it is a disadvantage to store produce in jute sacks because of the danger of increase in moisture content.

The main disadvantage of storing produce in B-twill jute sacks is that insects are able to move freely into and out of the sacks through the meshes of the jute, and therefore can gain ready access to the contents at all times. Contamination of produce by insects is of economic importance, not only because the produce is reduced in nutritive value and fouled by excretions and secretions of the insects, but also because of direct losses in weight. Therefore methods of minimising infestations are important, in particular those preventing cross infestation from one parcel of produce to another and from residual infestations in the fabric of buildings.

Insecticidal control measures can be used under certain conditions but they are costly and their application is time consuming and requires skilled labour. Many of the chemicals available are toxic to man and domestic animals and may impart a taint to produce.

Under temperate conditions in the laboratory, Parkin[1] and Jewett & Price[2] have shown that sacks impregnated with 5·0% DDT give good protection against *Calandra granaria* (*Sitophilus granarius*), *Tribolium castaneum*, *Ptinus tectus*, *Oryzaephilus surinamensis* and *Ephestia elutella*, but finely ground and fatty commodities stored in the impregnated sacks become highly contaminated with the insecticide, to a dangerous extent, after only 4 months' storage.[3]

Insect species in different parts of the world appear to vary in their resistance to an insecticide and some species develop an increased resistance towards many of them. Although the use of new insecticides appears to overcome this problem, resistance may soon develop and in the last resort mechanical barriers may be required.

Protection of produce by mechanical barriers would seem to be highly suitable providing the commodity is not infested. Therefore the barrier should be permeable to fumigants if it is a sack, but impermeable if it is a cover to a stack. In addition it should be impermeable to water vapour, and be competitive in price and as strong as jute.

Previously, experiments have been carried out at this laboratory to test materials which might suitably replace the jute sack and give mechanical protection to the contents from a few species of insect.[4, 4a] As new materials became available, a reconsideration of the problem was considered desirable. A range of 19 cheap, strong materials, representing 7 types (see Appendix A), was investigated for resistance to penetration by nine species of insects. The susceptibility of a material was judged by the number of days taken to penetrate it by the insect. With certain multi-ply materials only some of the ply were perforated and the suitability of these materials is discussed.

Experimental

Apparatus

The apparatus, which was similar to that used by Gostick,[4] consisted of two glass rings each $2\frac{1}{2}$ in. in diameter and $\frac{13}{16}$ in. deep. The material to be tested was folded to simulate folds occurring in sacks, into a pocket $\frac{1}{2}$ in. deep, held open by a glass bead inserted into the pocket, and then securely fastened by means of adhesive tape between the two rings (Fig. 1).

The food commodity, on which the insect species is commonly found, was sterilised and conditioned to 70% R.H. It was then packed into one side of the apparatus (food side) so that it was pressed firmly, as it would be in a sack, against the material held between the two

* The Calcutta jute sacks produced at the present time have a lower bursting strength than those used prior to 1949

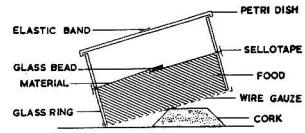

FIG. 1.—*Apparatus used for testing the resistance of certain materials to insect penetration*

glass rings. The food was held in position by means of a piece of 60 mesh wire gauze placed over the end of the glass ring. Test insects were enclosed under the Petri-dish without food (non-food side) (Test *a* below) and in food (Test *b* below). The whole apparatus was held at about 30° to the horizontal on two corks so that air could circulate freely ; it was kept at 30°C, and 70% R.H.

Insects used in the tests

Many different species of insects infest tropical produce. Only the more common and frequently occurring species were chosen for this experiment (Table I), and were cultured by the Insectaries of the Pest Infestation Laboratory with the exception of *Tribolium castaneum* which were provided by the Insecticide Section. The age of the insects best suited to survive under starvation conditions was determined by tests described in Appendix B. Insects of a known age were obtained by sieving a freshly emerging culture then allowing emergence to occur over a known period of time and sieving off the required insects. These were then either used immediately in the tests or kept on fresh culture media until they had reached the required age (see Tables II and III).

Tests

(a) *Insects not in the food*

This test was designed to simulate the most rigorous conditions that could occur on the outside of a sack, i.e., to determine whether the insects could penetrate a material into a food commodity while under starvation conditions. It might be argued that insects would be weaker under these conditions and in any case that they are unlikely to encounter such conditions in warehouses. Nevertheless insects occur on the outsides of sacks where there is very little food, and a field scale experiment in Trinidad (carried out by Mr. G. Stell) indicated that paddy

Table I

Insects used and their culture conditions

Insect	Culture medium	Rearing temperatures, ° C, at 70% R.H.	Insect	Culture medium	Rearing temperatures, ° C, at 70% R.H.
Bostrichidae			Silvanidae		
Rhizopertha dominica Fabricius	Wheat	30°	*Oryzaephilus mercator* Fauvel	Miller's offal, rolled oats and dried yeast 9 : 9 : 1	25°
Anobiidae			Phycitidae		
Lasioderma serricorne Fabricius	Miller's offal* and dried yeast 18 : 1	25°	*Ephestia elutella* Hübner	Miller's offal and glycerine 5 : 1	25°
			Ephestia cautella Walker	Miller's offal and glycerine 5 : 1	25°
Curculionidae					
Sitophilus oryzae Linnaeus	Wheat	25°	Dermestidae		
			Dermestes maculatus Degeer	Fish meal, dried yeast and bacon	25°
Tenebrionidae					
Tribolium castaneum Herbst	Wheat flour	25°	*Trogoderma granarium* Everts	Wheat and wheat feed	30°

* Miller's offal is mostly wheat husk with a small quantity of endosperm

Table II

Insects used under starvation conditions, their age and the food commodities with which they were associated in the tests

Insects in order of their ability to penetrate	Stage	Age	Food commodity	Viability,* days	Replacement period, weeks
Rhizopertha dominica Fabricius	Adults	1–4 weeks	Rice	7	2
Dermestes maculatus Degeer	Larvae	Instar I–III	Fishmeal	—	No deaths
,, ,, ,,	,,	,, III–VI	,,	7	1
Lasioderma serricorne Fabricius	Adults	0–7 days	Miller's offal	7	1
,, ,, ,,	Larvae	Instar III	,, ,,	7	1
Sitophilus oryzae Linnaeus	Adults	0–4 weeks	White flat dent maize	0–6	1
Tribolium castaneum Herbst	Adults	4–6 weeks	Shelled groundnuts	8–10	2–3
Oryzaephilus mercator Fauvel	Adults	0–6 weeks	,, ,,	0–6	1
Trogoderma granarium Everts	Larvae†		Whole wheat	—	No deaths
Dermestes maculatus	Adults	?	Fishmeal	—	No deaths
Ephestia elutella Hübner	Larvae	small	Cocoa beans	6–11	1
		large			
Ephestia cautella Walker	Larvae	Instar IV–V	Shelled groundnuts	7	1

* Viability means that deaths began to occur after this number of days.
† All *T. granarium* larvae were reared at 30°. They were then conditioned as described in the text.

Table III

Insects confined in the food during tests*

Material†	Insects			
	Larvae		Adults	
2 (*a*) Two-ply kraft paper	*E. cautella*	—	—	*O. mercator*
3 (*a*) Five-ply kraft and polyethylene	*E. cautella*	—	—	—
4 (*b*) Two plies of bitumen-union, low-stretch creped kraft	—	*L. serricorne*	*T. castaneum*	—
5 (*a*) Two-ply creped kraft	*E. cautella*	—	—	—
5 (*c*) Soft wax creped kraft union	—	*L. serricorne*	*T. castaneum*	—
6 (*a*) Polyethylene 0·005 in.	*E. cautella*	*L. serricorne*	*T. castaneum*	*O. mercator*
6 (*c*) High-viscosity polyethylene	—	*L. serricorne*	*T. castaneum*	*O. mercator*
7 'Melinex'	—	*L. serricorne*	—	—

* See Table II
† See Appendix A

stored in six-ply paper sacks became infested by insects boring their way into the sacks, penetration having been achieved after 2 months' storage.

The insects, usually 50 adults or 25 larvae, were placed in the apparatus as described above, and inspections for penetration through the material and mortality of the insects were made on the first, second, fourth and seventh day after setting up. Those species which died were replaced by living insects at regular intervals (Table II) until the materials had been exposed for approximately three months.

(b) *Insects in the food*

These tests were designed to find whether some of the insects which had been unable to penetrate materials under starvation conditions [see (*a*) above] could do so if they were confined on the food side of the material. The whole apparatus was propped on corks with the food side uppermost.

The species of insect and the materials tested are recorded in Table III and the results are given in the appropriate sections.

(c) *Limited tests with* Trogoderma granarium *and* Dermestes maculatus

Trogoderma granarium

Polyethylene is being used in the malt trade as a liner to jute sacks because not only does it help to keep the malt at a constant moisture content but it is thought to resist the penetration of the Khapra beetle, *T. granarium*.

Three tests were designed to determine whether the larvae of *T. granarium* could penetrate polyethylene (0·005 in.) under different conditions of temperature when in different stages of development.

Test I.—Third instar larvae (provided by Dr. D. H. Burges) were used, one-seventh of them feeding and the remainder in diapause having been in that condition for nearly a year at 20° or at 30°.

During the test the apparatus was exposed to the following different temperatures for varying periods: 43 days at 30°, 21 days at 40°, 33 days at 20°, 16 days at 35°.

Test II.—Large and small larvae which had been resting for 49 days at 30° were then placed at 20° for 7 days to stimulate activity. They were set up in the apparatus and kept at 35° for 5 weeks.

Test III.—Third instar feeding larvae were used and kept at 35° for the duration of the test.

Dermestes maculatus

D. maculatus is one of the insects causing a great deal of contamination in stockfish imported into Nigeria. Among the suggestions for controlling the pest was the use of a better covering material than the light weight jute in which the fish are wrapped. Therefore the larvae and adults of *D. maculatus* were exposed to the following materials: *

1 Jute ' heavy '
4 (*d*) Hessian reinforced bitumen-union creped kraft paper
6 (*a*) Polyethylene (0·005 in.)
(*b*) Polyethylene (0·0012 in.) on 10½ oz. jute
(*c*) High viscosity polyethylene 0·004 in. thick on 10 oz. jute

(d) *First instar level penetration*

Although some jute materials may not be penetrated by some adult insects, e.g., moths, the food lying beneath can become infested by newly hatched, 1st instar larvae from eggs laid either through the sacking or on the surface of the material.

In these tests, adults of *L. serricorne* and *E. cautella* were allowed to lay eggs on the B-twill jute which is ' open ' weave and *T. castaneum* and *L. serricorne* were exposed to the ' heavy ' jute. In all cases, eggs were laid on the surface of the jute on the non-food side and the 1st instar larvae which subsequently hatched crawled between the jute fibres into the food beneath. *E. cautella* adults were also able to deposit some eggs on the food side of the jute.

Results

The time taken for penetration of the materials depends on the insect as well as on the texture of the material and to a lesser extent on the presence of folds in the material. The detailed results of the experimental work have therefore been recorded for each material.

Since there were two replicates of each, penetration times for both have been given in Table IV. Frequently the times were similar but in a few cases there was a considerable divergence.

Jute (two samples of different weights of B-twill were used as controls)

All species of insects tested passed through the ' open ' weave of the control B-twill jute from the non-food side to the food side in 24 h., with the exception of *Lasioderma serricorne* which sometimes took 8 days.

A ' heavy ' jute sacking, more closely woven than the ' open ' weave, was tested. *Rhizopertha dominica* adults ate their way through in 23–28 days and *Dermestes maculatus* larvae made large holes in 35–42 days. Young larvae (2nd–3rd instar) of *Lasioderma serricorne* were unable to penetrate, nor were the adults, although the food became infested with 1st instar larvae hatched from eggs laid on the side of the material away from the food. *Ephestia elutella* larvae were unable to penetrate.

Kraft papers

These were penetrated by *R. dominica* and *L. serricorne* adults (Table IV) which bored through all layers into the food. *Sitophilus oryzae* adults penetrated three plies but were unable during

* See Appendix A

Table IV

Rate of insect penetration (in days) of various sack materials

Material	*Rhizopertha dominica*	*Dermestes maculatus* (larvae)	*Lasioderma serricorne*	*Sitophilus oryzae*	*Tribolium castaneum*	*Oryzaephilus mercator*	*Trogoderma granarium*	*Ephestia elutella* (larvae)	*Ephestia cautella* (larvae)
1 *Jute*									
(*a*) B-twill open weave (eggs and young larvae)	1	P†	8	1	1	1		1	
(*b*) ' heavy ' (eggs and young larvae)	23, 28	33, 40†	P		P			NP	P
2 Kraft paper									
(*a*) Two-ply	17, 17		53, 87	9, 11	NP	NP NP*			NP NP*
(*b*) Three-ply	21, 21		26, 89	59, 174	NP	NP		NP	
(*c*) Five-ply	38, 48		53, 53	NP	NP	NP NP*		NP	NP
3 Kraft paper with polyethylene laminate									
(*a*) Five-ply	15, 17		50, 75	NP	NP			NP	NP NP*
(*b*) Five-ply + bitumen	15, 17		NP P*	NP	NP			NP	NP
4 Bitumen-union kraft papers									
(*a*) Triple laminate of glazed kraft and bitumen	5, 8		15, 33	12, 12	NP			NP	
(*b*) Two plies of bitumen-union low-stretch creped kraft	9, 53		31, 67 43*	52, 52	NP NP*			NP	
(*c*) Sisal-reinforced bitumen-union glazed kraft	9, 9		10, 36	8, 36	82, 84			NP	
(*d*) Hessian reinforced bitumen-union creped kraft	17, 20	2	22, 36	59, 60	NP			NP	
5 Creped kraft and waxed papers									
(*a*) Two-ply creped kraft	5, 8		22, 67	38, 61	43, 58			NP	NP NP*
(*b*) Hard wax kraft union	5, 21		10, 19	4, 7	4, 14			NP	
(*c*) Soft wax creped kraft union	33, 33		43, 55 43*, 59*	17, 17	43, 43 NP*				
6 Polyethylene									
(*a*) 0·005 in.		2, 2†	20, 142 36*	6, 27	13, 20 59*, 205*	NP NP*	NP	NP	15, 26 NP*
(*b*) 0·0012 in. on jute	22, 37	22, 24	40, 61	23, 33	139, NP	—	—	—	—
(*c*) High-viscosity 0·004 in. on jute	8, 27	2, 9	NP 45*, 71*	NP	NP NP*	NP NP*	—	NP	—
7 ' Melinex ' (0·001 in. on jute)	P‡		NP*	28, 95					

P = penetrated NP = not penetrated
* insects penetrated by boring out of food into the non-food side
† adults did not penetrate ‡ tested by Miss Evans (P.A.T.R.A.)

this experiment (3 months) to bore further. *T. castaneum* and *O. mercator* adults and *E. elutella* and *E. cautella* larvae did not penetrate although they were able to damage the paper by roughening its surface and by making a hole through the first layer.

Two-ply kraft paper exposed to *O. mercator* breeding in the food for 6 months showed no penetration although the groundnuts were damaged to such a degree by the beetles that sufficient oil was released to seep into the paper. *E. cautella* larvae living in the food were unable to damage the paper.

Kraft papers with a polyethylene laminate

Two materials of this type were tested, one with and one without a bitumen bond between two of the five layers of kraft paper. The polyethylene was stuck to the first layer of paper and was exposed to the insects in all the tests.

R. dominica adults bored through both materials into the food (Table IV). *L. serricorne* adults penetrated the five-ply kraft without the bitumen bond but did not completely penetrate the bitumen-bonded material. On closer examination it was found that holes had been made through all the layers but they were not sufficiently large to allow the passage of insects. Some *L. serricorne* adults which were an accidental infestation of the food, made large holes through four of the plies including the bitumen-bonded layer, thus making it clear that the bitumen did not prevent *L. serricorne* adults from penetrating.

Those adults which did not penetrate either material in the time available, i.e., *T. castaneum* *S. oryzae* and *O. mercator*, were able to roughen the surface and make holes in the first layer, i.e., the polyethylene. *E. elutella* larvae did not cause any damage under these conditions but *E. cautella* larvae, living in the food, were able to make holes in the paper layer bonded to polyethylene in 98 days. They caused no damage from the non-food side.

Bitumen-union kraft papers

Four materials were tested (Table IV, 4) all of which were penetrated by *R. dominica*, *S. oryzae* and *L. serricorne* adults boring into the food and by the last-named also boring out of the food through the two plies of bitumen-union, low-stretch creped kraft. *T. castaneum* adults only bored into the food through the triple laminate of glazed kraft and bitumen but adults confined

Table V

% of adults whose ages ranged over consecutive 7-day periods and were observed to be still living under starvation conditions on the days stated

	Days of inspection	Initial age of adults, days						
		0–7	7–14	14–21	21–28	28–35	35–42	42–49
R. dominica	7th	97	100	98	97	98	86	79
	14th	16	16	0	8	3	4	1
	21st	0	5	—	0	0	0	0
T. castaneum	7th	91	99	100	92	80	95	—
	14th	2	44	53	8	16	51	—
	21st	0	9	24	1	2	32	—
L. serricorne	7th	100	14	—	—	—	—	—
	14th	28	0	—	—	—	—	—
	21st	0	—	—	—	—	—	—
O. mercator	1st	100	99	100	100	99	—	—
	2nd	90	97	100	88	76	70	—
	3rd	34	97	21	16	9	6	—
	4th	13	80	9	1	0	0	—
	5th	0	49	0	0	—	—	—
	6th	—	18	—	—	—	—	—
	7th	—	0	—	—	—	—	—
S. oryzae	1st	98	100	100	100	100	100	—
	2nd	98	99	99	99	100	99	—
	3rd	92	94	92	83	92	91	—
	4th	65	75	81	75	65	53	—
	5th	21	—	—	—	—	9	—
	6th	2	—	—	—	0	0	—
	7th	0	0	0	0	—	—	—

in the groundnuts caused so much damage to them in 205 days that oil was absorbed by the two plies of bitumen-union, low-stretch creped kraft being tested, causing the bitumen to bleed. None of these materials was penetrated by *E. elutella* larvae from the non-food side.

Hessian reinforced bitumen-union creped kraft (Table IV, 4*d*) was tested for penetration by *Dermestes maculatus* and the old larvae of this species penetrated by making large holes in the material in 2 days ; younger larvae took 7 days to penetrate ; adults were unable to penetrate the material in 21 days although they passed through the holes made by the larvae.

Creped kraft and waxed papers

Two of these materials were bonded with wax, the third was not waxed. All the insects tested (Table IV, 5) bored through the three materials into the food.

Since the two-ply creped soft wax (Table IV, 5*c*) was fairly resistant to penetration by *L. serricorne* and *T. castaneum*, when these insects were confined on the side away from the food, it was decided to confine some adults in the food. Many *L. serricorne* larvae developed in the food and penetrated the material in 59 days. The *T. castaneum* did not penetrate in 7 months.

E. elutella larvae made a hole in one layer of a replicate of the two-ply creped kraft paper on the side away from the food. They did not penetrate any of the layers of the other creped kraft materials although there were indications of very slight gnawings which roughened the exposed surfaces of the paper. *E. cautella* did not penetrate the two-ply creped kraft material from either side in 3 months.

Polyethylene (polythene) (three samples tested)

Polyethylene film (0·0012 in.) stuck on to 10½ oz. jute and which is highly extensible (Table IV, 6*b*), and high-viscosity polyethylene (0·003 in.) stuck on to 10 oz. jute with 0·001 in. liquid polyethylene (Table IV, 6*c*) were exposed to the insects, from the jute side, in the expectation that this would facilitate penetration. The ordinary polyethylene (0·005 in.) (Table IV, 6*a*) was not tested for penetration by *R. dominica* since these adults had been able to penetrate the relatively tougher high-viscosity polyethylene.

The high-viscosity polyethylene (*c*) was not as easily penetrated as the ordinary polyethylene, the only insects able to bore into the food being *R. dominica* adults and *D. maculatus* larvae. Adults of *L. serricorne* were able to bore away from the food side either just after emergence or on emergence from the pupal case, at which time three or four hundred pupae were present and were adhering, two or three layers deep, to the polyethylene, i.e., a very heavy infestation.

The ordinary highly extensible polyethylene (0·001 in.) stuck to jute, appears to have been particularly resistant to *R. dominica* and *D. maculatus* when compared with the high-viscosity polyethylene. This may have been due to the special resiliance or high extensibility of this polyethylene or to the special adhesive used to stick it to the jute.

It is interesting to note that *E. cautella* larvae penetrated 0·005 in. polyethylene but *E. elutella* did not.

Polyethylene terephthalate (' Melinex ') on 7 oz. jute

This film material (0·001 in.) belongs to the same group of plastics as Terylene fibres[5] and, compared with other plastics, it has a high tensile strength which can be 25–35 kg./sq. mm.

Tests on the penetration of ' Melinex ' (0·001 and 0·005 in.) by *R. dominica* adults were made by Miss Evans of the Printing, Packaging & Allied Trades Research Association, who has observed that penetration was complete in a minimum of 9 days (personal communication). In these tests *L. serricorne* adults breeding in the food were unable to bore through 0·001 in. thick ' Melinex ' and 7 oz. jute in 3 months but *C. oryzae* penetrated into the food in 28 and 95 days. From this it would seem that ' Melinex ' is slightly more resistant to penetration than is polyethylene.

Discussion

It is well known that uninfested produce stored for any length of time in a jute sack is liable to become infested with insects. This fact has been amply supported by the results of this experiment.

Possible replacements or reinforcements of jute, tested in these experiments, were penetrated by at least one species of insect. It would seem, as demonstrated by Gerhardt & Lindgren,[6] that the thick materials are more resistant to insect penetration than the thin ones. However, physical properties other than thickness are obviously important since some plastic films and aluminium foil, thinner than kraft paper, are more efficient mechanical barriers than is paper.

The insect species tested differed in their powers of penetration. *Rhizopertha dominica* adults made holes through all the materials and *Lasioderma serricorne* adults through all except 'Melinex' on jute. *Sitophilus oryzae* adults penetrated all but three of the materials tested which were (1) five-ply kraft paper with a polyethylene laminate, (2) five-ply kraft paper with one-ply of bitumen-union and a polyethylene laminate and (3) high-viscosity polyethylene. *Tribolium castaneum* adults were unable to penetrate any of the kraft papers although they were able to make holes through light-weight paper wax and bitumen-union. They also penetrated muslin used for protecting stacks of groundnuts in the Gambia.[7] *Oryzaephilus mercator* adults and *Ephestia elutella* larvae did not penetrate any material tested but *E. cautella* larvae penetrated 0·005 in. thick polyethylene, though they did not penetrate two-ply creped kraft and kraft papers. The two Dermestid beetle larvae behaved quite differently from each other. *Dermestes maculatus* larvae made holes with ease and rapidity through the materials tested, whereas *Trogoderma granarium* larvae did not penetrate polyethylene. The adults of *D. maculatus* did not penetrate the materials tested.

Penetration usually took place round the edge of the glass rings used in the tests and not very frequently within the fold—possibly because these were somewhat flattened and the insects could not creep inside. The density of insects to which the materials were exposed was much greater than that normally seen on the outsides of sacks. The effect of this on the behaviour of a species was not investigated in this work.

The materials were generally exposed to insects for 3 months, which is about the minimum storage period of any commodity. In a few cases, where one of a pair of replicates had been penetrated, the test period of the other was prolonged. In the majority of cases, 3 months was adequate to show whether or not a material could be penetrated by a particular species.

Starving insects were used in most cases in the tests in order to expose the materials to the most rigorous conditions. It is possible that starving insects are weaker than those fully fed, but the results show in only one instance, where five-ply kraft paper (one-ply of bitumen-union) and a polyethylene laminate, was exposed to *L. serricorne* adults, that the starving insects did not penetrate while insects in the food penetrated. This result is probably anomalous in any case because *L. serricorne* adults are short-lived and are not known to feed.

If it is decided to store produce in sacks made of materials through which insects do not penetrate, then it is essential that the commodity stored in them should be completely free from infestation, i.e., it should be fumigated. It has been demonstrated that infested groundnuts stored in paper sacks become more seriously damaged than if stored in jute sacks.[8] Therefore, materials from which sacks are made must be permeable to fumigants. They must also be strong and able to withstand being dropped from a considerable height.[9, 10]

Another factor to be taken into consideration is the possibility of effecting a good closure; most stitching machines used for sewing jute are adequate for stitching paper but the stitches and holes in paper sacks require to be sealed, e.g., by covering with adhesive tape or by filling with an adhesive. Spear sampling is frequently carried out on commodities stored in jute sacks, and if it is necessary, then the walls of the sacks should be composed of a material which can be easily mended (e.g., by the use of an adhesive patch) after the spear has been withdrawn.

Paper sacks have given some protection from cross infestation in field trials. Paddy storage experiments carried out by Mr. G. Stell in Trinidad (personal communication) and by Iyengar *et al.*[11] in India have shown that insects bore through paper sacks. It is possible therefore that protection of a particular commodity against a certain insect could only be obtained if the mechanical barrier were impregnated with a suitable long-lasting insecticide.

The results of the experiment carried out in this laboratory at one set of conditions (30° and 70% R.H.) provide a basis for full-scale field trials under conditions where many variable factors play a part.

Recommendations

1. Use multi-ply kraft paper as sacks or as a liner to jute sacks against *Tribolium castaneum* and *Sitophilus oryzae*.
2. Use 0·005-in. polyethylene as a sack liner for malting barley.
3. Fumigate infested produce immediately after the sacks have been filled.
4. Employ a method of patch sticking when carrying out spear sampling.
5. Avoid the use of hooks for handling sacks.
6. Investigate the efficacy of a combined mechanical barrier and an insecticide deposit against certain species of insects.

Appendix A

Paper and other sack materials tested for penetration by insect pests of stored food in the tropics

Material tested	Specifications	Approximate cost of one sack
1 Jute B-twill		
(*a*) ' open ' weave	16 oz. per sq. yd. Weft 8, Double warp (cocoa bean sack)	1*s.*–1*s.* 10*d.*
(*b*) ' heavy ' jute	30 oz. per sq. yd. Weft 12, Single warp 13	—
2 Kraft papers	Each ply 37 lb. D.C.‡	1*s.* 1*d.*–1*s.* 2*d.*
3 Paper laminated with polyethylene		
(*a*) five-ply kraft paper with a polyethylene (0·0025 in.) laminate	Each ply 37 lb. D.C. 0·0025 in. thick laminate	1*s.* 6*d.*
(*b*) five-ply kraft paper (one ply of bitumen-union) and a polyethylene (0·001 in.) laminate	Outer ply 36/37 lb. wet strength kraft 2nd ply 52/54 lb. bitumen-union kraft (each 18 lb. + 18 lb. bitumen) 3rd ply 36/37 lb. kraft 4th ply 36/37 lb. kraft inner ply 36/37 lb. kraft + 0·001 in. polyethylene	—
4 Bitumen-union kraft papers		
(*a*) triple laminate of glazed kraft and bitumen	19 lb. D.C. paper 21½ lb. D.C. bitumen 19 lb. D.C. paper 21½ lb. D.C. bitumen 19 lb. D.C. paper } bursting strength 108 lb. Mullen tests	1*s.* 6⅜*d.*
(*b*) two plies of bitumen-union low-stretch creped kraft	30 lb. D.C. low-stretch crepe 24 lb. D.C. bitumen 30 lb. D.C. low-stretch crepe } twice	—
(*c*) sisal-reinforced bitumen-union glazed kraft	Each ply 20¾ lb. D.C. Machine glazed two layers of unspun sisal reinforcing high-m.p. bitumen laminate	1*s.* 3½*d.**
(*d*) hessian-reinforced bitumen-union creped kraft	1st ply 38 lb. D.C. high wet-strength creped kraft, 2¾ oz. hessian, bitumen 2nd ply 38 lb. D.C. pure creped kraft	4*s.* 4*d.*
5 Creped kraft and waxed papers		
(*a*) two-ply creped kraft	Each ply 21 lb. D.C. Stretch at right angles to direction of crepe 34%	—
(*b*) hard wax kraft union	Each ply 19 lb. D.C. } bursting strength 65 lb. on Mullen tests Wax 12–14 lb. D.C.	1*s.* 4½*d.*
(*c*) soft wax creped kraft union	Each ply 36 lb. D.C. } bursting strength 45 lb. on Mullen tests Wax 30 lb. D.C.	1*s.* 6½*d*
6 Polyethylene (polythene)		
(*a*) 0·005 in. thick		3*s.* 0*d.**
(*b*) 0·0012 in. thick on 10½ oz. jute	Has great extensibility	1*s.* 1*d.**†
(*c*) high-viscosity 0·004 in. thick (0·003 in. keyed on cold with 0·001 in.) on 10 oz. jute	High tensile strength	3*s.* 2*d.**†
7 Polyethylene terephthalate (' Melinex ') (0·001 in.) on 7 oz. jute	Terylene film and is very tough	3*s.* 9*d.**†

* Cost of 2 sq. yd. † cost of jute

‡ Weight in lb. of 480 sheets of Double Crown paper (standard size of printing paper 20 in. × 30 in.)

Appendix B

Tests on the age at which insects should be used when exposed to materials under starvation conditions

In most of the tests carried out, materials were exposed to starving insects. Since the insects died before the material had been exposed for the chosen period of 3 months, the dead insects were replaced at regular intervals by living ones. The age of the insects used in these tests was determined by their longevity under starvation conditions at a certain period in their adult life. To determine this the following tests were carried out all at 30° and 70% R.H.

Cultures containing newly emerged adults were cleared of all adults by sieving. More adults were allowed to emerge during the next 7 days by which time a sufficient number, the main stock, were available for tests. These were 0–7 days old and were separated from the food and the young stages. Two batches each of 50 of these 0–7-day-old adults were confined in empty jars and the remainder were put on to fresh food. After a further 7 days, when the adults in the food were 7–14 days old, two lots of 50 each were removed and starved and the remainder were placed on fresh food.

The starving adults were observed periodically for dead insects. At weekly intervals further batches of 50 were removed from the main stock and starved.

The species of insect on which these tests were carried out and the results are given in Table V (p. 183).

In some instances most of the adults of *R. dominica*, *T. castaneum*, *O. mercator* and *S. oryzae* had died a day or two before the replacement period [see Table II]. Consequently the exposure period of the material to all the insects was somewhat less than the stated 3 months.

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FACTORS INFLUENCING UPTAKE OF COPPER FROM BRANDY BY ION-EXCHANGE RESINS

By B. C. RANKINE

Copper was efficiently removed from un-aged brandy by cation-exchange but not at all by anion-exchange. In aged brandy partial removal was obtained with both cation and anion resins.

Caramel and tannin both formed complexes with copper, and their stability increased with decrease in acidity. Very low removal of copper from these compounds was obtained at pH values above 5, and in some experiments copper was actually taken up from the cation-exchange resin.

Tasting tests indicated that ion-exchange treatment of brandies produced a slight reduction in quality.

Plastic tubing (P.V.C.) produced a permanent haze in brandy after dilution, and its use cannot be recommended.

The copper content of 33 representative Australian brandies was 0·7–12 p.p.m. with a mean value of 3·4 p.p.m.

Introduction

Excess copper is undesirable in brandy because it can cause a flocculent deposit,[1] and also because at least one state importing Australian brandy (Singapore) has recently imposed a legal limit of 5 p.p.m. on brandy in their 1957 Food and Drug Regulations.

Apart from this legal aspect, copper does not appear to play an important rôle in Australian brandy as deposition is not frequently encountered and copper is not toxic in the amounts present.

However as Singapore imports a considerable portion of Australian brandy exports it is important that more information should be obtained on copper in brandy and means of reducing it, if necessary.

It was thought that an investigation of the treatment of brandy with ion-exchange resins to remove copper would be fruitful, in view of the removal obtained in wine[2] and the anticipated absence of copper-complexing compounds in brandy. Rentschler & Tanner[3] and Garino-Canina[4] have reported removal of metals from brandy by ion-exchange.

Removal of copper by traditional fining methods is unsatisfactory, but Lafon & Couillaud[5] obtained satisfactory removal with carbon used as an ion-exchanger, followed by a milk fining. Negre & Cordonnier[6] found that calcium and sodium phytate could remove copper from brandy, although the removal was more effective under alkaline conditions.

In order to obtain more information on the copper content of Australian brandies generally, a survey of 33 representative samples was undertaken. These were obtained from the major licensed brandy distillers.

Experimental

Ion exchange

Two brandies were selected for investigation :

(1) a young acid brandy sampled shortly after distillation and not aged ;
(2) an aged brandy consisting of a blend of 4-, 5- and 6-year brandies aged in 65-gal. oak casks and ready for sale.

Both brandies were double pot-distilled from wine made mainly from Doradillo grapes. Details of their composition, determined by methods described in the A.O.A.C.,[7] are shown in Table I. The young brandy was selected as an extreme example of copper contamination and in comparison with typical Australian brandies its acidity is very high.

Both brandies were brought to the laboratory and treated similarly by passing down columns of cation and anion resins. The cation resin was Zeo-Karb 225 (Permutit Co., London) which was sieved before use to obtain beads of 20–40 B.S.S. mesh size. The resin was used in both the sodium and hydrogen forms by regeneration with sodium chloride and HCl respectively. The anion resin (De-Acidite G, Permutit Co. Ltd., London) was sieved as above and used in the hydroxyl form by regeneration with aqueous ammonia.

Table I

Composition of brandies used in the ion-exchange investigation

	Young brandy	Old brandy
Ethanol, % by vol.	60	62
,, proof spirit	5 O.P.	8 O.P.
pH	2·8	4·0
Total acid, mequiv./l.	20	7
Fixed acid, ,,	1	2
Volatile acid, ,,	19	5
Aldehydes, mg./l.	81	88
Caramel, g./l.	1·5	1·5
Copper, mg./l.	26	4·8
Iron, ,,	0·5	0·8

Laboratory ion-exchange resin columns were prepared in 25-ml. burettes with the resin (10–13 ml.) resting on a pad of glass wool. The flow rate of brandy down the columns was adjusted to 18 resin volumes per h.

Samples were withdrawn every 22 bed volumes and analysed for pH by glass electrode, and copper by the colour developed with diethyldithiocarbamate measured spectrophotometrically after wet digestion with HNO_3, H_2SO_4 and perchloric acid. All analyses were carried out in duplicate.

Equilibration experiments were then carried out by shaking 80 ml. of brandy, or solutions of pure brandy components in 60% ethanol, at various pH values between 3 and 6 (HCl or KOH), in 100-ml. Jena glass reagent bottles with 5 g. of air-dried cation-exchange resin for 3 h. at 20 $\pm$ 2° C. This technique was used in a previous investigation[8] and the shaking time and resin quantity have been found adequate to ensure that equilibrium is reached.

After being shaken, the solutions were decanted from the resin and analysed for pH and copper content as above.

Copper content of Australian brandies

The 33 representative Australian brandies were analysed for copper and pH as described above, and for total acidity by the method described in the A.O.A.C.[7] The lead content was also measured and is reported elsewhere.[9]

Results and discussion

Column experiments

The progressive removal of copper was measured during passage through the ion-exchange resin columns. The results are shown in Table II, with the changes in pH.

Although 120 resin volumes of brandy were treated it was apparent that the resins were not exhausted of their exchange capacity but, as the progressive changes were considered of more importance, the actual volumes of brandy treated to the break-through point of the resins were not measured.

The interesting feature of the results in Table II is the difference shown between the young and the old brandy in the amount of copper removed by both resin types. The cation resin efficiently removed the copper in the young brandy in both the hydrogen and sodium form of the resin, whereas removal of copper by the anion resin was quite unsuccessful. In the old brandy efficient removal took place only with the cation resin in the hydrogen form.

Equilibration experiments

It was decided to study the influence of pH on the extent to which copper can be complexed, and also to investigate the nature of the complexing agents by using pure samples of brandy constituents. Rather than use column ion-exchange, which leads to greater difficulties in pH control and interpretation of results, batch equilibration experiments were used.

Consideration was given to the components of brandy which might complex copper and two were selected for testing: caramel, an additive used for colouring, and tannic acid, representing the tannic constituents which are extracted from wooden cooperage during maturation.

Table II

Progressive removal of copper from brandies by column ion-exchange

Resin volumes 10–13 ml. Cation resin: Zeo-Karb 225
Flow rate 18 resin volumes/h. Anion resin: De-Acidite G

Bed volumes	Cation exchange (Na)			Cation exchange (H)			Anion exchange (OH)		
	Cu, p.p.m.	% removed	pH	Cu, p.p.m.	% removed	pH	Cu, p.p.m.	% removed	pH
Young brandy									
Untreated	26	—	2·78	26	—	2·78	26	—	2·78
0–22	0·7	97	3·28	0·1	99	2·61	26	0	5·00
22–44	—	—	3·00	—	—	2·59	—	—	3·64
44–66	0·2	99	2·90	0·1	99	2·57	—	—	3·55
66–88	—	—	2·82	—	—	2·56	26	0	3·35
88–110	—	—	2·77	—	—	2·55	—	—	3·26
110–132	0·1	99	2·75	0·1	99	2·55	26	0	3·19
Mean	0·3	99	—	0·1	99	—	26	0	—
Old brandy									
Untreated	4·8	—	4·02	4·8	—	4·02	4·8	—	4·02
0–22	1·3	75	4·27	0·1	98	3·15	2·3	52	5·53
22–44	1·1	77	4·08	—	—	3·06	—	—	5·43
44–66	—	—	4·05	0·1	98	3·05	2·7	44	5·34
66–88	1·0	79	4·04	—	—	3·03	—	—	5·16
88–110	—	—	4·03	—	—	3·02	—	—	5·12
110–132	1·2	76	4·02	0·1	98	3·00	2·7	44	5·06
Mean	1·1	77	—	0·1	98	—	2·6	46	—

Acetic acid was also tested as a check on the experimental procedure, because it is an important constituent of brandy and is unable to complex copper.

A number of experiments were carried out with solutions of caramel and tannic acid in ethanol and also as additions to brandy, and the results of these experiments have been brought together in Table III. Each of the solutions shown was divided into several 80-ml. samples and each sample was adjusted to a different pH value in the range pH 3–6, which is the extreme pH range of brandy. The sodium form of the cation resin was used so that the pH change produced by contact with the resin was minimised.

The results obtained with pure solutions showed that copper was completely removed from 60% ethanol both in the absence and presence of 13 mequiv./l. of acetic acid (approaching the quantity present in the young brandy).

Caramel, however, exerted a binding action on copper, which was dependent on pH, and, in view of the experiments in which caramel was added to the young brandy, accounts for the behaviour of the latter. The old brandy showed a considerably stronger binding action for copper particularly above about pH 5–5·5. It is apparent that the presence of caramel can account for binding of copper in the young brandy, but only partially account for the strong binding action exerted by the old brandy.

In view of the results of the experiments in Table III the significant difference in composition between the young and the old brandy is wood age. During maturation in small oak cooperage various tannin constituents are known to be extracted into the brandy. These tannin constituents are of indefinite and complex composition, but to test whether they exerted a binding action on copper experiments with Merck reagent grade tannic acid (the purest form of tannic acid available) were carried out.

The results indicated that above about pH 5–5·5 tannin exerts a significant binding action on copper as shown by its addition to the young brandy.

It would appear that the strong binding action of the old brandy on copper is due to the presence of caramel and wood extractives. One important finding is the strength by which copper is bound at pH values above about pH 5·5, where copper was actually removed from the ion-exchange resin and taken up by the brandy. (An analysis of the resin after several thorough regenerations showed that it contained 35 p.p.m. of copper presumably present as an impurity.)

Table III

Influence of pH and certain brandy components on removal of copper from brandy by cation-exchange resin

80 ml. of solution, 5 g. Zeo-Karb 225(Na) shaken 3 h. at 20 ± 2°

Solutions	pH at equilibrium	Copper, p.p.m. Before shaking	Copper, p.p.m. After shaking	Cu removed, %	Solutions	pH at equilibrium	Copper, p.p.m. Before shaking	Copper, p.p.m. After shaking	Cu removed, %
Ethanol 60% (v/v)	3·76	4·8	0	100	Young brandy	3·37	26	0·4	98
	4·63	4·8	0	100		4·21	26	1·7	93
	5·18	4·8	0	100		5·38	26	5·7	78
	5·44	4·8	0	100		5·98	26	10·2	61
Ethanol 60% (v/v) + 13 mequiv./l. acetic acid	3·66	4·8	0	100	Young brandy + 0·1% tannic acid (Merck)	3·94	26	1·5	94
	4·52	4·8	0	100		4·78	26	3·0	88
	4·93	4·8	0	100		5·67	26	17·1	34
	5·84	4·8	0	100					
Ethanol 60% (v/v) + 1·5 g. of caramel/l.	3·80	4·8	0	100	Young brandy + 1·5 g. of caramel/l.	3·40	26	1·0	96
	4·78	4·8	0·4	92		4·19	26	3·1	88
	5·41	4·8	0·9	91		4·99	26	5·9	77
	5·86	4·8	1·2	75		6·05	26	12·0	54
Ethanol 60% + 1·5 g. of caramel/l. and 0·1% tannic acid (Merck)	4·65	4·8	0·2	96	Old brandy	3·71	4·8	0·6	88
	5·08	4·8	0·4	92		4·50	4·8	1·0	79
	5·88	4·8	1·2	75		5·24	4·8	2·7	44
						6·20	4·8	7·7	−60*
As above but with 0·5% tannic acid	4·58	4·8	0·2	96	Old brandy + 0·1% tannic acid	4·22	4·8	0·9	81
	5·19	4·8	0·4	92		4·82	4·8	1·8	63
	5·97	4·8	2·2	54		5·59	4·8	3·9	19
					Old brandy + 1·5 g. of caramel/l.	4·44	4·8	1·9	60
						5·22	4·8	3·9	19
						5·94	4·8	7·3	−52*

* Negative value implying that copper was removed from the ion-exchange resin

In view of the complex and indefinite composition of both caramel and tannin no attempt was made to elucidate the nature of the complex formed between these constituents and copper.

Influence of ion-exchange treatments on taste

The brandy samples which were passed through the ion-exchange columns were compared with the control samples by a panel of four tasters. The general comment was that the treatments seemed to produce a reduction in quality, and in the old brandy a loss of wood character was observed.

However, should brandy need to be treated by ion-exchange resins the results of this investigation show that the treatment should be carried out soon after distillation before addition of caramel or contact with wood, and accordingly the action of ion-exchange treatment in reducing the 'aged-in-wood' character would not apply.

Haze produced by plastic tubing

An interesting side effect was observed after brandy was passed through plastic tubing into ion-exchange columns. After passage through the columns the brandy was quite clear, but on dilution to normal bottling strength of 39% ethanol by volume (32° under proof) a heavy and permanent haze was formed.

It was found that the haze only formed after the brandy had passed through P.V.C. (polyvinyl chloride) plastic tubing and was not related to the ion-exchange process. Tests of several samples of P.V.C. tubing, both beverage-grade and otherwise, gave similar results, and indicated the undesirability of using P.V.C. plastic tubing for handling spirits.

Discussion with plastic technologists revealed that flexible plastics of the P.V.C. type contain plasticisers and stabilisers which may sometimes be dissolved by the appropriate solvents.

The inference from the observations and tests was that brandy containing 60% ethanol by volume dissolved out some constituent of the plastic tubing to form a stable clear solution, but on diluting the brandy to bottling strength the lowered solvent concentration precipitated the plastic constituent which remained as a heavy stable opalescent haze, consisting under the microscope of very small particles a fraction of 1 μ in diameter.

Nature of copper in brandy

Whilst the investigation was designed to study the factors influencing uptake of copper from brandy by ion-exchange resins, some information may be inferred on the nature of copper in brandy.

Table IV

Copper content (p.p.m.) of Australian brandies

Distiller	Age in years	Alcoholic strength		Total acid,	pH	Cu,
		Proof spirit (British)	% (v/v)	mequiv./l.		p.p.m.
A	3	34 U.P.	38	5·3	3·42	4·0
	4	29 U.P.	40	9·1	3·22	4·6
B	4	33 U.P.	38	3·6	4·04	2·7
	4	15 O.P.	66	4·4	4·24	2·5
C	3	33 U.P.	38	2·7	4·20	0·8
	Blend 5, 6, 14	32 U.P.	39	4·2	4·25	0·7
D	Blend 4, 5, 7 & 9			2·6	4·57	1·0
E	3	30 U.P.	40	4·6	3·58	3·2
F	3	34 U.P.	38	2·7	4·00	0·8
G	2	30 U.P.	40	4·6	4·22	0·8
H	—	32 U.P.	39	3·5	4·35	0·9
I	3	33 U.P.	38	5·3	3·30	5·3
	5	33 U.P.	38	5·8	3·43	3·7
J	—	31 U.P.	39	3·0	4·10	4·9
K	3	6 O.P.	61	9·5	3·47	8·5
	—	32 U.P.	39	8·4	3·68	7·3
L	1	10 O.P.	63	2·5	4·01	6·6
	2	10 O.P.	63	2·1	4·60	0·8
	3	9 O.P.	63	3·0	4·58	1·7
	6	33 U.P.	38	2·8	4·24	7·0
	14	25 U.P.	43	8·0	4·28	12·0
M	4	33 U.P.	38	2·8	4·04	2·3
	5	25 U.P.	43	4·9	4·08	1·6
N	1	0·4 O.P.	57	1·7	4·39	1·0
O	3	32 U.P.	39	2·6	4·14	1·1
	5	30 U.P.	40	4·3	4·01	1·9
	15	30 U.P.	40	7·5	3·92	1·7
P	3	31 U.P.	39	4·8	3·60	6·5
	4	25 U.P.	43	9·3	3·77	6·9
Q	3	21 U.P.	45	2·4	4·06	0·7
	4	20 U.P.	46	3·1	4·13	1·1
Range of values: 0·7–12; mean 3·4						
Samples obtained from United Kingdom						
P	—	30 U.P.	40	7·9	3·51	4·2
	—	10 O.P.	63	7·6	3·89	3·7
Distilled water		—	—	—	—	0·0
R	—	31 U.P.	39	4·7	4·05	0·9
	—	10 O.P.	63	6·1	4·52	1·4
Distilled water		—	—	—	—	0·0

It would appear that in un-aged brandy without addition of caramel, copper is present as a simple cation, as it can be readily removed with a cation-exchange resin but not at all with an anion-exchange resin. When caramel is added a copper–caramel complex is formed, the stability of which depends on pH. After the brandy is aged in wood the complex tannin materials further complex copper and make its removal with cation-exchange resin more difficult. The complex formed is partly anionic since it is partly taken up by an anion resin, and the copper remaining can be mainly removed by subsequent passage through a cation-exchange resin.

Entry of copper into brandy

Considerable information is available on the methods of entry of copper into cognac from the work of Lafon & Couillaud,[5] who found that all the copper entered as contamination during and subsequent to distillation, and the copper content of the wine being distilled was not important, because copper is not entrained in the vapour.

Contamination mainly occurred from copper condensers on the stills, particularly if not properly cleaned, and the extent of contamination varied during distillation, being high initially, low during most of the distillation and very high in the ' queue ', or the relatively high-boiling fraction (tails) distilling after most of the ethanol, and containing a high proportion of volatile acid.

Subsequent cellar handling with brass equipment, such as pumps, produced an increase in copper content, e.g., 1 l. of cognac left in a brass pump for 24 h. increased in copper content by 150 p.p.m.

After brandy has been placed in wooden maturation casks the copper content does not remain stationary. The copper–tannin combination forms and is removed from solution as a flocculent brown precipitate. This reaction is dependent on pH and is most complete above pH 4·2, progressively less so between pH 4·2 and 2·5 and does not occur below pH 2·5. The results reported above serve to support and amplify this finding.

As brandy matures in wood its acidity increases, due mainly to extraction of acidic compounds from the wood, the copper–tannin precipitate slowly redissolves with time and the copper content gradually increases.

Copper content of Australian brandies

The results of the analyses are shown in Table IV. The range of values from 0·7 to 12 p.p.m. is considerable and it is interesting to observe that eight of the samples exceed the limit set by the State of Singapore (5 p.p.m.).

The United Kingdom samples were obtained from two of the principal Australian exporters. Most of the brandy is exported in bulk and broken down with distilled water and bottled in England, and accordingly samples of the bulk brandy and distilled water were also obtained.

As a matter of interest the copper content of the brandies was compared with the total acidity and a highly significant correlation was observed (the correlation coefficient with 29 degrees of freedom was 0·62), indicating that the extent of copper contamination is closely related to the total acidity of the brandy.

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The Australian Wine Board arranged with licensed brandy distillers for the samples of Australian brandies and Mr. J. F. Burgoyne of Messrs. P. B. Burgoyne & Co. Ltd., London, kindly arranged the collection and shipment to Australia of the bottled and bulk brandy samples and distilled water.

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LEAD CONTENT OF AUSTRALIAN BRANDIES

By B. C. RANKINE

The lead contents of 37 Australian brandies ranged, with one exception (0·25 p.p.m.), from <0·01 to 0·06 p.p.m. with a mean of 0·029 p.p.m. These included samples of Australian brandies bottled in the United Kingdom.

The significance of the results in view of the proposed British legal limit of 0·5 p.p.m. is discussed.

Introduction

For many years lead has been known to be a cumulative poison and its maximum content in foods and beverages has been subject to legislation. Apparently lead is one of the most toxic of the trace elements and serves no beneficial function in human metabolism.

In 1954 the British Ministry of Food recommended revised limits of 1·0 p.p.m. for lead in wines, and recently a limit of 0·5 p.p.m. for spirits has been proposed.

As the United Kingdom is one of Australia's export markets for brandy, a limit of lead in brandy is of importance to the Australian industry, and consequently this investigation of Australian brandies was made at the request of the Australian Wine Board, which represents the export interests of the wine industry.

Experimental

Collection of brandies

With the co-operation of the Australian Wine Board, a total of 31 samples was obtained from 17 licensed distillers and, as far as possible, each sample represented the finished product as sold. Consequently the samples are probably a representative cross-section of Australian brandies available for sale at present. Information on age of the brandy, type of still, alcohol content and other relevant data was obtained with most samples.

In addition samples of Australian brandy from two shippers were obtained from the United Kingdom. These comprised samples from high-strength bulk brandy as shipped (most brandy is shipped in bulk to U.K.), samples of the bulk brandy broken down to bottling strength with distilled water and also samples of the distilled water used for dilution.

Method of analysis

The method of analysis was that described by the Analytical Methods Committee of the Society for Analytical Chemistry.[1] Care was taken to purify the reagents as described in the method, and in addition all acids were purified by distillation through Pyrex glass or silica.

The organic matter in the brandy aliquots (100 ml.) was destroyed by wet digestion with HNO_3 and H_2SO_4 and method A was used for the separation of lead. All analyses were carried out in duplicate. The recovery of added lead was found to be 95–98% and replicate analyses on the same sample agreed to within 0·3 μg. in a 100-ml. brandy sample, corresponding to 0·003 p.p.m.

The total acidity was determined by the method described by the A.O.A.C.[2]

Results

The results of the analyses are shown in Table I.

Although the overall range of lead content is <0·01 to 0·25, with a mean of 0·036 p.p.m., one brandy produced by distiller J contained much more than any of the other brandies, and with this exception the range is <0·01–0·06, mean 0·029 p.p.m. The distiller J could give no reason

Table I

Lead content in p.p.m. of Australian brandies

Distiller	Age in years	Alcoholic strength		Total acid, mequiv./l.	pH	Pb, p.p.m.
		Proof spirit (British)	% (v/v)			
A	3	34 U.P.	38	5·3	3·42	0·03
	4	29 U.P.	40	9·1	3·22	0·03
B	4	33 U.P.	38	3·6	4·04	0·06
	4	15 O.P.	66	4·4	4·24	0·04
C	3	33 U.P.	38	2·7	4·20	0·02
	Blend 5, 6, 14	32 U.P.	39	4·2	4·25	0·025
D	Blend 4, 5, 7 & 9	32 U.P.	39	2·6	4·57	0·025
E	3	30 U.P.	40	4·6	3·58	0·045
F	3	34 U.P.	38	2·7	4·00	0·01
G	2	30 U.P.	40	4·6	4·22	0·015
H	—	32 U.P.	39	3·5	4·35	0·05
I	3	33 U.P.	38	5·3	3·30	0·025
	5	33 U.P.	38	5·8	3·43	0·02
J	4	31 U.P.	39	3·0	4·10	0·25*
	4	31 U.P.	39	3·0	4·15	0·03
	2	31 U.P.	39	0·9	4·93	0·01
	1	31 O.P.	75	1·0	5·71	0·01
	1	18 O.P.	67	1·0	5·34	0·01
K	3	6 O.P.	61	9·5	3·47	0·015
	—	32 U.P.	39	8·4	3·68	0·055
L	1	10 O.P.	63	2·5	4·01	0·025
	2	10 O.P.	63	2·1	4·60	0·035
	3	9 O.P.	63	3·0	4·58	0·03
	6	33 U.P.	38	2·8	4·24	0·035
	14	25 U.P.	43	8·0	4·28	0·055
M	4	33 U.P.	38	2·8	4·04	0·01
	5	25 U.P.	43	4·9	4·08	0·01
N	1	0·4 O.P.	57	1·7	4·39	0·015
O	3	32 U.P.	39	2·6	4·14	0·02
	5	30 U.P.	40	4·3	4·01	0·03
	15	30 U.P.	40	7·5	3·92	0·015
P	3	31 U.P.	39	4·8	3·60	0·01
	4	25 U.P.	43	9·3	3·77	0·035
Q	3	21 U.P.	45	2·4	4·06	0·025
	4	20 U.P.	46	3·1	4·13	0·04
	Range of values: All branches: 0·01–0·25; mean 0·036 Omitting J*: 0·01–0·06; mean 0·029					
	Samples obtained from United Kingdom					
P	—	30 U.P.	40	7·9	3·51	0·015
	—	10 O.P.	63	7·6	3·89	0·045
Distilled water		—	—	—	—	0·01
R	—	31 U.P.	39	4·7	4·05	0·02
	—	10 O.P.	63	6·1	4·52	0·025
Distilled water		—	—	—	—	0·01

for this high value and it may have been due to contamination during sampling, as the four other samples obtained later as a check were uniformly low in lead content.

The lead content of the various brandies could not be correlated with age, type of still or total acidity: The total acidity was higher in the older brandies, as would be expected, due to solution of acid constituents from the oak maturation casks.

The copper content of the brandies was also determined and the results are reported separately,[3] but it is interesting to observe that copper and lead contents show no correlation, although both arise from contamination from metallic equipment.

Discussion

Only limited information could be found in the literature on the lead content of brandies. Bagchi *et al.*[4] reported 0·05 and 0·06 p.p.m. for two brandies and Liendo[5] reported 0·04 p.p.m. for one Peruvian brandy. De Almeida[6] examined 15 samples of distilled spirits, mostly Portuguese, and found lead contents ranging from traces to 0·6 p.p.m. with a mean of 0·27 p.p.m. ; he considered the spirit to be a contributing factor in the lead content of Portuguese fortified wines. However, samples of Australian fortifying spirit examined by Rankine[7] had uniformly low lead content (0·01–0·04 p.p.m.).

The results of the present survey indicate that the lead content of Australian brandies is gratifyingly low, and well within the proposed British legal limit of 0·5 p.p.m.

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Mr. J. F. Burgoyne of Messrs. P. B. Burgoyne & Co. Ltd., London, kindly arranged the collection and shipment to Australia of the bottled and bulk brandy samples and distilled water.

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A SEMI-MICRO TECHNIQUE FOR CRUDE FIBRE DETERMINATION

By R. M. BREDON and C. D. JUKO

The semi-micro technique of Bredon has several advantages over the standard method of the Fertilisers & Feeding Stuffs Regulations. The former gives satisfactory results and, with proper equipment, can give four times as many crude fibre determinations as the latter in the same time.

After application of the Dougall corrections for altitude, results by the semi-micro method showed comparatively small errors as compared with those by the official method (determinations at 3750 and 6800 ft. altitude), and are acceptable for routine analyses.

Introduction

The standard ' Weende ' method of analysis of crude fibre, devised by Henneberg[1, 2] a hundred years ago, was originally intended as a measure of the indigestible part of foodstuffs and crude fibre was assumed to represent the structural framework of the plant or product. Kellner[3] and later workers have shown, however, that the fraction representing crude fibre is far from being wholly indigestible.

Although studies have been made of the exact chemical composition of crude fibre when derived from different feedingstuffs and also of the relationship between the crude fibre constituents as they occur in the fraction and in the original plant material,[4, 5] the nature of the crude fibre is not known and its chemical composition is not consistent.

The fraction obtained from foodstuffs after the Weende treatment consists mainly of cellulose and lignin, together with small amounts of pentosans and minerals. The cellulose and lignin content of fibre, however, does not represent the total amount of these substances present in the original foodstuff. Recovery of the cellulose in crude fibre may be anything from 40 to 88% of the original, while recovery of lignin is even more variable, with an extreme range of 4–67%[4] (see also Bredon[6]). Crude fibre high in lignin is not necessarily obtained from the most highly lignified materials.

The crude fibre fraction does not represent a constant fraction of the plant constituents and the fibre from one material is not necessarily comparable in composition with that from another. At the same time it has been shown that there is a significant relationship between the crude fibre content and the digestibility of animal feedingstuffs.[7, 8] The crude fibre figure is also used for calculating nitrogen-free extractives and starch equivalent. It is therefore used as an indication of the quality of a feedingstuff and was incorporated in the U.K. Feeding Stuffs Act, 1926.[9] The present position can therefore be summarised by noting that the crude fibre figure of any feedingstuff, whilst not denoting a chemical entity, is still very useful in a general way for routine work as being an indication of the probable feeding value of the material. The lack of a chemical entity in crude fibre makes it impossible to effect any considerable changes in the method of determination if the values are to be comparable throughout the world, and any improvements must produce results directly comparable with the standard Weende method.

The standard method, however, is slow and its accuracy leaves considerable scope for improvement. It has been shown[10, 10a] that there are considerable differences between crude fibre values obtained from replicates of the same feedingstuffs when analysed in different laboratories and by different workers.

The experimental work described in this paper has been undertaken to investigate the problem of crude fibre determination in relation to East African conditions. The first part deals with the adaptation of the semi-micro technique previously developed by Bredon,[6] to the conditions of East Africa and comparison of the results obtained with the standard method. In the second part a comparison between these two methods is carried out at different altitudes.

Experimental

Milling of samples

Uniform milling of samples is very important. To obtain uniformity of material from tropical grasses, a Christy & Norris hammer mill used with a sieve (supplied by Messrs. A. Gallenkamp & Co. Ltd.) having conically shaped holes of 0·8 mm. diameter was found to be most satisfactory.

The ' standard ' method referred to below is that given in the Fertilisers & Feeding Stuffs Regulations.[11]

Part I. The semi-micro technique[6]

The sample of feedingstuff is dried, milled and defatted by extraction in a Soxhlet apparatus with light petroleum (b.p. 40–60°) and 0·25–0·30 g. of the sample is weighed into a transparent silica tube of 50 ml. capacity. The number of samples which can be dealt with simultaneously depends upon the number of buckets of the centrifuge, but with our apparatus twelve samples can be examined simultaneously.

Twenty-five ml. of 1·25% v/v H_2SO_4 are added to each tube and placed in a multiholder immersed in a water-bath. The water-bath is brought to boil within 10 min. and a further 1 ml. of the acid is then added by a pipette with a thin jet to wash down the material which might have accumulated at the side of the tube. Evaporation of the reagent is minimised by placing glass bulbs over the mouths of the tubes. Boiling is continued for 35 min. (see later) and the multiholder is occasionally shaken in order to ensure mixing of the contents of the tubes without removal from the water-bath. The tubes are then removed from the water-bath and are immediately centrifuged. The supernatant is removed with a pipette which has a fine nozzle (slightly bent at the end) attached to a suction pump. The residue is washed once with hot water, centrifuged and the supernatant removed. Centrifugation for 5 min. at 3500 r.p.m. (2800 g.) is usually sufficient.

The same procedure is repeated with 1·25% NaOH, but now the sediment is washed twice to eliminate the alkali, centrifuging each time. The tubes are then dried at 105° for 8 h. or overnight. They are then cooled in a desiccator and weighed. The samples are ignited by placing the whole batch in an electric muffle furnace maintained at 600° until all organic matter is destroyed. When cool the tubes are again weighed. Adjustment is made for the weight of fat which was removed prior to analysis. The whole determination is carried out in the same silica tubes without removing the residues.

Development of semi-micro techniques for crude fibre determination and comparison of results obtained with the standard method under East African conditions

The results obtained originally with the semi-micro technique[6] gave slightly higher results for crude fibre than those with the standard method of the Fertilisers & Feeding Stuffs Regulations[11] (see Table I). An attempt has been made to reduce these differences and to find out if the technique is satisfactory when used at higher altitudes. Two grasses were examined by the semi-micro technique with various times of digestion and the results obtained were compared with those by the standard method.

The results (Table II) indicate that the most comparable values are obtained when digestion in acid and alkali is extended to 40 min. instead of the 35 min. of the original semi-micro technique. These results were verified by examination of twelve feedingstuffs which were chosen to represent a cross-section of various types of materials on which crude fibre is likely to be determined.

Table I

A comparison of the original semi-micro technique with the standard method of crude fibre estimation

Material	Crude fibre, % Standard method[11]	Semi-micro technique	Difference	Material	Standard method[11]	Semi-micro technique	Difference
Barley meal	4·6	5·3	+0·7	Red clover	14·4	15·1	+0·7
Dwarf beans*	5·0	5·2	+0·2	Grass (new growth)	17·8	19·6	+1·8
Weaning meal	6·5	6·6	+0·1	Silage	19·5	19·8	+0·3
'Lobel' calf nutlets	6·7	8·1	+1·4	Grass (middle stage growth)	21·1	22·1	+1·0
Barlic fattening meal	7·0	7·9	+0·9	Grass (late Autumn)	28·1	29·4	+1·3
Crushed oats	9·8	9·9	+0·1	Hay*	31·7	32·6	+0·9
Bean meal	11·6	11·7	+0·1	Straw*	38·8	39·9	+1·1

* Private communication

Table II

Comparison of results of standard method with those obtained by semi-micro technique at various times of digestion

Time of digestion, min. Elephant grass	Semi-micro technique	Standard method	Time of digestion, min. Mixed grass	Semi-micro technique	Standard method
30	31·14	30·22	30	44·69	43·31
35	30·44		35	43·96	
40	30·26		40	43·24	
45	29·95		45	42·30	
50	29·89		50	41·37	

Crude fibre was determined by both methods in quadruplicate and the standard deviations were calculated for each sample. These results are shown in Table III. To compare the values obtained by both methods, the mean values of replicates were used. The Feeding Stuffs Regulations[11] specify a permissible error of the total crude fibre when determinations are made and, because of this, the differences in the results of the semi-micro technique were tabulated as percentages of the figure obtained by the standard method.

It can be seen from the results that the semi-micro technique, in some cases, produces a higher standard deviation than the standard method but the difference between the crude fibre contents of the samples is small and does not exceed 3%, which is well within the limits of allowed error of 12%.[11] Table IV shows the errors of results of crude fibre determination between two workers for both methods. The results, although not statistically conclusive, indicate that there is a better reproducibility of results obtained from the semi-micro technique than from the standard method but, in both cases, the results are acceptable.

Part II. Comparison of semi-micro techniques and the standard method of crude fibre determination at different altitudes

In East Africa an additional error to the normal error of crude fibre determination is created by the various altitudes at which determinations are carried out. The extent of this error in relation to the standard method was investigated by Todd[12] and Dougall[13] and an equation for the correction factor was suggested by Dougall which makes the results comparable with those obtained at sea level. To find out if the semi-micro technique produces comparable results to the standard method at higher altitudes than Entebbe (3750 ft.) the determinations were carried out by the same worker, by both methods, at an altitude of 6800 ft. in the Laboratories of the East African Agriculture & Forestry Research Organisation, Muguga. The results were corrected to the values which would be obtained if determinations were conducted at sea level, using the altitude correction factor calculated from the equation suggested by Dougall.[13] The factors calculated from the equation were 0·9633 for Entebbe and 0·9286 for Muguga. The results of crude fibre corrected in this manner are shown in Table V.

Table III

Comparison of semi-micro technique with standard crude fibre method

Sample	Standard method		Semi-micro method		Difference of semi-micro method from standard method	Difference of semi-micro method from standard method expressed as % of standard crude fibre
	Mean of replicates	Standard deviation	Mean of replicates	Standard deviation		
Millet seeds	4·82	0·221	4·88	0·027	+0·06	+1·2
Russian comfrey	10·83	0·167	10·99	0·403	+0·16	+1·5
Maize bran	12·01	0·427	12·11	0·623	+0·10	+0·8
Faeces	27·82	0·227	28·44	0·797	+0·62	+2·2
Maize fodder	29·27	0·332	29·42	0·550	+0·17	+0·6
Elephant grass	30·22	0·327	31·09	0·809	+0·87	+2·9
Rhodes grass hay	35·69	0·323	35·38	0·375	−0·31	−0·9
Maize silage	40·14	0·808	40·35	0·355	+0·21	+0·5
Mixed grass	40·16	0·388	40·62	0·431	+0·46	+1·1
Rhodes grass hay	41·56	0·214	40·64	0·205	−0·92	−2·2
Mixed grass	43·31	0·185	43·35	0·158	−0·06	−0·0

Table IV

Difference in crude fibre results obtained by two operators by the standard method and semi-micro technique as determined at Entebbe

Sample	Standard method		Difference A–B	Semi-micro method		Difference A–B
	Operator A	Operator B		Operator A	Operator B	
Millet seeds	4·82	4·18	+0·64	4·88	4·83	+0·05
Russian comfrey	10·83	10·59	+0·24	10·99	11·17	−0·18
Faeces	27·82	26·95	+0·87	28·44	28·21	+0·23
Elephant grass	30·22	30·61	−0·39	31·09	30·64	+0·45
Maize silage	40·14	40·09	+0·05	40·35	40·34	+0·01
Rhodes grass hay	41·56	41·66	−0·10	40·64	40·54	+0·10

Table V

Results for crude fibre obtained by the semi-micro technique and standard method at Muguga (altitude 6800 ft.) and Entebbe (altitude 3750 ft.)

Results are expressed as % and corrected for the effect of altitude

Sample	Entebbe		Muguga	
	Standard	Semi-micro	Standard	Semi-micro
Millet seeds	4·64	4·70	4·72	4·46
Russian comfrey	10·43	10·59	10·71	11·09
Maize bran	11·57	11·67	11·76	11·90
Faeces	26·80	27·40	27·07	27·22
Maize fodder	28·20	28·34	27·72	28·19
Elephant grass	29·11	29·95	28·73	29·41
Maize silage	38·67	38·88	39·40	38·40
Rhodes grass hay	40·04	39·15	40·25	39·05

The variations between the four determinations for each sample are between 0·5 and 4·8% but only two figures are above 3%. As an additional variable was introduced by the use of a correction factor based on a regression equation, the results are in close agreement.

Discussion and conclusions

The standard crude fibre method is entirely empirical and, as such, cannot be basically modified.

Two main criticisms of this method are as follows:

(1) the inaccuracy of the results; (2) the limitations in the number of determinations that can be carried out simultaneously.

In the method as recommended by the Fertilisers & Feeding Stuffs Regulations it is possible for many errors to creep in. Thus there is no specification of the source of heating which is to be used for digestion. Some workers use electric hot plates while others use a naked flame of varying intensity and it has been shown by Nordfelt *et al.*[5] and Hall *et al.*[10] that the method of heating can contribute considerably to the error of the method.

It is usual in British laboratories to mark the level of acid and alkali on the beaker and then to maintain the level by addition of hot water as the reagent evaporates. American workers use reflux condensers, but Hall *et al.*[10] have shown that these can produce results differing from those obtained by British workers.

There is no general agreement about the method of filtering of fibre residues. The equipment used for filtering varies from alundum crucibles to linen or poplin cloths of various weaves for filtration after acid digestion. Dougall[14] advocates the use of Terylene cloth for roughages and Whatman No. 54 filter paper for concentrates; whereas the Fertilisers & Feeding Stuffs Regulations[11] specify cotton cloth or filter paper for filtration after acid digestion and an ordinary filter paper after alkali digestion. Some laboratories wash the final residue with 1% acid followed by alcohol and ether, while others use hot water only, although official recommendations advocate washing with water, 1% HCl followed again by water and then alcohol followed by ether. This lack of standardisation of the method causes considerable variations. Hall *et al.*[10] have shown that the difference between the results of nine laboratories was, in one case, as much as ±17·3% of the crude fibre figure. This figure is much higher than any difference obtained in this laboratory between the two methods.

The semi-micro technique now described standardises the procedures for the determination of crude fibre and therefore eliminates many of the errors. Thus the use of boiling water as the means of heating ensures that the temperature of the reagents at the same altitude is constant. Centrifugation is adopted in place of filtration, which eliminates the losses of residue through the cloth used for filtering and also losses of the residue which is inevitably left behind on the cloth or filter paper. As the temperature of the reagents remains just below boiling point and the glass bulbs placed on the top of the tubes act as reflux condensers, negligible amounts of the reagents are lost by evaporation.

The main source of error in the semi-micro technique is in the method of sampling. The quantity of material used for analysis is 0·25–0·30 g. against 2–3 g. used in the standard method. This error is minimised by the use of well powdered samples. Although the effect of milling

has not been separately investigated it has been observed that large particles do not compact well under centrifugation and cause difficulty when removing the supernatant liquor.

Despite this error, the total error of the semi-micro technique as shown in Table III is small and within the limits specified under the Feeding Stuffs Regulations. The technique is therefore acceptable for routine analysis. A statistical analysis of results given in Table V proves that there is no significant difference between the standard method and the semi-micro technique ($P > 0.05$) or between determinations of crude fibre by either methods at the two altitudes ($P > 0.05$). It also shows that the correction factor for altitudes as suggested by Dougall is applicable to both methods.

The limited number of simultaneous determinations of crude fibre possible by the standard method depends on the time required by an average worker for filtering and washing the fibre. On average a sample can be put for digestion every 6 min. and six samples can be dealt with simultaneously. Thus, when the filtration of the last sample after the acid digestion is completed, the first sample for filtering after the alkali digestion is ready. By this means, six samples can be ready for drying in slightly less than 2 h. With the semi-micro method and a twelve-bucket centrifuge, twelve samples can be digested. Consequently, 24 samples can be ready for drying in $2\frac{1}{2}$ h. In both cases the time for final filtration and washing after alkali and, in the case of the semi-micro method, time for centrifuging has been taken into account. The increased output of the semi-micro technique makes the method superior to the standard method when large numbers of crude fibre determinations are required. The authors found that, instead of using silica tubes, which are expensive, for determination of crude fibre, it is possible to use Pyrex tubes for digestion and to transfer the final residues into silica crucibles for drying and ashing. This produces the same results but delays the determination and the possibility arises of an additional error.

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CHEMICAL INVESTIGATION OF SEEDS OF SELECTED TROPICAL PLANTS. I.—Component Acids of the Fats or Oils

By A. MACKIE and D. G. MIERAS

The component acids of the fats or oils of Pinang mabuk, *Monodora myristica*, *Xylopia aethiopica*, *Celosia argentea*, *Anogeissus schimperi*, *Mangifera indica* and *Carapa procera* were investigated. The acids were determined qualitatively and quantitatively by gas chromatography. The results are compared with those obtained for seeds of other plants of the same families.

Introduction

The component acids of the fats or oils from the seeds* of the following plants were investigated: (a) Pinang mabuk (P.m.), a variety of betel nut (*Areca catechu*, Palmae); (b) *Monodora myristica*, Dun. (M.m.) (Anonaceae); (c) *Xylopia aethiopica* (X.a.) (Anonaceae); (d) *Celosia argentea* (C.a.) (Amarantaceae); (e) *Anogeissus schimperi* (A.s.) (Combretaceae); (f) *Mangifera indica*, L. (M.i.) (Anacardiaceae); (g) *Carapa procera*, D.C. & C. (C.p.) (Meliaceae).

With the exception of Pinang mabuk (from Malaya) all the other seeds are of West African origin. These seeds were selected with the view to isolating any vermicidal principle, since they have been used by natives as anthelmintics.

In the literature are described the acids of the seed fat of the betel nut (of which Pinang mabuk is a variety) and of *M. indica* (by three authors[1]): otherwise little is known of the chemistry of these seeds. The first part of this investigation was concerned with the isolation of the fats or oils and the determination of the component acids.

Several methods for the determination of the latter were examined, viz., (i) the vacuum distillation of the methyl esters, (ii) reverse-phase partition chromatography, (iii) gas chromatography.

Experimental

Mineral matter

The ash content of the dried seeds was determined and the ash examined for metals on a Hilger Semi-quartz Spectrograph. The results are given in Table I.

Table I

Ash content of seeds

Plant	Ash, %	Metals present
Pinang mabuk	1·5	K, Mg, Na (spectrum of ash of betel nut identical)
Monodora myristica	2·2	Na, Mg (K, Cu, Fe traces)
Xylopia aethiopica	2·6	Na, Mg, Ca (K, Cu, Fe traces)
Celosia argentea	3·4	Na, Mg (K, Cu, Fe traces)
Anogeissus schimperi	3·4	Na, Mg, Ca (K, Li, Cu, Fe traces)
Mangifera indica	4·4	K, Na, Mg (Li, Cu, Fe traces)
Carapa procera	4·7	Na, Mg, Ca (K, Li, Cu traces)

Isolation of fat or oil

The seeds were finely ground and extracted in 100–250-g. portions (Soxhlet, 6 h.) with light petroleum (b.p. 40–60°) and allowed to macerate overnight. After removal of the solvent, the residues were examined.

If the residues were oils, they were steam distilled to ascertain the amount of volatile matter. The non-volatile residues were then examined and the results tabulated (Table II).

Saponification

The fat or oil was saponified with 10% ethanolic KOH and the unsaponifiable matter and fatty acids were isolated by the standard procedure. The characteristics of the fatty acids are shown in Table III.

* In the strictly botanical sense, parts of the fruit were, in some cases, included with the seed.

Table II

Properties of the fats and oils from the seeds

Fat or oil from	Fat, % wt. of seeds	Steam volatile matter, %	Sap. value	Iodine value	Setting point, °C	Melting point, °C	Unsaponifiable matter, % wt. of fat
P.m.	8·0% pale yellow fat	—	223·8	28·7	34·0	40	0·8% yellowish-brown waxy solid
M.m.	22·0% amber oil	3·5	172·2	84·6	—	—	10·0% pale brown liquid
X.a.	27·0% pale yellow fat	1·5	202·3	46·4	23·7	38	2·5% yellow-brown waxy semi-solid
C.a.	8·0% yellow-green oil	—	249·5	97·2	—	—	6·6% pale yellow semi-solid
A.s.	0·5% dark green sticky semi-solid	—	89·5	43·5	—	—	42·0% yellow-green semi-solid
M.i.	14·0% pale yellow fat	—	191·2	45·1	38·6	35–42	3·0% yellowish-brown solid
C.p.	13·0% off-white fat	1·0	200·8	61·8	—	20	3·0% yellowish-brown solid

Table III

Characteristics of the mixed fatty acids from the fats and oils

Fat or oil from	Appearance	Sap. equiv.	Iodine value	Setting point, °C	Melting point, °C
P.m.	Pale yellow solid	237·2	30·5	34·6	40·0
M.m.	Dark viscous oil	331·6	103–75*	—	—
X.a.	Almost white solid	276·4	39·7	21·2	33–38
C.a.	Almost white solid	208·7	89·8	34·7	35–36
A.s.	Dark green semi-solid	555·2	65·6	—	20–23
M.i.	White solid	293·8	38·8	41·5	55–57
C.p.	Yellowish-brown solid	283·0	63·8	31·3	32–36

* Iodine value decreased from 103 to 75 in 3 months

Preparation of methyl esters

The mixed fatty acids were converted into esters by the usual procedure by refluxing with absolute methanol containing 1% of conc. H_2SO_4 and then removal of any unesterified acids. They were stored under nitrogen until required for gas chromatography.

Determination of the fatty acids by gas chromatography

The apparatus was a Pye Argon Chromatograph. Celite (80–120 mesh), previously washed successively with HCl and water, was coated with ethylene glycol polysuccinic ester[2] in the ratio 3 parts of ester to 8 parts of Celite in chloroform. The solvent was removed and the residue heated (45 min.) *in vacuo* at 200°. The packed column was conditioned overnight at 180° with an argon flow rate of 60 c.c./min.

During the determination of the methyl ester mixtures, the apparatus was run at a temperature of 172—177°; gas flow rate 42–37 c.c./min.; detector voltage 1250 V; sensitivity ×3; chart speed 45 in./h. The sample size was 0·1 μl.

The acids were determined quantitatively and qualitatively by the graphical method as used by Farquhar *et al.*[2]. Table IV shows the results obtained.

The gas chromatographic technique was found to be much superior to other techniques tested. In the initial stages of this research the acids from Pinang mabuk were determined qualitatively by separation of the methyl esters[3] and the acids from *M. myristica* by reverse-phase partition chromatography.[4] Although the results obtained by these techniques were similar to those obtained by gas chromatography, the latter technique could determine quantitatively not only acids present in quantity, but also minute traces.

Discussion

The major component acids in the seed fats are as follows:

(a) Pinang mabuk, lauric (15·9%), myristic (50·6%), palmitic (14·8%)
(b) *M. myristica :* oleic (37·2%), linoleic (49·7%)
(c) *X. aethiopica :* palmitic (38·6%), oleic (41·6%)
(d) *A. schimperi :* palmitic (29·4%), oleic (15·4%), linoleic (38·6%)
(e) *M. indica :* stearic (47·8%), oleic (38·2%)
(f) *C. procera :* palmitic (31·3%), oleic (49·3%), linoleic (11·9%)

When Pinang mabuk (see Table IV) is compared with the ordinary betel nut, it is found that there are only slight differences in the amounts of the component acids ; Pathak & Mathur[5] found the following component acids in the fat from betel nut (*Arecu catechu*) : capric (0·2%), lauric (16·6%), myristic (44·9%), palmitic (13·8%), stearic (2·0%), lauroleic (0·3%), myristoleic (0·6%), palmitoleic (7·8%), oleic (7·4%) and linoleic (6·4%). Myristic acid is thus predominant in the fat from both sources.

Table IV

Fatty acids present in the seed fats

	P.m. %	M.m. %	X.a. %	A.s. %	M.i. %	C.p. %
Saturated acids						
capric C_{10}	trace	—	—	—	—	—
lauric C_{12}	15·9	—	—	—	—	—
tridecanoic C_{13}	—	—	—	trace	—	—
myristic C_{14}	50·6	0·2	0·3	2·4	—	0·2
palmitic C_{16}	14·8	3·8	38·6	29·4	6·5	31·3
margaric C_{17}	0·3	—	0·3	trace	—	—
stearic C_{18}	3·4	5·3	9·1	6·8	47·8	5·0
nonadecanoic C_{19}	3·1	—	—	—	—	—
arachidic C_{20}	—	1·4	—	4·1	2·7	0·9
heneicosanoic C_{21}	4·9	—	3·6	—	—	—
Unsaturated acids						
Mono palmitoleic C_{16}	—	—	—	1·4	—	1·0
oleic C_{18}	4·9	37·2	41·6	15·4	38·2	49·3
Di hexadecadienoic C_{16}	—	—	—	trace	—	—
linoleic C_{18}	6·9	49·7	3·2	38·6	4·4	11·9
Tri linolenic C_{18}	1·1	2·5	3·3	1·9	0·5	0·4

The higher content of unsaturated acid from betel nut compared with that from Pinang mabuk is noteworthy. Amongst the acids with an odd number of carbon atoms present in Pinang mabuk, appreciable amounts of nonadecanoic (3·1%) and heneicosanoic (4·9%) have been detected besides a small amount of margaric acid (0·3%).

The content of the component acids of the seeds examined did not vary much from other species of the appropriate family as recorded by Hilditch[1] except in the few cases noted.

M. myristica and *X. aethiopica*, both members of the Anonaceae, show considerable differences in component acid content ; e.g., *X. aethiopica* contains approximately 10 times the amount of palmitic acid and only 1/16 the amount of linoleic acid as does *M. myristica*.

X. aethiopica seeds contained at least twice the amount of palmitic acid and not more than one-quarter of the amount of linoleic acid present in other members of the Anonaceae. *M. myristica* seeds, on the other hand, contained almost one and a half times the amount of linoleic acid as would be expected from the Anonaceae.

A. schimperi has five times the amount of arachidic acid and not more than half the amount of oleic acid stated for members of the Combretaceae.

The *M. indica* examined was obtained from Ghana : it was found that the amounts of the component acids did not differ much from the figures quoted for *M. indica* from other sources.[1]

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PROTEIN AND LIPID CONSTITUTION OF SOME PAKISTANI PULSES*

By B. E. BAKER, J. A. PAPACONSTANTINOU, C. K. CROSS and N. A. KHAN

Nineteen fatty acids have been determined by gas chromatography in the light petroleum extracts of five species of pulses commonly grown in West Pakistan.

The dispersion of the nitrogenous constituents of the same five pulses by water and by sodium chloride solutions ranging in concentration from 0·005N to 1·0N, has been studied. Two minima were observed in the peptisation curves obtained with mash, mung, masur and gram but only one minimum was observed with lobia.

Introduction

The importance of the pulses in the nutrition of the people of India and Pakistan has stimulated considerable interest in the constitution of these materials. The amino-acid composition of the pulses (gram, *Cicer arietinum*; mung, *Phaseolus mungo*; mash, *Phaseolus radiatus*; masur, *Lens esculenta*; lobia, *Vigna sinsensis*) most commonly grown in India and Pakistan, has already been reported,[1–9] but there has apparently been no systematic study of the salt peptisation of pulse proteins[10–12] or of the fatty acid composition of the oils from these pulses. The present investigation was undertaken in view of the sparsity of information in these fields.

Experimental

The samples of pulses used in these experiments were supplied by the Director of Agriculture, Northwest Frontier Province, West Pakistan.

Preparation of oil-free pulse meal

The seeds were ground in a Mikro-Samplemill (Pulverising Machinery Co., Summit, N.J., U.S.A.) fitted with a screen that had openings 1 mm. in diameter. The results of the proximate analyses of the pulses have been reported previously.[1] The meals were dried at 25° in a vacuum desiccator containing P_2O_5 and were then extracted (Soxhlet) with light petroleum (b.p. 30–60°) for 5 h. The solvent was removed from the meals by means of a water aspirator and they were passed again through the Mikro-Samplemill. The meals were then extracted (Soxhlet) with light petroleum for 15 h. and again freed of solvent.

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Fatty acid analyses of ether extracts

The combined ether extracts of each pulse meal were heated on a steam bath to remove most of the solvent, the last traces of which were removed by means of a water aspirator. Calculations based on the quantity of oil that had been isolated from each pulse meal showed that the pulses contained the following amounts of petroleum-extractable material: gram, 4·30%; masur, 1·42%; lobia, 2·04%; mung, 1·44%; and mash, 2·14%.

The samples of oil were first saponified by refluxing with 0·3N-KOH in 95% ethanol and the fatty acids were then liberated in a separatory funnel by the addition of 1·0N-HCl acid. The fatty acids were extracted with light petroleum, the ether evaporated and the fatty acids esterified with diazomethane. The esterified samples were analysed by gas chromatography with a butanediol–succinic acid column (6 ft.) and a diethylene glycol–succinic acid column (4 ft.), both operated at 200° and at a gas flow rate of 120 c.c. (helium) per min. Table I shows the fatty acid composition of the five pulse oils.

Extraction of protein by water and by neutral salt solutions

The oil-free meals (2·5 g.) were shaken for 30 min. in centrifuge bottles (250 ml.) with 100 ml. of solvent on a mechanical shaker (ERWEKA G.m.b.H, Type KUI) and the mixtures then centrifuged (International Centrifuge, Size 2, Model R) for 15 min. at 1900 r.p.m. The supernatant liquids were decanted and the residues washed with 20-ml. portions of the solvent and the wash-waters were combined with the extracts. The extraction and washing procedure was repeated twice and the resultant extracts and wash-waters were combined with those obtained in the first extraction. The volume of each combined extract plus wash-water was made up to 500 ml. The nitrogen content of each composite sample (500 ml.) was determined by Kjeldahl analysis. Table II shows the percentages of the total N (average of three determinations) extracted by water and by the various NaCl solutions.

Discussion

The results reported in Table I show that the five pulse oils differ markedly in their fatty acid composition. Collin *et al.*[13] demonstrated that the oleic acid, linoleic acid and linolenic acid contents of soya-bean oil vary from one variety of soya-bean to another and with environmental conditions. The same is undoubtedly true for the pulse oils analysed, but when the variations due to these factors are taken into consideration, it is evident that the pulse oils are quite different in fatty acid composition. Worthy of note is the relatively high cerotic acid content of mung and the high behenic acid content of mash. Miki & Sera[14] analysed a sample of mung oil extracted from *Phaseolus mungo* grown in Manchuria and obtained values for the palmitic, stearic and linoleic acid contents in general agreement with the values reported in the present paper. Miki & Sera, however, reported a value for oleic acid which is three times greater and a value for linolenic acid which is five times smaller than that reported in the present paper.

Table I

*Composition of the ether-extractable portion of pulses**

	Lobia	Mung	Gram	Masur	Mash		Lobia	Mung	Gram	Masur	Mash
C_{12}	N.D.	N.D.	N.D.	T	0·1	$C_{18}=$	12·2	6·4	19·3	36·0	20·8
C_{13}	N.D.	N.D.	N.D.	T	0·2	$C_{18}^{2}=$	27·4	32·6	62·9	20·6	16·3
C_{14}	0·3	0·4	0·3	1·1	0·6	$C_{18}^{3}=$	12·3	14·4	3·3	1·6	35·7
C_{15}	0·1	0·2	N.D.	0·6	0·5	C_{20}	0·9	0·9	T	2·3	N.D.
isoC_{16}	N.D.	0·1	N.D.	0·3	T	$C_{20}=$	N.D.	0·2	N.D.	1·8	N.D.
C_{16}	33·4	28·1	12·7	23·2	14·1	$C_{20}^{2}=$	N.D.	N.D.	N.D.	0·1	N.D.
$C_{16}=$	0·3	0·1	0·1	0·3	0·9	C_{22}	4·0	2·4	N.D.	2·7	9·3
C_{17}	0·6	0·1	T	0·1	0·5	$C_{22}^{2}=$	N.D.	N.D.	N.D.	2·8	N.D.
C_{18}	7·1	7·8	1·5	4·6	4·3	C_{24}	0·2	N.D.	N.D.	N.D.	N.D.
						C_{26}	1·1	6·3	N.D.	1·7	3·8

* The data are expressed in wt.-% of total eluate. The subscript below C shows the number of carbon atoms in the straight acids. The exact configuration of the carbon skeletons of the iso-compounds is not known. Two horizontal bars next the C indicate a double bond, and the superior figure is the number of double bonds.

T indicates trace
N.D. indicates that the fatty acid was not detected

Table II

Effect of salt concentration on peptisation of pulse protein

Normality of NaCl solutions	% of total nitrogen extracted				
	Lobia (*Vigna sinensis*)	Mash (*Phaseolus radiatus*)	Mung (*Phaseolus mungo*)	Masur (*Lens esculenta*)	Gram (*Cicer arietinum*)
0 (water)	56·37	23·24	57·18	45·13	46·59
0·005	51·72	22·70	34·87	42·06	42·65
0·010	48·53	28·92	35·64	48·47	37·99
0·025	34·56	27·30	32·56	39·55	42·65
0·050	32·60	22·70	25·13	30·92	35·84
0·075	33·58	21·08	32·05	31·20	34·77
0·100	34·31	28·12	40·76	38·72	44·09
0·250	54·41	56·22	68·46	61·00	55·76
0·500	63·24	67·57	69·49	71·31	73·12
1·000	68·23	68·65	70·51	71·31	75·27

It will be noted from Table II that water extraction removed about twice as much nitrogen from masur and gram and about two and one half times as much nitrogen from lobia and mung as it did from mash. One may conclude from these results either that mash contained less albumin than did the other four pulses or that more of the globulin fractions were extracted from the foul pulses than from the oil-free mash meal.

The effects of salt concentration on the peptisation of the nitrogen constituents of lobia are similar to those observed by Smith *et al.*[15] in their studies on soya-bean and tepary bean. With the other four pulses, however, two minima are observed in the curves plotted from the results shown in Table II, the first at a salt concentration of 0·005N with mash, mung and masur, and at 0·1N with gram ; the second at a salt concentration of 0·075N with mash, mung and gram, and at 0·005N with mung. The various extracts are being examined by electrophoresis to obtain more precise information on the nature of the protein constituents of these pulses.

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THE SHRINKAGE OF CHERRIES IN JELLY

By MARGARET J. ANTHISTLE

It is demonstrated that cherries gradually shrink if set in a gelatin jelly and that the main factor governing the extent of the shrinkage is the gel pH. The pH of minimum shrinkage depends on the isoelectric pH of the gel and the results are discussed in the light of Donnan's theory of membrane equilibrium.

Introduction

The work described here followed the observation that cherries set in a gel containing gelatin, sugar and citric acid, sterilised by heat treatment, began to shrivel after a few weeks' storage. No reports relating directly to the subject could be found and the experimental work suggested itself after a consideration of Donnan's theory of membrane equilibrium. The influence was investigated of gel strength, of gel pH and of gel type as defined by isoionic pH, on the extent of shrinkage.

Experimental

Preparation of the gels

Several litres of gelatin solution were prepared at a time by dissolving gelatin crystals in a small amount of boiling distilled water, adding sugar if required and making up to volume with cold distilled water. The pH values of portions of the solution (measured with a Pye Cambridge pH meter) were then adjusted by the addition of solid malic acid, or in a few cases with a small amount of hydrochloric acid.

Preparation of the fruit in jelly

The cherries used were taken from cans of cherries (pH 4·0) in sugar syrup. In the first experiments (Tables I and II) a pack canned in 35° Brix syrup gave cherries of soluble solids 24·5–25·5%. In the later work cherries canned in a lighter syrup were used (soluble solids content about 12–14%). In the initial work A1 plain cans were used as containers for the fruit in jelly. These were later replaced by small glass jars with metal caps. Four cherries were weighed into each container, covered in hot gelatin solution and processed immediately.

Sterilisation process

Cherries in cans were covered with 280 ml. of gelatin solution at 175° F and given a process consisting of a 5 min. exhaust at 185° F followed by a cook of 12 min. at 212° F. When glass jars (holding 120 ml. of gelatin solution) were used a separate exhaust was not given. The gelatin was filled at 180° F and the jars were closed immediately and cooked for 10 min. at 212° F. The products were cooled and stored at 15° C. These processes gave good keeping quality at all the pH values used but the packs were not intended for human consumption. In a commercial pack some adjustment of the process may be needed and the gelatin must be heated to 200° F before filling to avoid any chance of bacterial spoilage.

Examination of products

After suitable storage intervals the cherries were removed, wiped free from gelatin and reweighed.

Measurement of isoionic and isoelectric pH values of the gelatins

The mixed bed deionisation method[1] was used for measurements of isoionic pH. A 5% solution of gelatin was passed at a rate of 250 ml. of solution per h. per 100 ml. of resin, through a column of Biodeminrolite maintained at 35° C. In the case of the acid-processed gelatin the eluate was collected in an evacuated flask to minimise contamination with CO_2. Measurement of the pH of the eluate gave the isoionic pH of the gelatin.

Viscosities were plotted against pH values to determine the isoelectric pH—the pH of minimum viscosity. All measurements were made with 3% solutions at 35° in a U-tube viscometer and the pH values of the gelatin were adjusted with HCl or KOH as required.

Results

Shrinkage was estimated as the percentage change in weight of the cherries. Under the experimental conditions used it could be estimated to within ±0·5%. From general observations it may be said that a weight loss of less than 10% has no obvious effect on the appearance of the fruit, losses between 10 and 15% are accompanied by slight shrinkage and above 15% loss shrinkage is noticeable.

Tables I and II show the results of the initial work. Table I shows that the shrinkage of fruit increased with the gel concentration and that at both concentrations a large amount of shrinkage occurred between pH 2·5 and 3·5 whereas small changes, usually slight swelling, occurred between pH 4·5 and 5·6. Table II shows that at pH 4·0, which is a convenient value from a commercial point of view, shrinkage was negligible both in the presence and absence of sugar. Sugar in the gel at pH 3·5 considerably reduced the shrinkage.

Table III shows the results of experiments carried out under slightly different conditions, with cherries of lower soluble solids content (12—14%) set in gelatin and packed in glass jars. In this Table are also indicated the effects of two gelatins, one lime-processed (isoionic pH 4·9) and the other acid-processed (isoionic pH 9·05), and of various additives to the jelly. There is a general trend towards more marked shrinkage than that previously experienced, although this was not noticeable from the appearance of the fruit apart from that set in the high-isoionic gel. This gel caused weight losses over the whole pH range from 3·4 to 5·6, and shrivelling at the lower pH values was very rapid and was noticeable after a few days' storage.

The shrinkage becomes less as the pH value of the gel increases over a certain pH range, but the pH for minimum shrinkage is not clearly defined. From the earlier work (Tables I and II) it was thought to lie between pH 4·0 and 4·5 for the lime-processed gelatin, but in the later work it is evident that the upper limit for minimum shrinkage can lie above pH 4·5. The

Table I

Changes in weight of cherries set in gelatin gels

Gelatin in solution, % w/v	Original pH of gel	Decrease in weight of fruit, % Storage time			Comments
		6 weeks	9 weeks	12 weeks	
3	2·5	9·9	6·5	10	Gel did not set. Slight shrinkage
3	3·5	17	27	24	Soft gel. Noticeable shrinkage
3	4·5	−1·0	−4·1	−1·9	Firm gel. Cherries firm and round
3	5·6	−4·2	−6·3	−5·5	Firm gel. Cherries firm and round
5	2·5	34	27	—	Gel did not set. Noticeable shrinkage
5	3·5	37	52	46	Soft gel. Shrinkage very marked
5	4·5	−0·4	−2·2	−0·8	Very firm gel. Cherries firm and round
5	5·6	−1·2	−0·1	+1·5	Very firm gel. Cherries firm and round

Table II

Changes in weight of cherries set in a gel containing sugar and gelatin

Gelatin in solution, % w/v	Sucrose in solution, % w/v	pH of gel		Decrease in weight of fruit, % Storage time		Comments
		Original	Final pH	6 weeks	9 weeks	
3	nil	3·5	3·4	12	26	Soft gel
	6·5			7·8	17	Soft gel
	13·0			6·7	13	Soft gel
3	nil	4·0	4·0	−0·5	+0·7	Firm gel
	6·5			−0·6	—	Firm gel
	13·0			+0·4	−10	Firm gel
3	nil	4·2	4·1	−5·9	+0·2	Firm gel
	6·5			−2·6	−2·2	Firm gel
	13·0			−3·1	−3·4	Firm gel
3	nil	4·5	4·4	−3·7	−1·6	Firm gel
	6·5			−2·2	−3·4	Firm gel
	13·0			−3·3	−3·9	Firm gel

Tables I and II show the results of experiments using gelatin of isoionic pH 4·9 and cherries of high soluble solids content (24·5–25·5%) packed in plain cans. Adjustment of pH was made with malic acid.

Table III

Changes in weight of cherries set in a 3% gelatin gel containing various additives

Isoionic pH of gelatin	pH adjusted with	Other additives to gelatin	Original pH of gelatin	Decrease in weight of fruit, % Storage time	
				7 weeks	27 weeks
4·9	Malic acid	None	3·4	25	—
,,	,, ,,	,,	3·8	19	—
,,	,, ,,	,,	4·0	19	—
,,	,, ,,	,,	4·2	2·8	—
,,	,, ,,	,,	4·5	2·4	—
4·9	Hydrochloric acid	None	3·4	23	27
,,	,, ,,	,,	3·8	—	—
,,	,, ,,	,,	4·0	4·2	3·0
,,	,, ,,	,,	4·2	−1·1	−2·4
,,	,, ,,	,,	4·5	−3·9	−2·8
4·9	Malic acid	Sodium chloride (to give a 0·1M solution)	3·4	5·2	—
,,	,, ,,	,, ,,	3·8	—	—
,,	,, ,,	,, ,,	4·0	4·0	0·2
,,	,, ,,	,, ,,	4·2	3·6	4·0
,,	,, ,,	,, ,,	4·5	−4·0	0·4
9·05	Malic acid	None	3·4	54	—
,,	,, ,,	,,	3·8	—	—
,,	,, ,,	,,	4·0	40	—
,,	,, ,,	,,	4·2	38	—
,,	,, ,,	,,	4·5	17	—
,,	,, ,,	,,	5·6	4·0	—

pH of minimum shrinkage is higher in the acid-processed gel and probably lies somewhere in the region of pH 7·0. Insufficient experiments have been made to allow any definite conclusions to be drawn about the effects of HCl or NaCl on shrinkage, but it seems likely that sodium chloride will reduce shrinkage at the lower pH values.

When the low-isoionic gels were heated with HCl or malic acid to give a pH value less than 4·0 they became turbid. This change did not depend upon the presence of cherries and it could be due to heat-coagulable protein in the alkali-processed gelatin used.

The viscosity determinations showed aqueous solutions of the lime-processed gelatin to have an isoelectric pH which coincided with its isoionic pH of 4·9. With this gelatin made 0·02M with respect to malic acid (the concentration of malic acid required to give pH 4·0) and with lime-processed gelatin from actual jars of cherries in jelly it was difficult to obtain reproducible measurements of viscosity, and it was not possible to make an accurate determination of the isoelectric pH.

Discussion of results

The most likely cause of shrinkage of the cherries is a difference in osmotic pressure on either side of the cherry skin which would cause an osmotic flow of liquid from the cherry into the gel. This can be thought of in terms of Donnan's theory of membrane equilibrium. If the gelatin sets it will act as its own membrane since the gelatin ions will be held rigidly; if it does not set the cherry skin (provided that it is undamaged) will form the membrane, permeable to all the ions present except to the large ions of gelatin itself. In either case the gelatin ions not being free to diffuse will prevent the equal distribution of other ions on either side of the cherry skin. This will give rise to an abnormally high osmotic pressure and therefore to an increased flow of liquid through the skin. This is in many ways analogous to the case of an acid solution of gelatin separated by a membrane from a solution of the same acid. Bolam[2] and Glasstone[3] applied Donnan's and Van't Hoff's equations for osmotic pressure in dilute solution to show that for solutions on the acid side of the isoelectric pH: (1) the osmotic pressure in the jelly will be greater than that in the solution of acid (so that by analogy shrinkage of the fruit due to the passage of water into the gel will be expected), (2) the osmotic pressure difference (and hence the shrinkage) will depend on, and will fall to a minimum at, the isoelectric pH of the gel,

(3) the presence of a neutral salt in the jelly will reduce the osmotic pressure difference at all pH values (and so should help to reduce shrinkage of the fruit).

The results support this theory in that the shrinkage depends on the isoelectric pH of the gel and becomes less as that pH is approached. It would be expected that the actual pH of minimum shrinkage would depend upon the solids content of the cherries and the presence of pectin in the fruit. Because of anion adsorption the isoelectric pH values of gels used are likely to be lower than their corresponding isoionic pH values. The dependence of shrinkage on gel strength is in accordance with ordinary osmotic pressure considerations.

It is intended to carry out further experiments with a deionised gelatin over a wide pH range and to investigate the salt effect more fully. In the meantime the work shows that any noticeable shrinkage may be avoided by the use of an alkali-processed gelatin adjusted to pH 4·0.

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Fruit & Vegetable Canning & Quick Freezing Research Ass.
Chipping Campden
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ENZYMIC CHANGES IN LAMBS' LIVER DURING STORAGE. I

By D. N. RHODES and C. H. LEA

The rates of autolytic degradation of the phospholipids, neutral lipids and protein of lambs' liver have been measured during storage at 37°, 15°, 1°, −10° and −20°, and at 15° and 1° after frozen storage at −10° or −20° for 8 or 14 weeks.

Chemical changes in the fresh liver proceeded about 13 times faster at 37° than at 15°, and about 3·5 times faster at 15° than at 1°. At −10° and −20° hydrolysis was hardly detectable after storage for 24 weeks. At 15° the onset of measurable autolytic change was delayed for about 2 days, but no lag period was observed when the material had previously been held at −10° or −20°. This activating effect of freezing on the hydrolytic enzymes was much less marked when post-thawing storage was at 1°.

The degradation of the glycerophospholipids results from the simultaneous removal of the two fatty acid residues, followed by breakdown of the water-soluble phosphate ester moiety to orthophosphate. The fatty ester linkages of the triglycerides were split even more rapidly than those of the phospholipids, indicating the presence of a true lipase in lambs' liver.

Fresh liver remained organoleptically acceptable for up to 10 days at 1° or 1–2 days at 15°, times which were not significantly affected by frozen pre-storage at −10° or −20° for 8 or 14 weeks. No significant deterioration could be detected by tasting tests after storage for up to 25 weeks at −10° or −20°.

Introduction

The commercial handling of lambs' liver for consumption in the United Kingdom falls into two phases: frozen transport from abroad at temperatures between −10° and −20° for

periods of approximately 6–8 weeks, and the holding of thawed imported and fresh home-killed material at chill temperatures during retail distribution for 2–7 days. To facilitate rapid freezing, livers for overseas transport are packed in seamed cans with a minimum of headspace and avoiding the inclusion of pockets of air. Frozen storage can, therefore, be regarded as very largely anaerobic. After being thawed the liver is normally exposed to air.

In vivo, animal liver is the site of intense metabolic activity and rapid changes in the composition of the lipids, proteins and carbohydrates induced by continued enzymic activity might be expected after death, even at the low temperatures of chilled or frozen storage. Although some work has been published on biochemical aspects of the degradation of the lipids of liver during autolysis, mainly at 37° (see Discussion), no information appears to be available on autolytic changes under the conditions of practical handling. The present work has, therefore, been designed to investigate such changes and to relate them to the organoleptic acceptability of the foodstuff.

Experimental

Materials

Livers were obtained from lambs slaughtered in commercial abattoirs in November and December, 1959. The livers were recovered from the carcasses between 15 and 30 min. after death and storage treatments were commenced within a further 90 min.

Storage conditions

Frozen storage.—Livers were cut into seven pieces of roughly equal weight (about 70 g.) and sealed in 5-oz. cans with seamed lids. The cans were exposed to an air blast at −20°, which cooled the centre of the piece to −10° in 2 h. The cans were then transferred to cold storage rooms at −10° or −20°.

On the following day the headspace gas in the cans was replaced with nitrogen by evacuation and flushing three times through a pin hole in the lid which was then sealed with solder. The seams of the cans were rendered completely gas-tight by quickly dipping in molten Woods metal under a layer of lactic acid, a process shown to cause no thawing of the liver.

Post-thawing storage.—After several intervals, cans were removed from frozen storage and thawed by immersion in ice water or running tap water for 2 h. The lids were then punctured to allow ingress of air and the cans sealed in a large polythene bag to prevent loss of moisture during subsequent storage at 1° or 15°.

Chilled storage.—To investigate changes in fresh liver at temperatures above freezing, 50-g. pieces of liver were held at 1° and 15° in sealed polythene bags which prevented desiccation while permitting access of oxygen. For chemical examination only, 5-g. pieces of liver were sealed in small polythene bags containing 1 ml. of 0·1% aqueous sodium ethyl mercurithiosalicylate ('Thiomersil') as bactericide. This reagent was used in preference to toluene–chloroform which has been found to inhibit tissue phospholipase activity.[1]

Methods

Tasting tests.—Some difficulty was encountered in standardising the procedure for cooking liver samples. In the method finally adopted, slices about 1 cm. thick were laid on the bottoms of glass dishes and the tops sealed with aluminium foil. The dishes were steamed for 20 min., after which the pieces were turned over and cooked for a further 10 min. Any fluid exuded from stored samples ('drip') was added to the dish before cooking.

Samples were scored for odour, flavour and texture by a panel of 5–8 persons using a hedonic scale from 1 to 9, 5 representing neither like nor dislike, 6 to 9 like slightly, moderately, very much and extremely, and 4 to 1 dislike in similar degrees. The average of the scores is given. The panel was given no preliminary training, and their reactions can therefore be considered to be no more critical than those of average consumers.

Extraction of soluble constituents.—The basis of the chemical examination was the analysis of those constituents of the tissue extractable into either the organic or the aqueous phase of a chloroform–methanol–water mixture. The procedure was based upon that of Folch *et al.*[2]

in which lipids are extracted from tissues by a mixture of chloroform and methanol, and the extract washed free of water-soluble material by shaking with a critical volume of water. In the method used in the present work the washing step was incorporated into the extraction step, so that the non-lipid substances present in the tissue could be simultaneously extracted into the aqueous phase.

Wet liver (5·0–5·5 g.) was weighed accurately into the 100-ml. cup of a top-drive homogeniser (Nelco) and 50 ml. of methanol–chloroform (1 : 2 v/v) and 8·0 ml. of water (or 7·0 ml. when 1·0 ml. of antiseptic solution was present in the sample) were added. The glass cup was fitted to the homogeniser under a cap made from polythene to fit as closely as possible to the driving axle of the blades and to rest firmly on the rim of the cup. Loss of the solvent by evaporation was thus reduced from 5 ml. when the open cup was used to less than 0·5 ml. Samples were homogenised for 4 min. and the whole homogenate decanted into a 100-ml. centrifuge cup and spun at 2000 r.p.m. for 10 min. (M.S.E. major). Two clear homogenous liquid layers were produced, separated by a tightly packed layer of disintegrated tissue. The upper layer was simply poured off and the exposed sides of the tube carefully wiped. The wad of tissue was then displaced with a glass rod and the lower layer poured through a filter paper to remove any particles of debris. In sampling stored liver which had exuded drip, a proportionate volume of the fluid, based on the weights of the sample and the whole piece, was added to the sample before homogenisation. The extracts were stored at 1°; after 1 day the upper phases deposited a fine white precipitate which was filtered off before analysis.

The efficiency of the extraction of the lipids was assessed by the measurement of the lipid P recovered from 5·0-g. samples of the same liver after homogenisation for 1, 2, 3 and 4 min. The yields (0·392, 0·368, 0·384 and 0·396 mg. respectively) indicate that no advantage was to be gained by a longer treatment. Moreover, when the tissue wad was twice more re-extracted with fresh solvent under the same conditions the lipid P appearing in the extracts was no greater than that calculated from the volume of the previous extract mechanically held in the wad.

To determine the actual volumes of the two phases obtained, known amounts of elaeostearic acid and ammonium sulphate were added to standard runs and the concentrations in the phases measured by ultra-violet absorption and alkaline distillation respectively. The volumes found were 25·0 ml. in the aqueous phase and 36·5 ml. in the organic phase when 5·0 g. of liver were taken. Variation in the temperature of the solvents between 10° and 30° during homogenisation and separation did not significantly affect the volumes of the phases. The compositions of the upper and lower layers were chloroform : methanol : water 3 : 48 : 47 and 86 : 14 : 1 respectively.[2]

Folch *et al.*[2] demonstrated the almost quantitative retention of some natural lipid mixtures in the organic phase of this system during the washing procedure, but a further study was made of the behaviour of the lipophobic lysophospholipids which could be present as partial degradation products of the diacyl-compounds. Under the standard extraction conditions detailed above, 95% of added lysophosphatidylcholine appeared in the organic phase when pure solvents were used. The partition ratio was not affected by the presence of 0·003M-calcium or magnesium chlorides or 0·05M-sodium chloride in the water taken, additions originally recommended.[2]

No significant loss of the lipids present in the liver would, therefore, be expected from the organic phase of the extracting mixture. However, an arbitrary fractionation of the water-soluble components must result from the presence of approximately 50% of methanol in the aqueous phase. For example, whereas oligosaccharides, proteins and higher peptides would be insoluble, simple sugars, amino-acids, phosphate esters and salts would be expected to be dissolved at the concentrations encountered.

pH.—In preliminary experiments on liver stored for various times, pH measurements were made with a glass electrode on the aqueous phase of the extracts and compared with those on homogenates of the whole tissue. The values obtained on the extracts were found to be 1·0 ± 0·1 units higher than on the homogenates over a range 7·3 to 5·5. All determinations reported were made on the aqueous extracts.

Phosphorus was determined by the method of Allen[3] and inorganic phosphate by the same method without digestion with perchloric acid.

Total N was determined by a micro-Kjeldahl procedure and *amino-N* by reaction with ninhydrin.[4]

Total solids.—Solvents were removed from aliquots in flasks and the residue dried under 0·1 mm. Hg vacuum at 70° for 30 min.

Carboxylic esters.—Fatty acids esterified in glycerides or phospholipids were estimated colorimetrically by reaction with hydroxylamine and ferric iron.[5] The molar colour yield given by methyl palmitate was found to be unaffected by the presence in the reaction mixture of 0·5 ml. of chloroform, methanol or of the mixed solvents of the organic phase described above. The method was therefore simplified by omitting the evaporation step.

Free fatty acids.—The release of free fatty acids from glyceride or phospholipid esters was followed by titration of the dry matter recovered from the total solids determination on the organic phase. The residue was dissolved in 10 ml. hot 75% ethanol and the amount of 0·01N-NaOH required for the phenolphthalein end-point after neutralisation to methyl red was taken as a measure of the fatty acid content.

Model experiments with mixtures of HCl and palmitic acid gave satisfactory estimates of the latter showing that inference by the strongly acidic phosphate groups of phospholipids would be eliminated by the differential titration procedure.

Since the amounts of free fatty acids present in the samples used were small and because the end-points could not be observed precisely in the coloured solutions of badly deteriorated samples, the titration method proved to be generally less accurate than the measurement of the decrease in ester value, although the two measurements correlated well. The titrations were, therefore, discontinued when this point had been established.

Drip.—The volume of fluid released from the liver tissue during post-thawing or chilled storage was measured by decantation from the solid. For muscle it is known that an appreciable time is required after thawing for the exudation of drip resulting from damage by freezing and this was confirmed with the present material, the volumes after thawing being 6·6, 8·5 and 9·8 ml./100 g. of liver after 2, 3 and 18 h. respectively. From these figures it appears that most of the drip resulting from freezing damage was released within a few hours of thawing.

Presentation of results.—Analyses for P, N, amino-N and carboxylic esters are given in mmoles throughout, and all values are calculated to the basis of 100 g. of wet liver.

Results

Sampling and experimental errors

Two approximately 5-g. samples, taken from different lobes of each of three livers, were analysed for the amounts of total P, inorganic P, total N and total solids in the aqueous phase, and for total P, total N, carboxylic esters and total solids in the organic phase. The mean deviation for each pair of results was calculated as a percentage of the mean and the averages of these values for the eight measurements made on each liver were ±1·0, 0·8 and 2·0% in the three cases. This close agreement between duplicate analyses indicated little variation in the composition of liver within the organ.

Analysis of fresh liver

The composition of lambs' liver has been given as protein 21%, fat 4% and water 71%.[6] In the present work eleven livers were sampled for analysis as soon as possible after death. The results showed no great variation in the amounts of the various lipid and non-lipid extractable solids in ten of these; in the eleventh case the amount of dry matter soluble in the aqueous phase was markedly lower, although the other values were within the normal range (Table I).

The total lipids of the fresh livers amounted to 5·7% of the wet weight and had a mean N/P ratio of 1·03, esters/P ratio of 2·45 and dry solids/P ratio of 1073 mg./mmole. If the phospholipids are assumed to contain two ester groups per atom of P and to possess a molecular weight of 800 these figures indicate the presence of about 20% by weight of triglyceride fat in the lipids.

The total N content of the extractives soluble in the aqueous phase was 4·08% by weight. Of this N, 28% reacted with ninhydrin indicating the presence of free amino-acids or peptides. In addition to salts, the remainder of the extractives was presumably carbohydrate, and in the

Table I

Analyses of fresh lambs' livers

	Unit per 100 g. of liver	Mean of 10 livers ± S.D.	Liver No. 11		Unit per 100 g. of liver	Mean of 10 livers ± S.D.	Liver No. 11
Organic extract				*Aqueous extract*			
Total P	mmoles	5·34 ± 0·39	5·30	Total P	mmoles	5·39 ± 0·36	5·59
Total N	mmoles	5·48 ± 0·33	5·34	Inorganic P	mmoles	1·20 ± 0·21	1·58
Carboxylic esters	mmoles	13·1 ± 2·1	12·6	Total N	mmoles	16·5 ± 0·8	17·6
Dry solids	g.	5·73 ± 0·71	—	Dry solids	g.	5·66 ± 0·68	3·03

case of liver 11 (Table I) this fraction comprised a much smaller proportion of the total solids. About 22% of the P soluble in the aqueous phase was inorganic phosphate in the fresh materials and the total P content of the extractable solids was 2·95%.

The total P extracted in both phases was 10·73 mmoles/100 g. (= 332 mg./100 g.). The total of the solids extracted amounted to 11·4% of the wet weight of the liver.

Pre-storage changes

In four experiments small pieces of the livers were removed with scissors and dropped into liquid nitrogen within 6 min. of the death of the animal. Further samples were taken from the same livers after the normal handling period (about 1·5 h.). The analyses by the most sensitive criteria of hydrolytic action were compared (Table II). No significant changes had occurred in the lipids, but considerable increases in the inorganic phosphate and the total solids of the aqueous phase were found. The production of free phosphate was paralleled by a significant drop in pH. The increase in the total solids soluble in the aqueous phase was not accompanied by any change in the total N and was, therefore, probably due to the appearance of soluble sugars resulting from the post-mortem breakdown of liver glycogen.

Storage experiments I and II

In the first two storage experiments the rates of autolytic changes in pieces of intact liver at 37°, 15° and 1° were established. In Expt. II (the results of which are given in Figs. 1 and 2) 5-g. pieces from the same liver were placed in polythene bags with 1 ml. of 0·1% 'Thiomersil'. Although the antiseptic delayed the growth of micro-organisms the last points on the curve may have been affected by bacterial growth. In separate experiments samples were held at 15° for periods of up to 8 days with and without 1 ml. of 0·1% Thiomersil or of 0·025% Aureomycin solution saturated with sorbic acid.

The results showed that the changes in lipid P and esters, soluble-P in the aqueous phase, inorganic P, total N and amino-N were unaffected by the presence of the antibacterial agents. It can be concluded, therefore, that the antiseptic did not affect the activity of the enzymes of

Table II

Hydrolytic changes in liver during the immediate post-mortem period

	Mean of 3 livers		One liver	
Sampling time (min. after death)	6	90	3	120
Organic extract		(mmoles/100 g. liver)		
Carboxylic esters	12·9	13·0	16·6	16·8
Total P	5·08	5·43	4·90	4·61
Aqueous extract				
Total P	5·07	5·47	4·84	5·18
Inorganic P	0·95	1·21	1·00	1·47
Total N	15·2	15·9	16·7	16·7
pH	7·81	7·33	7·80	7·31
		(g./100 g. liver)		
Dry matter	4·48	5·58	3·92	6·58

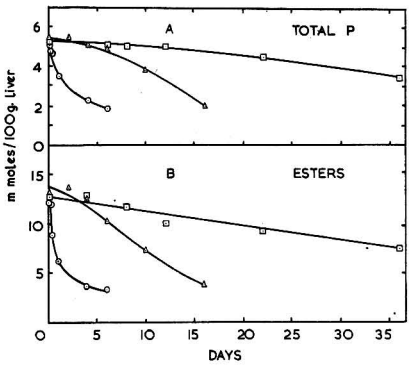

FIG. 1 (left).—*Changes in the lipids of liver during storage in air at* 1° (□), 15° (△) and 37° (○) (Expt. II)

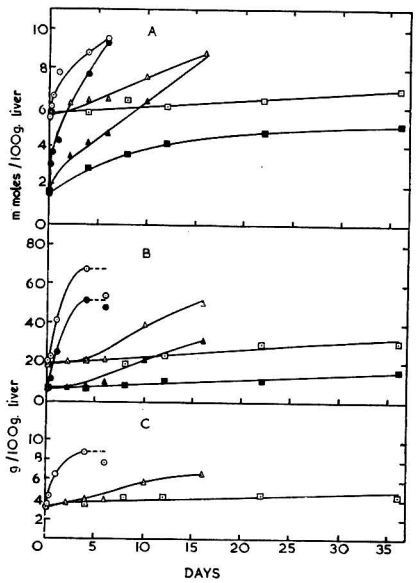

FIG. 2 (right).—*Changes in the extractives in the aqueous phase of liver during storage in air at* 1° (□), 15° (△) *and* 37° (○) (Expt. II)

A total P open symbol; inorganic P closed symbol
B total N open symbol; amino-N closed symbol
C dry matter

the tissue and that microbial metabolism made no appreciable contribution to the hydrolytic changes observed.

Results on liver from another animal (Expt. I) followed closely those given in Figs. 1 and 2 except with respect to the increase in water-soluble solids and total-N. In experiment II at 37° the total solids rose from 3·0 to 8·4 g./100 g. of liver and the total N from 17 to 67 mmoles/100 g. (Fig. 2); in experiment I the corresponding figures were 7·5 to 16 and 17 to 140. The extent of these changes at 15° and 1° showed similar differences between the two experiments.

Effect of temperature

The rates of the reactions at the three temperatures were estimated by measurement of the times taken for various degrees of change (Table III), a method which limits the comparisons to the early parts of the curves owing to the slow rate at the lowest temperature. The results showed that at 37° the degradative changes proceeded about 13 times faster than at 15° and at 15° about 3·6 times faster than at 1°. These figures cannot be used to compute a valid temperature coefficient for the enzymic reaction because the curves at 15° show clear evidence of a lag period before the onset of hydrolysis, a feature which was also present in the 37° results although it cannot be seen on the reduced scale of Figs. 1 and 2. It is possible that the slow changes at 1° were still in a lag phase at the conclusion of the experiment.

Changes in the lipids

Total lipids.—Since the fatty acids make up the bulk of the weight of the lipids and remain soluble in the organic phase after release by hydrolysis the total extractable solids in this phase changed very little and results are not recorded.

Lipid P and esters.—The destruction of the phospholipids and the breakdown of triglyceride fat were shown by the marked changes in the lipid-soluble phosphorus and in the total carboxylic esters (Figs. 1A and 1B). The ratio of esters/P rapidly decreased from the initial ratio of 2·4 to about 1·7 at 37° and 1·9 at 15° and then remained steady, suggesting that the triglyceride esters hydrolysed more rapidly than those in the phospholipids (Fig. 3A). This was also shown by plotting the ester value against the lipid P (Fig. 3B); in this figure the later points lie on

Table III

Times taken for various amounts of chemical change in liver during storage in air (Expt. II)

	Amount of change/100 g. liver	Temperature of storage				
		37°	15°	1°	37°/15°	15°/1°
		(Days)			(Ratio of rates)	
Organic extract						
Total P	−1·0 mmoles	0·6	7·3	25	12	3·4
Esters	−5·0 mmoles	0·8	9·0	31	11	3·4
Aqueous extract						
Total N	+10 mmoles	0·6	7·0	18·5	12	2·7
Total P	+1·0 mmoles	0·35	6·8	33	19	4·9
Total solids	+1·6 g.	0·5	7·6	28	15	3·7

straight lines which have a slope of 2·1 ester groups/P, as would be expected for the simultaneous removal of the two fatty esters from a glycerophospholipid, whereas the early points lie on lines of much greater slope.

Total N.—Measurement of the true lipid N was rendered difficult in stored samples by the presence of large quantities of free amino-acids, part of which appeared in the organic phase of the extract by association with the phospholipids. Although these contaminating nitrogenous substances could be removed by further washing or by chromatography[7] the process was too tedious for routine application to the many samples examined.

Changes in the non-lipids

Nitrogen and total solids.—Whereas a maximum of 3 mmoles of phospholipid N could have been rendered water-soluble during the whole reaction period studied, 15 times this quantity appeared in the aqueous extracts (Fig. 2B). This increase in total N was paralleled by a similar large increase in amino-N. The relation between the two was linear, showing a ratio of amino-N/

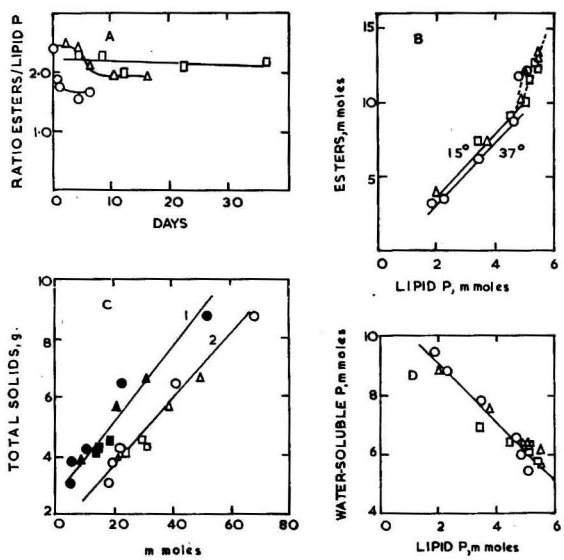

FIG. 3.—*Relationships between various chemical criteria* (Expt. II)

1° □, 15° △, 37° ○ (units per 100 g. liver)
C curve 1, amino-N curve 2, total N

total N of 0·89. Both increases correlated closely with the increase in total solids (Figs. 2C and 3C), the slopes of the correlation lines giving ratios of 112 and 125 mg. of dry matter/mmole of total N and amino-N respectively. These results showed that the solids soluble in the aqueous phase released during storage could be accounted for in terms of amino-acids of average molecular weight of between 112 and 125. Such a figure excludes the presence of any appreciable amount of peptides and two-dimensional paper chromatography in butanol–acetic acid–water and phenol–water showed spots corresponding to eleven of the common amino-acids but only traces of spots which may have been dipeptides.

The maximum increases in water-soluble solids at 37° amounted to 9 and 5% of the wet weight of liver in Expts. I and II, respectively, representing 35 and 25% of the total protein originally present.

Phosphorus.—As most of the phosphorus present in liver was extracted in the two solvents used, the correlation between the disappearance of lipid P and appearance of water-soluble P was good, the slope of the best fit line being −1·0 (Fig. 3D). The water-soluble esters initially present and those produced by the decomposition of the phospholipids during storage were rapidly hydrolysed further to give inorganic phosphate (Fig. 2A), indicating a high level of phosphoesterase activity.

Frozen storage

Samples were examined on removal from frozen storage at −10° and −20° at intervals up to 25 weeks, during which period the changes, as measured by the chemical criteria, were small. Since six livers in all were used to provide the samples, the results have been given in terms of differences from the appropriate initial value and compared with the mean of values obtained on the fresh livers (Table IV).

Table IV

Chemical changes in liver during frozen storage

Storage Temp., °C	Time, weeks	No. of samples examined	Soluble in aqueous phase: Total P	Soluble in aqueous phase: Inorganic P	Soluble in organic phase: Total N	Soluble in organic phase: Total P	Soluble in organic phase: Esters
			(mmoles/100 g. liver)				
Initial values		9	5·39	1·20	16·5	5·34	13·1
				Increase		Decrease	
−10	8	4	0·31	1·19	1·7	0·01	−0·2
−10	14	5	0·12	1·63	1·5	0·61	−0·2
−10	24	5	0·14	1·62	1·9	0·54	0·1
−20	8	2	−0·12	0·20	0·9	0·04	−0·8
−20	14	5	0·09	1·12	2·1	0·36	0·9
−20	24	4	−0·13	1·09	0·0	0·43	0·1

Chemical changes.—Only the increase in inorganic phosphate soluble in the aqueous phase represented a significant change from the initial value. This increase must have derived largely from hydrolysis of the water-soluble phosphate esters present in the fresh material since the amount of glycerophosphate esters released from the lipids during storage was much smaller. Even after 24 weeks the loss of lipid P amounted to only about 10% with no apparent corresponding decrease in the carboxylic esters.

Organoleptic changes.—Nine livers were tasted by the panel within 3 h. of excision in batches of three on different days. The mean of the marks for flavour ranged between 6·5 and 7·5 with a mean of 7·2. The stored samples were offered to the panel in groups of two or three and, since it was not possible to include fresh liver as a control with all long-stored samples owing to cessation of supplies, no controls were included at any stage.

The results for flavour score on samples stored for periods up to 24 weeks at −10° or −20° showed that the panel considered the stored material to be no less acceptable than when fresh, the samples being marked mainly within the limits of scatter of the initial values (Fig. 4).

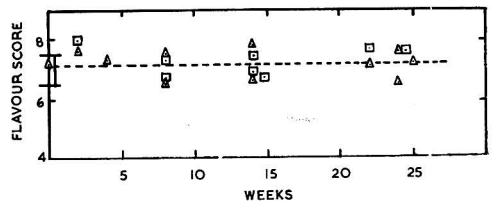

FIG. 4.—*Changes in flavour of liver held in nitrogen at* −10° (⊡) *or* −20° (△)

Post-thawing storage

After 8 and 14 weeks at −10° and −20°, samples were thawed and examined after further storage for 0, 2, 4, 6 and 12 days at 15° or 0, 4, 8, 12 and 24 days at 1°. In order that the taste panel should compare all of the edible samples from each of the higher storage temperatures at one time, the dates of removal from frozen storage were varied by up to 3 days at 15° or 6 days at 1° about the correct removal date. In view of the slow rates of change at the freezing temperatures the effects of these slight variations in frozen storage time can be neglected.

Four livers were used in this series of experiments, each providing samples for complete post-thawing storage after 8 and 14 weeks in the frozen state; the curves are labelled L5, 6, 8 and 9 to identify the origin of the material. Comparison of the results has been facilitated by adjusting the curves to a common origin, in the case of lipid P and ester value by expressing the figures as % of the appropriate initial value, in other cases by the addition of a small constant throughout the curve.

Organoleptic changes (Fig. 5).—Liver held at 1° after frozen storage for 8 or 14 weeks at either −10° or −20° showed no greater rate of deterioration than did fresh material. In all cases the panel flavour score began to drop below the initial values after about 8 days, but the samples were still judged acceptable (flavour score 6) after 12 days at 1°.

At 15° the rate of deterioration was rapid, the panel rejecting all samples as completely inedible (score 3) after 3 days and as unacceptable (below 6) after 1–2 days. The effect of frozen pre-storage on the rate of deterioration at 15° was again insignificant.

The decline in the acceptability of liver stored at 1° or 15° was due mainly to the appearance of a bitter flavour which was usually detectable in samples marked 6 by the panel, and which became extremely unpleasant in samples of lower score.

Since the release of free amino-acids was quantitatively the most marked of the chemical changes measured, a test was made of the effect on the flavour of cooked liver of the addition of these substances. Seventeen amino-acids, in the proportions present in bovine serum albumin, were mixed in 10 ml. of aqueous solution with 50 g. of fresh minced liver to give final total concentrations ranging from 2 to 20 mmoles/100 g. of liver. The samples were cooked by steaming for 30 min. and tasted. The panel was just able to detect the presence of the

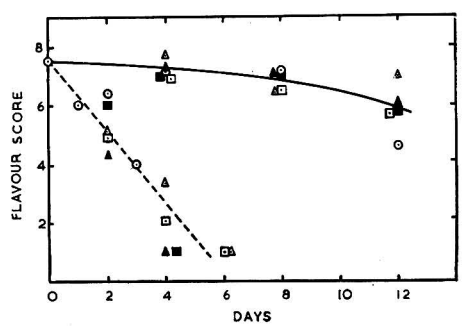

FIG. 5.—*Changes in flavour of liver held in air at* 1° (solid line)

After storage at −10° (□) for 8 weeks (open) or 14 weeks (closed)

After storage at −20° (△) for 8 weeks (open) or 14 weeks (closed). Fresh liver (⊙)

amino-acids at the two higher concentrations but no comment was made indicating the presence of any particular off flavour in any sample (Table V). The solution of amino-acids itself, at a concentration of 100 mmoles/100 ml. had only a slightly salty ' meat extract ' flavour, with no traces of the bitterness characteristic of the stored liver.

Similar tests were made of the effect on flavour of additions of mono- and dibasic potassium phosphates at the concentrations of inorganic phosphate which developed in the deteriorated liver samples. Again the changes in flavour were quantitatively small and were not related by the panel to the particular off flavour induced by storage.

Chemical changes. (a) *Drip.*—No drip was released from fresh liver during storage for 12 days at 1°. At 15° none was found until after 4 days, and by 8 days the maximum amount had developed (Table VI). After frozen storage the amounts of drip released were very variable and showed no clear correlation with pre-thawing storage conditions. After 8 weeks at −10° or −20° drip developed on thawing and appeared to increase somewhat during the first few days' storage at either 1° or 15°.

(b) *pH.*—Acidity developed in the aqueous extractives during storage of fresh liver more rapidly at 15° than at 1°, the ratio, calculated from the times to drop 0·3 pH unit being 5·0 (Fig. 6). The rates of deterioration of thawed and fresh samples were identical when held at 1°. At 15° samples previously held at −20° showed a more rapid decrease in pH than those held at −10°, with the fresh samples in between, but these differences are more likely to have been due to variation between the individual livers used than to any real effect of the pre-storage treatments.

Fig. 7 shows some of the changes in the extractable solids during storage at 1° and 15° of fresh liver and liver previously held at −10° or −20° for 8 weeks. Results after 14 weeks were generally similar and have not been included.

Lipid P (Fig. 7A).—Fresh liver stored at 1° showed a slow loss of organic P over 12 days and the rate was not greatly affected by frozen pre-storage for 8 or 14 weeks.

At 15° the rate of phospholipid hydrolysis in the fresh liver was slow during the first 4 days and then accelerated sharply (cf. Expts. I and II). Frozen pre-storage eliminated this lag period entirely, the loss of lipid P commencing immediately after thawing. After both 8 and 14 weeks' frozen storage the rate of loss was greater in liver from −10° than from −20°.

Carboxylic esters (Fig. 7B).—The rates of disappearance of carboxylic ester groups at both 1° and 15° were considerably increased by the frozen storage treatments, an appreciable lag period again being present with the fresh liver. No consistent differences were found between frozen storage at −10° or −20° or between holding periods of 8 or 14 weeks.

Table V

Effect of addition of amino-acids on flavour of cooked minced fresh liver

Concn. of amino-acids (mmoles/100 g. of liver)	0	2·0	4·2	10·4	20·8
Mean flavour score	7·0	6·6	7·3	6·4	6·4

Table VI

Release of ' drip ' from liver held at 1° or 15°, fresh and after storage at −10° or −20° for 8 or 14 weeks

Frozen storage		Post-thawing storage				
Time, weeks	Temp., °C	Temp., °C	Time, days			
			2	4	8	12
			(ml./100 g. liver)			
None		1	—	0·0	0·0	0·0
		15	0·0	0·0	12·2	—
8	−10	1	—	6·6	3·4	9·4
8	−10	15	5·4	7·0	—	—
8	−20	1	—	4·5	6·7	7·7
8	−20	15	9·3	13·0	—	—
14	−10	1	—	6·6	11·3	7·7
14	−10	15	3·3	5·7	—	—
14	−20	1	—	5·9	5·4	8·8
14	−20	15	2·7	9·8	—	—

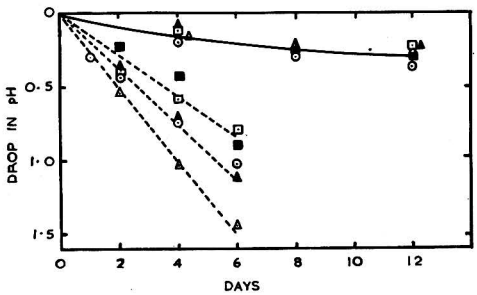

FIG. 6.—*Changes in pH of the aqueous extract of liver held in air at* 1° (solid line) *or* 15° (broken line)
After storage at −10° (□) for 8 weeks (open) or 14 weeks (closed)
After storage at −20° (△) for 8 weeks (open) or 14 weeks (closed). Fresh liver (⊙)

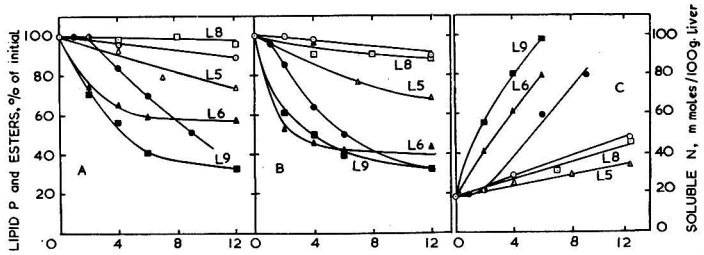

FIG. 7.—*Changes in liver held in air at* 1° (open symbol) *or* 15° (closed symbol)
After 8 weeks' storage at −10° (□), or −20° (△). Fresh liver (⊙)
A lipid P B esters C soluble N

Total N (Fig. 7C).—A marked induction period in the activity of the proteolytic enzymes in fresh liver held at 15° had been noted in Expts. I and II (Fig. 2B) and was replicated in two further experiments. After 8 weeks' frozen storage, however, the production of water-soluble N was rapid and immediate with no signs of any induction period. A similar result was found after 14 weeks.

During post-thawing storage at 1° the proteolytic changes were much slower and there was no appreciable difference between the fresh and frozen material.

Phosphorus.—The rates of production of total water-soluble P in these experiments were again exactly complementary to the losses of lipid P from the organic extracts and showed the same differences. The production of inorganic phosphate also moved parallel to the total P, as in Expts. I and II (Fig. 2A).

Examination of imported livers

A can of imported lambs' liver bought from a retail store contained seven whole organs weighing in all 10 lb. The can had been made ready for immediate handling and the temperature was −1·6° at the centre.

Pieces from two of the livers were cooked, together with one sample of fresh liver and one that had been in storage at −20° for 25 weeks. The tasting panel were unable to distinguish consistently between the four samples, marking them all within the normal range of a satisfactory standard. The mean score values were: English (fresh), 7·0; English (stored 25 weeks at −20°), 7·3; imported (1), 7·7; imported (2), 6·7.

Duplicate samples were analysed from each of five livers and, as in the case of the eleven fresh livers examined previously (Table I), remarkably small variations in the proportions of the extractable substances were revealed between individual livers (Table VII). No unequivocal comparison can be made between the analyses of fresh English and the imported livers

Table VII

Analyses of imported livers

	Mean of 5 livers ± S.D. (mmoles/100 g. liver)
Organic extract	
Total P	4·59 ± 0·20
Carboxylic esters	11·5 ± 1·0
Aqueous extract	
Total P	6·48 ± 0·28
Inorganic P	3·98 ± 0·14
Total N	30·3 ± 1·5
	(g./100 g. liver)
Dry solids	5·89 ± 0·83
pH	7·30 ± 0·06

owing to the wide difference in origin and storage history, but the differences that appear are all indicative of some enzymic degradation in the imported material, especially the proportions of inorganic P and total N soluble in the aqueous phase.

Discussion

The storage life of lambs' liver as measured by organoleptic tests was found to be about 10 days at 1° or 1–2 days at 15°, times which were not significantly affected when the liver had previously been held frozen at −10° or −20° for 8 or 14 weeks.

The extent of autolytic changes as measured chemically showed considerable variations at the end of the storage life. At 1° hydrolysis of the lipids averaged about 10% of the initial, the nitrogen soluble in the aqueous phase had increased by 20 mmoles/100 g. and the pH had decreased by 0·3 unit. At 15° changes in the lipid P and water-soluble nitrogen were almost nil during the first 2 days' storage of fresh liver, although the pH had dropped by 0·4 unit and the ester value by 15%. In thawed liver which had previously been held frozen, the chemical changes all proceeded rapidly from zero time and were already well advanced after 2 days at 15°. The lipid P and ester values, for example, had dropped by 30 and 45% respectively and the nitrogen soluble in the aqueous phase had increased by 30 mmoles/100 g. The analyses of imported frozen livers of unknown history also indicated that enzymic hydrolysis of lipid and protein had proceeded to a considerable degree, yet this material was not distinguished from fresh liver by the taste panel.

It appears from these data and from the negative results obtained in tasting experiments in which amino-acids or phosphates were added to minced liver, that the deterioration in flavour recognised by the tasting panel in the stored samples was not directly associated with any of the particular chemical criteria measured. Further work will, therefore, be necessary to elucidate the origin of the off-flavour which develops in liver during storage at temperatures above freezing. It is worth recording, however, that the development of bitter flavour in cheese has been attributed to the accumulation of certain bitter-tasting peptones produced by enzymic proteolysis,[8] and this may well be the case with stored liver, although most of the protein destroyed appears to be broken down completely to amino-acids. Bitter flavours can also be produced as a result of ' browning-type ' reactions between amino-acids or peptides and carbohydrates or carbohydrate degradation products.

The fact that considerable enzymic decomposition could occur in liver undetected by the taste panel may be due in part to a lack of training or experience on the part of members of the panel. It is likely that with training they would have become more discriminating, but the fact remains that these chemical changes did not result in easily detectable organoleptic changes.

Since no significant deterioration of liver in frozen storage at −10° or −20° was demonstrated by organoleptic or by chemical tests up to 24 weeks, it appears that the lower temperature offers no advantage in the commercial storage of the commodity for this period of holding. The present results demonstrate, however, that enzymic degradation proceeds at temperatures

above 0° much more rapidly in stored liver after thawing than in fresh, and emphasise the importance of expeditious handling and the maintenance of temperatures as close as possible to 0° during this time. In particular the exposure of sliced liver in unrefrigerated showcases, as is commonly practised by smaller retail butchers, is clearly undesirable.

The activating effect of frozen storage upon subsequent enzymic hydrolysis at temperatures above freezing will be described more fully in the following paper.[9]

The slow rate of hydrolytic change in the lipids of liver at −10° and −20° agrees with the observations of Cook & White[10] who found no significant changes in the free fatty acids of chicken subcutaneous fat during storage at −13·5° or −22° for periods up to 25 months.

The temperature-dependence of the activity of the lipolytic enzymes of the liver contrasts with the findings of Dyer & Fraser[11] and of Olley & Lovern[1] on the hydrolysis of the lipids of cod muscle. In the fish, lipolysis proceeded at about the same rate throughout the range 0° to −14° (~25% hydrolysis in 20 days) and at measurable rates at −22° and −29° (10% hydrolysis in 30 and 80 weeks, respectively). The rate of hydrolysis in the lambs' liver was comparable at 1° (25% hydrolysis in 25 days), but at −10° and −20° the lipid changes were not measurable after 22 weeks. The greater activity of the lipolytic enzymes of cod flesh at the lower temperatures may however be a characteristic of the species rather than a difference between cold- and warm-blooded animals since in halibut and rosefish-flesh lipolysis was very slow at −18°.[11]

The decomposition of liver lipids *in vitro* has been measured under various conditions encountered in biochemical studies. Fairbairn[12] found that cat or rat liver in pieces showed 8% loss of phospholipid fatty acids within a few minutes of excision. An unbuffered homogenate rapidly lost 15% of the phospholipid fatty acids and then remained stable, but autolysis proceeded much further in a homogenate buffered at pH 7·2. Fries *et al.*[13] found no hydrolysis in 1 h. but 30% destruction in 6 h. Fishler *et al.*[14] working with slices at 37° and pH 7·2 found 20% destruction after 6 h. Myers & Mendel[15] found 20% hydrolysis of total lipid of rat homogenate at 37° and pH 7·3 in 3 h. In the present work phospholipid hydrolysis in pieces of lambs' liver at 37° was almost linear over the first 24 h. after which time 30% had been destroyed. Overall lipolysis, as measured by disappearance of the carboxylic esters, was slow during the first 2 h. and then accelerated for 24 h., after which time 50% had been destroyed. Both reactions continued until, after 5 days, the rates had become very slow although 40% of the phospholipid and 20% of the acyl esters originally present still remained. Lipid hydrolysis at 15° and 1° was negligible during 24 h., and no great loss of lipid P or esters could be demonstrated in the immediate post-mortem period during cooling and handling.

At 15°, as at 37°, when hydrolytic decomposition of the lipids had ceased or had become very slow, 20–40% of the phospholipid still remained unattacked, whereas all the neutral fatty esters had been destroyed. The pH of the tissue had dropped by this time to 4·5–5·0 (corrected values). The known phospholipase of liver displays maximum activity at pH 6·5[16] and is greatly inhibited at pH 4·5, whereas animal lipases appear to be active over a wider range of pH.[17] The cessation of lipolysis before complete destruction of the phospholipids may, therefore, have been due to inactivation of the enzymes by the acid produced. Alternatively, in view of the rapid proteolysis proceeding in the tissue, the lipolytic enzymes may themselves have been destroyed by protease.

The route of decomposition of the diacylglycerophospholipids indicated by the present work is the simultaneous removal of the two fatty acids releasing the phosphate moiety as a water-soluble ester, in agreement with earlier work on liver and on fish flesh.[1, 11, 12] The two known mechanisms which could account for this result are (*a*) the action of a phospholipase releasing the phosphoryl-base and an $\alpha\beta$-diglyceride, followed by lipase attack on the diglyceride, or (*b*) the removal of the two fatty acids directly from the phospholipid molecule releasing a glycerophosphoryl-base. The former mechanism, however, is improbable because phospholipase C is believed not to be present in liver[16] and the required rates of phosphorus release and fatty acid disappearance would not agree with the observed kinetics. Direct removal of the fatty acids could be brought about by the consecutive actions of phospholipases A and B removing the β and α' acids respectively, or by the action of a phospholipase B alone under activating conditions similar to those described by Dawson[18] for the phospholipase B of *Penicillium notatum*.

A soluble enzyme preparation from acetone-dried liver has been shown to have considerable phospholipase B activity,[16] but was without action on pure phosphatidylcholine, indicating the probable absence of phospholipase A in the liver. It appears likely, therefore, that hydrolysis of the glycerophospholipids results from attack by the liver phospholipase B in the presence of activating acidic phospholipids.

Mammalian liver is known to be rich in esterases capable of promoting the hydrolysis of the esters of short chain acids, but it is reputed to contain no true lipase.[19] The rapid hydrolysis of the esters of the neutral fats, consisting mainly of triglycerides, which was observed in the present experiments indicates, however, a considerable lipase activity in lambs' liver.

The continued breakdown of the water-soluble phosphate esters to inorganic phosphate shows the presence of active phosphodiesterases, although Dawson[20] was unable to demonstrate the presence of the specific glycerylphosphorylcholine diesterase in extracts of acetone-dried sheep's liver.

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ENZYMIC CHANGES IN LAMBS' LIVER DURING STORAGE. II.*—Activation of the Autolytic Enzymes by Freezing

By D. N. RHODES

Autolytic degradation of the lipids and protein of lambs' liver at 15° is accelerated three- to four-fold by previous freezing and thawing of the tissue. The maximum activation is achieved after freezing for 5 days at −2° or 3 days at −10° and is unaffected by holding at −10° or −20° for periods up to 14 weeks. The effect is due to the elimination of a lag phase preceding the onset of enzymic hydrolysis. Possible mechanisms are discussed.

Introduction

In previous work[1] fresh liver was found to show only small changes in total carboxylic esters, lipid P and water-soluble N during the first few days of storage at 15°, and this lag phase

* Part I: preceding paper

was followed by a rapid enzymic degradation of both lipids and protein. When material had previously been held frozen at −10° or −20° for 8 or 14 weeks, however, hydrolytic changes commenced immediately on thawing and proceeded more rapidly.

Other examples of the activation of lipolytic enzymes by cooling have been reported. Lipolysis in ' naturally active ' milk can be accelerated simply by cooling, during which a soluble lipase is apparently irreversibly adsorbed on to the fat globule membrane.[2] Normal milk needs to be cooled, warmed to 36° and re-cooled to produce the same effect.[3] The phospholipase A of pancreas is extractable in an active form only if the tissue has previously been held frozen (e.g., at −12° for at least 14 days).[4] Olley & Lovern[5] and Dyer & Fraser[6] found that the rate of lipolysis in cod flesh was about the same at −14° as at 0°, suggesting that an increased activity due to freezing was balancing the retarding effect of the lower temperature. The activity of pancreatic lipase is known to fall off very rapidly below 0°.[7]

The present experiments were designed to explore the effect of exposure to freezing conditions on the activity of the autolytic enzymes of liver.

Experimental

The lipids and water-soluble constituents of the tissue were extracted in a biphasic solvent system containing chloroform, methanol and water. The extraction technique and the analytical methods used have been described in a previous paper.[1]

A freshly killed lamb's liver was divided into 5·0–5·5 g. pieces which were weighed, sealed in polythene bags and immersed in brine solutions at −10° or −2°, or held in air at 15°. Initial samples were extracted and treatments started at all temperatures 90 min. after the death of the animal. Samples stored at 15° were examined after 1, 2, 4, 6, 9 or 14 days. After being held at −10° for 1, 3 or 10 days or at −2° for 1, 5 or 10 days, three samples at each time were thawed in water and transferred to storage in air at 15°. These samples were examined after a further 2, 4 or 8 days.

Results

The breakdown of the phospholipids, total lipids and protein at 15° as measured by the disappearance of lipid P and total carboxylic esters and the appearance of water-soluble N respectively are shown in Figs. 1, 2 and 3. The curves have been returned to a common origin since it was known that the amount of change to be expected in 10 days at −10° would be negligible, and that at −2° small.[1] The rates of autolysis found after 3 or 5 days' frozen prestorage were virtually identical with those shown after 10 days, and have not, therefore, been reported.

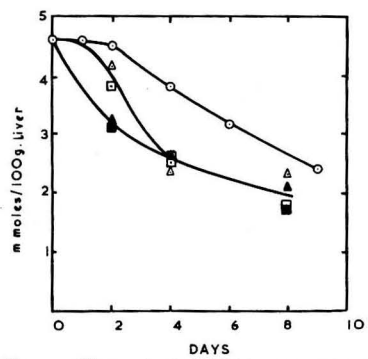

FIG. 1.—*Changes in the lipid P content of liver stored in air at 15°*

Fresh (⊙); after holding at −2° for 1 day (△), or 10 days (▲); after holding at −10° for 1 day (□), or 10 days (■)

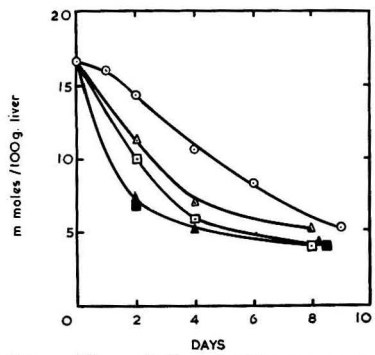

FIG. 2.—*Changes in the carboxylic ester content of liver stored in air at 15°* (Symbols as Fig. 1)

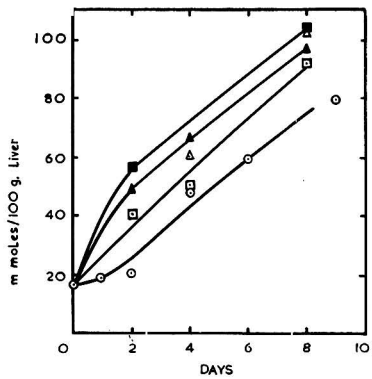

FIG. 3.—*Changes in the N soluble in the aqueous phase from liver stored in air at* 15° (Symbols as Fig. 1)

The induction period before the onset of enzymic degradation in the fresh liver was most clearly shown in the changes in lipid P (Fig. 1) and in water-soluble N (Fig. 3). A similar but less marked delay was found in the destruction of total fatty esters (Fig. 2). After the samples had been held at −10° for 3 or 10 days or at −2° for 5 or 10 days these induction periods were eliminated and rapid hydrolysis of both lipids and proteins commenced immediately on thawing. Even after 1 day at −10° or −2° subsequent autolysis at 15° was appreciably accelerated, but in these cases the induction periods were not completely removed.

No great differences were observed between the activating effects of the two temperatures of freezing, the maximum accelerations of lipid hydrolysis being identical in the two cases (Figs. 1 and 2). When the period of frozen storage was limited to 1 day, activation of lipolysis was slightly greater after exposure at −10° than at −2°. The lower temperature produced a slightly greater effect on the rate of proteolysis even after 10 days' exposure (Fig. 3).

The increased rates of autolysis observed in the present experiments, for samples from a single liver, amounted to a three-fold increase in loss of lipid P and a four-fold increase in destruction of total fatty esters (Table I, L12). These figures are of the same order as those derived from the earlier work[1] comparing the mean rate of deterioration in three experiments on fresh livers (Table I, L10, 11, 13) with those on two livers after storage at −10° or −20° for 8 or 14 weeks (Table I, L9, 6). It appears, therefore, that the effect of freezing at −10° is complete within 3 days and at −2° within 5 days, and that further frozen storage at −10° or −20° for periods up to 14 weeks results in no further enhancement of activity.

Table I

Comparison of rates of lipolysis at 15° *in fresh lambs' livers with those in livers previously held at* −2°, −10° *or* −20° *for up to* 14 *weeks*

Liver code no.	Frozen storage Temp., °C	Time, weeks	Days to destroy 30% lipid P	50% fatty esters	Ratio $\frac{\text{fresh}}{\text{stored}}$ Lipid P	Esters
L12	Fresh		5·7	6·0		
L12	−2	1·4	1·8	1·5	3·2	4·0
L12	−10	1·4	1·8	1·5	3·2	4·0
L10, 11, 13	Fresh		5·8	8·4		
L9	−10	8	2·0	2·0	2·9	4·2
L9	−10	14	2·8	4·0	2·1	2·1
L6	−20	8	2·7	3·8	2·2	2·2
L6	−20	14	4·0	2·0	1·4	4·2

Discussion

The apparent acceleration of autolysis in liver after freezing and thawing is mainly the result of the elimination of the lag phase which, in fresh material, precedes the onset of the rapid phase

of enzymic degradation of both lipids and protein. It seems unlikely that the effect of freezing is a result of purely chemical change since a period of 3 days at −10° is sufficient to produce the full activation, and it has been shown that the extent of enzymic hydrolysis in liver is very small at this temperature even after 24 weeks.[1] The formation and growth of ice crystals during freezing could, however, cause breakdown of the gross physical organisation of the tissue and the consequent dehydration of the cellular constituents might result in the denaturation of proteins[8] and in particular the disruption of lipoprotein complexes.[9]

Wattiaux & Duve[10] showed that many of the enzymes of liver are firmly bound in an inactive form in particulate components of the cells, and are released into solution only after physical treatments such as homogenisation, repeated freezing and thawing or the action of a detergent. The kinetics of the activation in the last case suggested that the enzymes are contained within a membrane which is ruptured by the active agent. Neither lipases nor protease were included in the studies of these authors, but it would seem likely that the activation of these enzymes by freezing is dependent on a similar mechanism. In this case the termination of the lag phase in the autolysis of fresh liver might be due to the release of the enzymes by the digestion of the membrane by the indigenous protease.

Acknowledgments

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EFFECTS OF SOME FUNGICIDES ON THE FLAVOUR OF FRUITS AND SYRUPS

By (the late) ALICE CRANG and G. M. CLARKE

The effects of the fungicides captan, Karathane (Dinocap), thiram and lime-sulphur on the flavour of fruits and acidified syrups have been assessed before and after bottling, canning and freezing. Captan at 5 p.p.m. increased the rate of corrosion of the cans and caused some taint in canned and bottled syrups, but was not detected before processing; when used as a spray on strawberries it caused slight taint of fresh or canned fruit in two yearly tests out of five. Karathane at 10 p.p.m. was not detected in the syrup and had no effect on the cans; it caused no taint in one year's trials on gooseberries and strawberries. Thiram at 2 p.p.m. increased the rate of corrosion of cans and caused distinct taint in canned, but not in bottled, syrups, although it was not readily detected before processing; it caused marked taint in much of the fruit tested over five years, especially after canning.

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Introduction

During the last 6 years, strawberries and gooseberries sprayed with fungicides at various stages in the growing season have been preserved and later assessed for flavour differences: whereas differences have been distinctly noticeable in some seasons[1] they have not been apparent in others. As other workers have found similar year-to-year variation with sprayed fruits,[2] the tests of fruit from field experiments have been supplemented by trials, without fruit, in acidified sugar syrups, to determine the levels at which fungicides may cause noticeable effects on flavour, and also by similar trials with added strawberry purée.

The first trial with canned syrups suggested that the taint observed may have been largely due to metal removed from the can; a further series was therefore prepared to include also syrups stored in glass preserving jars.

Experimental

Syrups

A 10% solution of cane sugar was acidified with citric acid and buffered with sodium citrate and dipotassium hydrogen phosphate to pH 2·7 (Series I–III) or pH 3·6 (Series IV–VIII); to 100 ml. of each were added 30 mg. of ascorbic acid immediately before processing. The following concentrations of fungicide were made from the basic syrup:

Captan 2·5, 5, 10 and 20 p.p.m. (from a powder containing 50% captan).
Karathane (*Dinocap*) 4, 8 and 10 p.p.m. (from a powder containing 25% Karathane).
Thiram 1, 2, 5 and 20 p.p.m. (from 'Fernide' containing 80% thiram).
Lime-sulphur 10 p.p.m. (from neat liquid).

Purée

Unsprayed strawberries grown at Long Ashton were macerated, and 60 g. added to each 100 ml. of syrup made as Series IV–VIII above.

Fruit

Strawberries.—In 1954–59, samples were collected at Luddington Experimental Horticulture Station from a spray trial for the control of *Botrytis*.[1] Treatments were:

1954 Captan 0·25%, thiram 0·4% (each sprayed 3 times); unsprayed control.
1955 Captan 0·25%, sprayed 1–4 times; captan dust (applied 4 times); thiram 0·4% (sprayed 4 times); unsprayed control.
1956–58 As 1955, but thiram sprayed 2 or 4 times.
1959 Thiram alone (0·4%) sprayed 3 times; thiram (0·4%) with Karathane (0·025%) or lime-sulphur (1%), sprayed once or twice.

In 1958, fruit was taken from a spray trial at Long Ashton which included unsprayed controls and Karathane sprayed at 0·025%.

Gooseberries.—Fruit grown in 1956 at Long Ashton included unsprayed controls and spray treatments with captan (0·1%) and Karathane (0·025%).

Processing methods

Syrup.—Plain and fruit-lacquered cans and glass preserving jars were used. The syrup was prepared with hot water in a stainless steel bucket; 800 ml. were placed in an A2½ can or a 2-lb. jar, and 400 ml. in a 1-lb. container. The fungicide was then added, and processing was carried out immediately, in the same way as for acid fruits. The syrups were stored at 25° for 3–6 months before examination.

Purée.—Fruit-lacquered cans, glass preserving jars and plastic deep-freeze boxes were used. Four hundred g. of purée (250 g. of syrup and 150 g. of macerated strawberries) were placed in a 1-lb. container and 10 ml. of fungicide solution (or water for the 'controls') added. Processing and storage were as for the syrups, the storage time being 4 months.

Strawberries.—Fruit from Luddington was transported by car directly after picking, and kept at 2–4° overnight. Representative samples of fresh fruit at the same stage of ripeness were then judged for flavour.

Canned fruit.—Plugged fruit (9 oz.) was canned with 6 fl. oz. of 40° Brix sugar syrup coloured with 4R dye (approx. 0·05%) in fruit-lacquered cans.

Frozen fruit.—Plugged fruit (9 oz.) was frozen with 6 fl. oz. of 40° Brix syrup (uncoloured) in polythene containers, and stored at −18° to −20°.

Method of tasting

Most of the samples were judged by the triangular tasting method (a group of three samples contained two treatments and one control or vice versa) two triangles being examined for each treatment at each session whenever possible. This is expensive of tasters' time and of control material, and for some samples the incomplete block method was used, a maximum of four treatments being compared with a control at one session.

Fruits or syrups were coded in random order for the incomplete block method ; for triangular tasting it has been shown[3] that the ordering of the samples affects the sensitivity of results, and the present authors have confirmed this for fungicide work. Triangles were therefore chosen with the restriction that a greater proportion should be of more sensitive orders (in particular, the odd sample appeared in the middle position infrequently).

Identical glass containers were used, those holding the syrups or purées being covered with coloured paper to mask any differences in appearance by which treatments might be distinguished.

Members of the tasting panel noted either the two like samples in the triangles or their order of preference in the incomplete blocks, and also recorded any taints : these were scored 0 (no taint), 1 (slight taint), 2 (taint) ; thus the nearer the average score for a sample approached 2, the more it was disliked. A score of 0 indicated that no taint was detected in a sample, whether or not it featured high in tasters' orders of preference.

Condition of containers

The syrups and purées were kept overnight at room temperature before being opened. The condition of the cans was examined, their air pressure noted, and the iron content of a representative number of syrups or purées was determined.

Results

The effects of the four fungicides on the flavour of the syrups and purées are shown in Tables I–IV. In each trial they were tasted against a control preserved and stored in similar containers.

The strawberries from Luddington were picked at two stages in the growing season—near the beginning and end—and while results were similar in most cases, there were some divergences which are noted below.

Table I

Effect of captan on flavour of syrups (I–VIII) and purées (IX–XI)

Series	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Captan, p.p.m.	20	5	10	2·5	5	5	5	5	5	5	5
Container*	PC	PC	FL	FL	—	G	FL	PC	G	FL	D
Storage (months at 25°)	6	6	6	6	0	3	3	3	4	4	4
No. of triangles											
Total	30	30	30	30	13	26	26	24	30	32	32
Correct	24	24	26	18	7	19	24	14	13	10	9
Incorrect	5	6	3	8	5	4	2	10	14	17	20
No decision	1	—	1	4	1	3	—	—	3	5	3
P†	0·1%	0·1%	0·1%	1%	—	0·1%	0·1%	1%	—	—	—
Taint (average score)											
Treatment	1·52	1·09	1·47	1·20	0·31	0·95	1·10	0·75	0·38	0·27	0·31
Control	0·00	0·17	0·16	0·40	0·19	0·13	0·28	0·42	0·48	0·32	0·39

* PC = plain can FL = fruit-lacquered can G = glass jar
D = plastic deep-freeze box
† P = level of significance of proportion correct

Table II

Effect of Karathane on flavour of syrups (I–VI) and purées (VII–IX)

Series	I	II	III	IV	V	VI	VII	VIII	IX
Karathane, p.p.m.	8	4	10	10	10	10	10	10	10
Container	PC	PC	—	G	FL	PC	G	FL	D
Storage (months at 25°)	6	6	0	3	3	3	4	4	4
No. of triangles									
Total	28	28	14	24	24	24	28	32	28
Correct	15	13	3	7	11	9	12	11	13
Incorrect	11	13	4	7	5	5	12	16	14
No decision	2	2	7	10	8	10	4	5	1
P	5%	—	—	—	—	—	—	—	—
Taint (average score)									
Treatment	0·21*	0·39*	0·21	0·22	0·17	0·30	0·67	0·11	0·55
Control	0·28	0·14	0·07	0·25	0·25	0·42	0·13	0·32	0·44

* An unpleasant after-taste was noted by three tasters; otherwise the taint was usually classed as a metallic taste, which was found in all syrups to the same degree.

Table III

Effect of thiram on flavour of syrups (I–VII) and purées (VIII–X)

Series	I	II	III	IV	V	VI	VII	VIII	IX	X
Thiram, p.p.m.	20	5	1	2	2	2	2	2	2	2
Container	PC	PC	FL	—	G	FL	PC	G	FL	D
Storage (months at 25°)	6	6	6	0	3	3	3	4	4	4
No. of triangles										
Total	30	30	30	14	26	24	26	30	30	30
Correct	28	27	22	5	4	22	20	7	20	21
Incorrect	1	3	8	6	10	—	6	11	7	4
No decision	1	—	—	3	12	2	—	12	3	5
P	0·1%	0·1%	0·1%	—	—	0·1%	0·1%	—	0·1%	0·1%
Taint (average score)										
Treatment	1·69	1·83	1·10	0·50	0·18	1·61	1·43	0·27	0·66	1·02
Control	0·11	0·03	0·22	0·07	0·07	0·00	0·13	0·36	0·46	0·27

Captan

Syrup.—It will be seen from Table I that in each series I–VIII the control syrup was preferred to that containing captan. In captan syrups stored for 6 months, taint was readily detected; some members of the tasting panel were less certain about those stored for 3 months; and in the fresh syrup it proved difficult to differentiate captan from the control (Series V).

Purée.—Captan at 5 p.p.m. could not be differentiated from control purées after any of the processing methods (Series IX–XI).

When five syrups from Series I and II were tasted in the order 0, 20, 20, 5, 0, the average taint scores were: 20 p.p.m. = 1·83; 5 p.p.m. = 0·60; control = 0·23.

Strawberries.—The effect has varied during the five years of the trials.

(1) In 1954, canned fruit contained 11–19 p.p.m. of captan,[1] and had a noticeable though slight taint, scoring 0·44 against 0·15 for the control. The panel correctly grouped the pairs in 28 of 53 triangles, 14 were wrongly paired, and in 11 triangles no difference was detected. Nor was any difference detected between jams made from captan-treated fruit and those from untreated fruit.

(2) In 1955, the incomplete block method of judging was adopted. One series of fresh fruit receiving captan dust four times had a marked taint (score 1·02), and this was apparent in frozen fruit also (1·06). No consistent taints appeared in captan-sprayed fresh fruit, or in canned or frozen fruit or jams.

(3) In 1956, unsprayed fresh fruit was liked less than that treated with captan, possibly because of strawberry mildew on some of the controls. The triangles were correctly grouped in 25 out of 62 canned, and 25 out of 60 frozen samples. Although there was a general preference for the controls, the taint scores did not reveal a strong difference.

Table IV

Tasting results for canned and frozen fruit treated with thiram and stored for 4 months

Fungicide	No. of sprays	No. of triangles				P_1*	Correct with control preferred	P_2*
		Total	Correct	Incorrect	No decision			
Canned fruit								
Thiram alone	2	58	33	22	3	0·1%	28	0·1%
Thiram+Karathane	1	54	32	20	2	0·1%	26	0·1%
,, ,,	2	56	34	20	2	0·1%	25	1%
Thiram+lime-sulphur	1	60	29	29	2	5%	19	—
,, ,,	2	60	40	13	7	0·1%	28	5%
Frozen fruit								
Thiram alone	2	54	20	28	6	—	8	—
Thiram+Karathane	1	52	27	21	4	1%	14	—
,, ,,	2	48	27	18	3	1%	11	—
Thiram+lime-sulphur	1	56	35	19	2	0·1%	22	—
,, ,,	2	56	20	32	4	—	13	—

* P_1 = significance level of proportion of correct triangles.
P_2 = ,, ,, ,, ,, ,, ,, ,, in which control preferred to treatment.

(4) In 1957 and 1958, with the incomplete block judging method, no marked difference appeared between any of the captan treatments and the control samples in either fresh or preserved fruit.

Gooseberries.—In 1956, no taint was found on gooseberries treated with captan.

Karathane (Dinocap)

Syrup.—Table II shows that no real difference was detected between the Karathane-treated syrups or purées and the corresponding control samples.

When five syrups from Series I and II were tasted in the order 4, 8, 4, 0, 8, the average taint scores were: 8 p.p.m. = 0·43; 4 p.p.m. = 0·50; control = 0·80. Thus, although discrimination was poor, the control syrup was disliked more than those containing Karathane.

Strawberries.—No taint was detected in samples of fresh fruit, or in canned or frozen fruit or jam, in the one-season trial of Karathane sprays alone. The 1959 results, when Karathane was combined with thiram, are noted in the section on thiram.

Gooseberries.—In the one-season trial, a difference was found between sprayed and unsprayed samples in one series of fresh fruit, where the control was preferred to the treated samples. In one series of canned fruit also, the treated fruit and the control could be differentiated, but there was no distinct preference for either, the difference being in the texture of the fruit.

Thiram

Syrup.—Table III shows that there was considerable dislike of thiram in the canned samples, but that this was less noticeable in the fresh syrup and not detected in the samples stored in glass containers.

Purée.—There was a similar dislike of thiram in deep-frozen and canned samples (although the ' control ' was not liked very well in the canned series, it was generally preferred to thiram) and again no difference was detected in bottled samples.

When five syrups from Series I and II were tasted in the order 0, 5, 0, 20, 5, everyone marked 20 p.p.m. ' definite taint ', everyone marked 5 p.p.m. 'definite taint ' or ' slight taint ', and only two tasters marked the first control sample ' slight taint '. Ten out of 15 tasters placed the sequence in the correct order. The average scores for taint therefore were: 20 p.p.m. = 2·00; 5 p.p.m. = 1·77; control = 0·07.

Strawberries.—The results in 6 years' trials are as follows:

(1) In 1954 thiram was correctly noted in 38 out of 42 triangles containing canned fruit, with a taint score of 1·50 against 0·09 for control. It was not detected in jam made from the same fruit.

(2) In 1955 in one series of canned fruit stored for 6 months thiram was marked heavily for taint; it was hardly perceptible, however, in three other samples stored for 3–5 months, or in fresh or frozen fruit or in jam.

(3) In 1956 no taint was observed in the fresh fruit, but some tasters recorded taints in canned and frozen samples. The average scores for taint were, for canned and frozen fruit in that order, fruit sprayed 4 times, 0·63, 0·40; fruit sprayed twice, 0·34, 0·34; unsprayed controls, 0·19, 0·28.

(4) In 1957 marked taint was found in fresh and canned fruit from the second picking of strawberries sprayed four times, but not in fruit from the first picking.

(5) In 1958, fresh fruit sprayed four times was disliked, but none of the canned or frozen fruit was scored heavily for taint.

(6) In 1959 in the canned fruit, thiram sprayed once or twice, alone or in combination with Karathane or lime-sulphur, was generally detected and disliked; this dislike was less definite in frozen samples, where correct decoding of the triangles was often not accompanied by any strong preference for the controls. Differences could not be detected consistently among fresh fruit samples, or between jams made from sprayed and unsprayed fruit. Table IV shows the tasting results for canned and frozen fruit stored for 4 months.

Lime-sulphur

Purée.—Table V shows that lime-sulphur at 10 p.p.m. caused taints in the purée stored in glass containers, and very strong taints in frozen samples; in the canned purée, the lime-sulphur samples were considered less pleasant than the controls, although discrimination in the triangles was poor.

Condition of containers after storage of syrups

Captan.—After 6 months' storage at 25°, the syrups containing 20 p.p.m. of captan had attacked the cans seriously: two of the fruit-lacquered cans had ½ lb./sq. in. pressure and the third was leaking. The two plain cans had 1½ and 3 lb./sq. in. vacuum, but were badly stained; the controls were unaffected. The iron contents of the syrups were 74 p.p.m. in the fruit-lacquered can and 16 p.p.m. in the plain can, compared with 5–8 p.p.m. in the controls.

Syrups containing 5 p.p.m. of captan stored for 3 months had stained the cans purple-black and contained black specks; the vacuum (3–4 lb./sq. in.) was similar to that of controls. The iron contents were 22 p.p.m. in the fruit-lacquered can, 11 p.p.m. in the plain can, and 9 p.p.m. in controls.

Purée containing 5 p.p.m. of captan showed 26 p.p.m. of iron after 4 months' storage.

Karathane.—There was little difference in the appearance of the cans containing Karathane and the control syrups; the iron content was 16 p.p.m. in plain cans and 9 p.p.m. both in fruit-lacquered cans and in the canned controls.

Thiram.—Thiram generally removed the tin layer from plain cans, leaving a dull grey surface with a black deposit in the syrups; the fruit-lacquered cans appeared normal. The iron contents of syrups containing 20 p.p.m. of thiram stored for 6 months were 88 p.p.m. in the plain can, 23 p.p.m. in the lacquered can, and 7 p.p.m. in each control can. Syrups and

Table V

Effect of lime-sulphur (10 p.p.m.) on the flavour of purées stored for 4 months at 25°

Series	I	II	III
Container	G	FL	D
No. of triangles			
Total	24	30	32
Correct	15	10	25
Incorrect	6	17	7
No decision	3	3	—
P	1%	—	0·1%
Taint (average score)			
Treatment	0·71	0·60	1·50
Control	0·17	0·22	0·15

purées containing 2 p.p.m. of thiram stored for 1–4 months generally had 25–30 p.p.m. of iron compared with 9–13 p.p.m. in the controls. Two fruit-lacquered cans containing syrup showed 78 and 105 p.p.m.

As would be expected, all the syrups and purées stored for 3 months in glass containers had less than 0·1 p.p.m. of iron; the same was true of samples preserved by deep-freezing.

Discussion

One of the difficulties in assessing whether insecticides or fungicides have adverse effects on the quality of fruit on which they are used is to obtain true control samples of the same variety, grown under otherwise identical conditions and equally free from pest or disease. Year-to-year variations in climate may also affect the strength of any off-flavours.

Although the use of acidified sugar syrups has the objections that no field weathering takes place and that interaction between fruit and fungicide is eliminated, it gives valuable information on the threshold values at which the material may cause taint and on its effect on containers during processing and storage. The addition of strawberry purée to the syrups removes some of these objections.

In these trials 5 p.p.m. of captan were easily detectable in processed syrup, especially in fruit-lacquered cans; it was less easily detected in fresh syrup, although in all tests the syrups were liked less than the controls. Its corrosive action on the metal cans was quite evident. The addition of strawberry purée appeared to conceal the taint, although iron contents remained considerable. In initial trials with sprayed strawberries,[1] residues of 11–19 p.p.m. of captan on the fresh fruit caused slight tainting of the fruit when canned, but not in fresh fruit or jam. In later trials, a significant amount of taint has sometimes been recorded in both fresh and preserved fruit.

Karathane in quantities of up to 10 p.p.m. had little effect on the flavour of the syrup or purée, and no apparent effect on the cans. It caused no taint in the strawberries and gooseberries sprayed in one season.

Thiram has a very unpleasant smell and taste, and was detected readily at 1 p.p.m. in canned syrup or purée; it also attacked the cans. Purée stored in deep-freeze was also heavily scored for taint when containing 2 p.p.m. of thiram. It is interesting, however, that the triangles were not correctly identified by the majority of tasters in fresh syrup, or in syrup or purée stored in glass jars, although the thiram syrup was scored more heavily for taint than was the control. Tasters often found their palates so affected that their power of discrimination was reduced, and only the largest differences could be detected with certainty. Strawberries sprayed with thiram showed considerable variation: taint was easily detected in canned fruit in two years and in frozen fruit in one year, and the samples were considered so objectionable that the fruit would not normally have been eaten. In other years, however, most members of the panel could not detect thiram in sprayed fruit, although even then a few tasters seemed especially sensitive to thiram and were able to identify triangles correctly. Other workers[4] noted no off-flavour in blackcurrants contaminated with 5 p.p.m. of thiram (applied as sprays) but found similar corrosion of the cans.

Lime-sulphur at 10 p.p.m. in purée was definitely detected in bottled and frozen samples, and although discrimination seemed less easy in canned purées the lime-sulphur was scored more heavily than control samples.

In the field trial of combined sprays, thiram appeared to produce a dominant off-flavour in the canned samples, but, as with the syrup tests, this did not show so strongly in fresh fruit or that preserved by other methods.

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LABORATORY AND FIELD TESTS FOR EVALUATING THE EFFICIENCY OF WETTING AGENTS USED IN AGRICULTURE

By R. DE B. ASHWORTH and G. A. LLOYD

A modified Shapiro's cotton tape test is a satisfactory laboratory method for examining the wetting properties of anionic and non-ionic wetters when used with agricultural sprays at high volume. It has been shown that the concentration of wetter which wets a standard length of cotton tape in 15 sec. is related to the degree of wetting of a cabbage leaf. The test may be standardised for leaves other than cabbage.

Introduction

A reproducible laboratory method capable of evaluating the efficiency of a wetter or formulation incorporating a wetting agent (' wetter ') on a leaf surface would clearly be helpful to all concerned with crop protection. Accordingly, work was begun by examining possible methods and contrasting their field and laboratory performance. Thompson,[1] working in this laboratory, has already shown that the Draves sinking-time test gives a better correlation with actual field performance at high-volume spraying than measurements of surface and interfacial tension. However, when the Draves test was subjected to rigorous collaborative tests by a number of laboratories represented on a Ministry Committee, results were variable owing to entrainment of air by irregular twisting of the cotton hanks when immersed in wetting solutions. This yarn was found to be of unbalanced construction, thereby retaining a twisting couple. With apparatus designed to give controlled winding of the hanks a few laboratories, experienced in the Draves test, obtained better agreement. Less variations in results between laboratories occurred when American cotton hanks prepared commercially from balanced yarn were used.

At this juncture trials were made by one of us (G. L.) with a modified Shapiro's test,[2] a test dependent on the wetting of a cotton tape of standard weave. Not only was it then possible to obtain good agreement between laboratories, even when the operators were unfamiliar with the test, but it was also found that results with anionic and non-ionic wetters could be closely related to the wetting of cabbage or other leaves which had been dipped or sprayed at high volume.

Experimental

In developing a laboratory procedure for evaluating the ability of wetters to wet leaves it is essential to compare results obtained in the laboratory with actual wetting of a leaf surface if such a test is to be of practical value. No standard simple field test for wetting a leaf surface was available and the following work was consequently undertaken.

(a) *Field test for evaluation of wetters*

A preliminary investigation showed that leaf wettability decreased in the order, nettle and laurel, apple, chrysanthemum, pear, broccoli, dandelion and cabbage. Since cabbage is a crop frequently sprayed in this country, is readily available all the year round and its leaf is difficult to wet, it seemed an appropriate choice for further work. However, any other leaf could be chosen as a standard for evaluation of laboratory results.

An obvious field test for wetting is to dip a fresh leaf into the spray tank, withdraw it immediately and assess the percentage of area wetted. In an attempt to standardise this procedure, black and white charts were prepared to simulate a leaf with 5, 25, 50, 75 and 95% of the area wetted. A panel evaluated the percentage of wetted area on the charts and it was found that reasonably reproducible results between members of the panel could be obtained only when 5 or 95% of the surface was wetted: intermediate values were erratic. Attempts to evaluate the percentage of leaf wetted by weighing the leaf before and after dipping were also useless, since weight differences appeared only to indicate sticking properties of wetting agents on different leaf surfaces.

To overcome the difficulty of deciding the percentage of leaf wetted a concentration of the wetter was selected which would just give 100% wetting of the leaf, and in this way the efficiency of wetters could be compared.

Recommended method

Transfer 400-ml. portions of solutions of wetting agents at suitable concentrations to beakers partly immersed in a water bath at 25° and remove foam or air bubbles from the surface of the solution. Immerse a freshly picked undamaged leaf in the test solution for 1 sec. and, after 5 sec., with the leaf held vertically make a visual estimate of the % area wetted. (At least five tests with leaves from different parts of the plant should be made, while care should be taken not to touch any surface with the leaf when withdrawing it from the beaker.)

To compare the wetting properties of a group of wetting agents for a leaf such as cabbage which is difficult to wet, determine the minimum concentration of wetting agent which produces 100% wetting of 80% of the number of leaves tested. The largest leaves from the middle of plants of the same age should be selected and the same variety of cabbage should be used throughout.

Results

Clearly any method of evaluation which depends on leaf dipping is open to the criticism that it is not comparable with spraying at high volume. An investigation of this point was accordingly kindly undertaken for the Joint Committee by Mr. M. R. Middleton who obtained the results shown in Table I.

The dipping procedure was as described above, while the spraying technique was carried out as follows:

An 'Aerograph' air brush was set up horizontally and connected to the compressed air supply. A section of leaf (2 in. × 2 in.) was held vertically by a paper clip 8 in. from the nozzle of the air brush. The air supply was switched on and 3 ml. of the wetting agent solution were put in the cup when a fine spray impinged on the leaf. The surface of the leaf was sprayed for 15 sec., the leaf reversed and the other surface sprayed for 15 sec. This time of spraying gave wetting to run off. The area of leaf wetted was assessed visually.

It will be seen in Table I that where three-quarters or more of the leaf surface was wetted, comparable results were obtained by dipping or wetting, but that spraying gave rather better wetting, particularly at low concentrations of the wetter. Nevertheless, results for the two procedures were regarded as sufficiently in agreement for the dipping test to be adopted as a standard for 100% wetting of a leaf by spraying at high volume.

In experiments carried out on cabbage, the variety January King was used throughout. In an extensive investigation it was found that as the plant increased in age a greater number of leaves difficult to wet were produced. In general, the youngest leaves at the stem apex and the oldest leaves at the base of the stem were wetted more easily than the middle larger leaves. The upper surfaces of cabbage leaves were of greater wettability than the lower surfaces. Wilted leaves showed an increase in wettability which became marked as storage time was extended. Significant changes in wettability were not observed with some plants grown under glasshouse conditions. Removal of the surface bloom by ether extraction produced a marked increase in wettability.

The range of results obtained by a number of laboratories using the leaf-dipping technique is given in the fifth column of Table V. (These results were not obtained on cabbage grown

Table I

Visual assessment of the wetting of a leaf surface (sugar beet) with a spraying and a dipping technique

Wetter	Concentration, %	100% wetted = 10 Dipping		100% wetted = 10 Spraying	
		Upper surface	Lower surface	Upper surface	Lower surface
W1	0·5	7	6	8	8
	0·1	6	4	8	8
	0·08	6	4	8	8
	0·06	4	3	8	8
	0·02	1	1	5	5
W3	0·06	9·5	9	9·5	9
	0·04	9	7	9	9
	0·02	7	4	9	8
	0·01	3	2	7	7
	0·005	1	1	6	6
W5	0·5	9	9	9·5	9
	0·1	6	5	9	8
	0·08	4	3	8	8
	0·06	2	2	8	7
	0·04	2	2	6	6
	0·02	1	1	5	6
W6	0·06	10	10	10	10
	0·04	10	9	9·5	9
	0·02	7	5	9	9
	0·01	3	2	7	6
	0·005	1	1	4	5

W3 and 5 are anionic wetters, while W1 and 6 are non-ionic

under identical conditions.) It has already been shown that the dipping test gives results comparable to high-volume spraying.

(b) *Laboratory evaluation of wetters*

In Shapiro's tape test a weighted length of standard cotton tape is dropped into a tall cylinder containing an aqueous solution of a wetting agent. The time required for the thread connecting the weight and the tape to relax is recorded as the sinking time. Changes were required in this test to make it applicable to wetters used for agricultural purposes.

Recommended method

The apparatus consists of a piece of No. 16 S.W.G. stainless steel wire about 3·5 cm. in length bent into the form of a two-pronged hook (Fig. 1). File the ends to sharp points and adjust the weight to exactly 0·500 g. Attach the hook with a length of nylon thread to a loop of wire secured to the centre of a flat cylindrical lead slug of 30–40 g. in weight. The distance between the base of the hook and the anchor should be 2·0 cm. Attach to the hook an unbleached cotton medium tape conforming to B.S. 1625 (1950) with 73 warp ends and 36 weft picks per inch which is suitable (obtainable from Haydn H. Levey & Sons Ltd., Bristol House, 19–20 Holborn Viaduct, London, E.C.1).

Prepare stock solutions of the agents to be tested to contain 5·00 g. of active ingredient per litre unless the solubility is so poor that less must be employed. Dissolve the wetting agent in about a quarter of the necessary water at the minimum temperature at which the material will dissolve and then dilute to the final volume with cold distilled water. Dilute suitable aliquots to 1000 ml. with distilled water and maintain at 25° ± 0·25°.

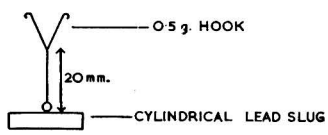

FIG. 1.—*Diagram of hook and anchor*

Where a standard wetter is required, dry sodium di-(2-ethylhexyl) sulphosuccinate at 95° for 2–3 h. and store in a desiccator. Make up a 0·5% solution in distilled water as a standard.

Determine the sinking time by pouring the dilute solutions for test into 1·5-litre beakers to ensure mixing and thence into the cylinders. If the more dilute solutions are tested first, the beakers and cylinders need not be rinsed and dried each time.

Place the cylinders in a water bath at 25° ± 0·25°. Leave the cylinders until all bubbles below the surface of the solution have risen to the top before sinking tests are made. Remove foam on the surface of the solution with a 100-ml. pipette or aspirator. The same diluted solution may be used many times without apparent exhaustion of the wetting agent. (This may not be applicable to cationic wetters.)

Place a ruler over a length of tape laid without tension on a flat surface and cut 22-cm. lengths as required. Insert the hook through the tape about 0·5 cm. from one end of a 22-cm. length. Hold the tape by the free end with the anchor and the bottom of the hook immersed in the wetting solution contained in a 500-ml. cylinder. Start a stop watch as the tape is released into the solution. Stop the watch when the buoyant tape definitely starts to sink to the bottom of the cylinder. At least five tests must be made for each concentration of wetting agent.

Determine the sinking time of each batch of tape by the standard procedure with a 0·02 g.-% solution of sodium di-(2-ethylhexyl) sulphosuccinate prepared by dilution of the stock 0·5% standard with distilled water. The mean of five determinations at 25° should normally give a sinking time of 15·0 ± 1·0 sec.

Plot the values for the concentrations of wetting agents expressed as g.-% w/v on the horizontal scale or X-axis of log.–log, graph paper. Similarly, plot mean sinking times in seconds on the vertical scale or Y-axis. Draw a smooth curve through the points and determine the concentration of wetting agent which corresponds to a sinking time of 15·0 sec. (Fig. 2).

A deviation of approximately ±7% may be expected for results evaluated by the log.–log. procedure.

In the case of cloudy solutions it may be difficult to observe movement of the tape when tests are made with emulsions or suspensions. Where great accuracy is not required, attach a short length of nylon thread to the free end of a length of tape and counter-balance over the side of the cylinder with a 20-mg. weight. Upward movement of the weight indicates the sinking time.

For greater accuracy use a square-sided vessel with side illumination from a powerful light source, or alternatively, wind a length of insulated single core copper wire closely round a 500-ml. cylinder to a height of one inch from the base of the cylinder. Similarly, construct a secondary coil of 4–5 times the number of turns on the primary. Connect the primary coil to a source of alternating current at 6–12 v and the secondary coil to a sensitive milliammeter. When a hook of iron wire enters the field produced by the primary coil, a current is induced in the secondary coil. Record the sinking time by observing the movement of the ammeter needle.

The sample of sodium di-(2-ethylhexyl) sulphosuccinate for the preparation of standard solutions should be of such purity that only 2-ethylhexanol is detected by gas chromatography. Additional analytical confirmation and specifications of purity are described in the Merck Index, 1952, 6th edn, pp. 146–147. [Sodium di-(2-ethylhexyl) sulphosuccinate may be obtained from Messrs. Hardman & Holden, Ltd., Manox House, Miles Platting, Manchester 10.]

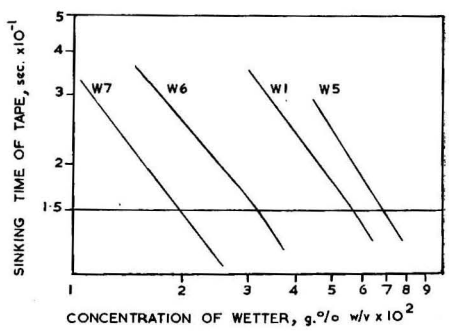

FIG. 2.—*Evaluation of wetting agents (log. × log. scale)*

Results

Replicate results with a 0·5-g. hook in the tape test, made by a single operator, are given in Table II.

Variations in mean sinking time with temperature are shown in Table III for two different wetters with the tape test.

The results showed that deviation of sinking time with temperature was greater with W6 (non-ionic) than with W3 (anionic), i.e., 0·5 and 0·3 sec./° C rise in temperature from 15 to 25°, respectively.

Collaborative studies

When the cotton tape test was subjected to collaborative study by nine laboratories, excellent agreement was obtained with the nine wetters employed (W3 and W7 were chemically the same wetter but W7 had been purified to some extent) (Table IV).

The sinking time of 15 sec. was arbitrarily selected as giving concentrations of the wetter required to wet a cabbage leaf by the leaf-dipping test. If it were required to standardise the test on the basis of wetting apple leaves, a longer period than 15 sec. would be selected and lower concentration of wetter would be used.

It will be noticed that the test readily distinguished between good wetters and those of lower quality (e.g., W2).

Table V shows a comparison of results obtained between the Draves test (with two different yarns), Shapiro's test, the leaf-dipping test (January King cabbage) and several prepared surfaces.

The agreement between the Shapiro tape test and the wetting of cabbage by the dipping test is surprisingly good when the natural variation that occurs in biological material is remembered.

A similar procedure to the leaf-dipping test was investigated on a collaborative basis with carnauba wax-coated strips. Since it is difficult to produce a standard surface and surface roughness is a complicating factor when comparing wetting agents, the results confirmed that a test based on the wetting of artificially prepared surfaces was not satisfactory as a basic method for evaluation of wetting agents.

Table II

Precision of the tape test

Wetter W_1, % w/v	Sinking times, sec.
0·05	14·5, 14, 15·5, 16, 15
0·0125	49, 50, 50, 50, 50
0·00625	92, 95, 91, 102, 100
0·003125	160, 163, 157, 143

Table III

Mean sinking times determined with wetters W3 and W6 for a range of temperatures

Sample	Concentration, % w/v	Temperature, ° C	Mean sinking time, sec.
W3	0·025	14·7	15·1
		25·0	12·5
		35·0	11·9
W6	0·040	15·0	17·8
		25·2	13·0
		34·4	11·6

Table IV

Collaborative results obtained by Laboratories A–I with the tape test

The results show the concentration of active compound (g.-% w/v) to give a sinking time of 15 sec.

Sample	A	B	C	D	E	F	G	H	I	Mean
W1*	0·052		0·056	0·063	0·045	0·059	0·060	0·058	0·062	0·057
W2	0·540									
W3	0·020		0·020	0·020	0·020	0·022	0·020	0·015	0·019	0·019
W4	0·045			0·057		0·063				0·055
W5	0·070		0·068	0·066	0·05	0·069	0·066	0·069	0·067	0·066
W6	0·029	0·03	0·030	0·032	0·026	0·037	0·037	0·032	0·031	0·032
W7	0·020	0·02	0·020			0·021	0·021		0·021	0·021
W8	0·080					0·106	0·074		0·21	0·117
W9	0·036	0·050	0·048		0·047	0·052	0·047			0·046
W10	0·190	0·110			0·198	0·207	0·175			0·176

*W1, W2, W4, W6, W9 and W10 are non-ionic wetters, while the others are ionic.

Table V

Comparison of results obtained by various test methods

Wetter	Concn., % w/v, per 15 sec. wetting time			Minimum concn., g.-% w/v, which gives 100% wetting of the surface indicated			
	Draves test		Shapiro	January King cabbage leaves	Carnauba wax-coated strips	Polythene film	Cabbage, wax-coated stripes
	American yarn	English yarn	B.S. 1625 tape				
W1	0·040	0·072	0·057	0·025–0·050	0·05–0·06	0·04	0·09
W2	0·420	0·560	0·540	>0·50	>0·50		
W3	0·018	0·025	0·020	0·025–0·050	0·05–0·08	0·09	0·15
W4	0·058	0·062	0·055	0·025–0·050	0·05–0·06	0·05	0·06
W5	0·052	0·076	0·066	0·050–>0·10	0·10–0·50	>0·50	>0·50
W6	0·035	0·050	0·032	0·020–0·050	0·03–0·05	0·02	0·05
W7	As W3						
W8	0·072		0·117	0·10–0·14	0·30		
W9			0·046	0·030–0·050	0·03		
W10			0·176	0·150–0·20	0·20		

Notes

(1) The results given in columns 1 and 2 were determined with 5-g. skeins and a hook of 4·5 g.
(2) The results in column 4 (January King cabbage leaves) were recorded as ranges of concentrations of wetters which will produce 100% wetting of cabbage plants from 6 to 20 weeks' growth respectively. The upper surface of a cabbage leaf is more easily wetted than the lower surface. In general, the leaves at the apex and base of the stem of a cabbage plant are more easily wetted than the larger middle leaves.
(3) All tests were carried out with solutions of wetters in distilled water.
(4) Throughout this paper the code letters used refer to the same laboratory or wetter.

Discussion

In his original paper Shapiro describes in some detail the basic work carried out on the tape test, so it is not proposed to dwell further on this aspect. Fogg[3] has used an advancing contact angle as a measure of the extent to which wetting takes place on leaf surfaces. The contact angle varies diurnally and with the position of the leaf on the plant—thus accounting for the range of concentrations obtained by different laboratories with the leaf-dipping test in Table V. Even so, the agreement between different laboratories using this test is surprisingly good. The ability of a solution to wet plant surfaces has been investigated by Juniper[4] with an electron microscope to examine the surfaces of the leaf, and this work would undoubtedly provide useful clues if it were deemed worth while to do more work on prepared surfaces.

It would seem desirable to indicate with some precision the limits of usefulness of the tape test. The results obtained show that a correlation between the tape test and the wetting of cabbage leaves by dipping or spraying at high volume to run off is reproducible for anionic and non-ionic wetters. Cationic wetters were not examined.

Clearly the tape test would need detailed investigation if it were intended to apply it to the examination of solutions to be used for application at low volume, if, indeed, it has any significance for such a purpose. Further, it does not give any indication as to the degree of leaf wetting desirable to give maximum biological efficiency for a particular pest. If the concentration of wetter used in a formulation designed to eliminate woolly aphid (*Eriosoma lanigerum*) on apple leaves wets only the leaves and not the aphid, the test must be standardised against the wetting of the insect if maximum biological efficiency is to be obtained.

The test gives no indication of the sticking properties of a particular formulation. By sticking property is meant the property of increasing the amount of spray retained on the leaf when a sticker is added to the formulation. Most wetters examined have little or no sticking properties, although there are exceptions. It is hoped to publish at a later date a paper on this aspect of formulation.

As already indicated, where two wetters both wet a leaf surface at economic concentrations no information will be obtained from the test as to which will give the better biological control when a given toxicant is added. Consequently, all that can be claimed for the test is that it will show in the laboratory whether a particular leaf will or will not be wetted by high-volume spraying in the field. In this respect it is of considerable practical application since high-volume solutions that do not wet leaves are of doubtful value.

Furmidge[5] has recently discussed the variation of phytotoxicity with the chemical structure of surface-active agents and has related wetting ability to the structure of the wetter. Clearly phytotoxicity is a factor which cannot be neglected in the examination of a wetter, nor, as Thompson[1] has shown, can the possibility of an ionic wetter being precipitated when used in conjunction with Epsom salts, to make good a magnesium deficiency, or sodium nitrate as a selective herbicide on beet.

Hartley[6] in a recent very interesting paper, discusses the difficulties of defining a biological specification which would be of assistance to the chemist in evolving a new formulation. In order to evaluate with greater exactness the biological efficiency of different formulations of the same active principle, standard tests for sticking, weathering, phytotoxicity, shelf life, optimum particle size and other properties will need to be agreed.

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MOISTURE DETERMINATION IN DATES BY FORCED VENTILATION INFRA-RED DRYING

By G. ZIMMERMANN

For quality control in the date drying industry, a method for moisture determination which was both simple and rapid, but none the less reliable, was required. The disadvantages of conventional methods which employ vacuum drying, refractive index, distillation and dielectric measurements are described. Comparison of results obtained by standard vacuum drying and by toluene distillation is made and the applicability of the latter as a reference method is established. Infra-red drying with forced ventilation gives highly reliable and reproducible results with five varieties of dates, a 5-g. sample of date meat and a 250-W lamp being used. Heating is for 10 min. at 50 W and then 15 min. at 60 W with a vacuum of 160 mm. Hg and a distance between lamp and sample of 30 mm.

Introduction

Experiments carried out by Nielsen *et al.*[1] showed that the moisture content of dates is of prime importance for good keeping quality at room temperatures without the development of mould or souring. The maximum moisture content of dates, above which such deterioration may occur, varies with variety. Rygg[2a] has shown that the moisture content of dates is related to their susceptibility to sugar spotting and browning.[2b]

For purposes of quality control in a date drying plant, the method for moisture determination must be simple, reliable and give reproducible results in a reasonably short time. Rygg[2c] suggested methods based on determination of refractive index or on distillation with a water-immiscible organic liquid of high boiling point. Schiller & Maier[3] prefer dielectric measurements.

In a comparison of methods based on the refractometer and distillation with toluene Rygg[2c] found for six varieties differences of up to 1% for the total soluble solids over a range of moisture content of between 17 and 38%. The same author recommended the addition of 0·3–1·4% to results for 9 varieties obtained by measurement of refractive index, based on results obtained by vacuum drying as the standard method.

Moisture determinations on date varieties, whether by distillation or oven drying, are not readily correlated with refractive index determinations on the same varieties, because of varying proportions of total soluble solids (as measured by the refractometer) to insoluble solids, which proportions are not even constant for any one variety over the period of growth and ripening. Furthermore, moisture determined by refractive index measurements is subject to a systematic error. According to Browne & Zerban,[4] solutions of sucrose and reducing sugars show different refractive indices for the same dry matter content. In the naturally drier varieties of dates, or in those artificially dried to a very high reducing sugar content in the dry matter, this fact may give rise to moisture results which are as much as 2% low.

Fretzer[5] states that distillation with toluene for approximately 1 h. gives consistent results, but, for rapid control purposes, this time is too long. According to Schiller & Maier[3] accurate moisture determination in dates with a dielectric moisture meter requires preparation of the sample under very high pressure (1500 p.s.i.) and the maintenance of constant temperature and voltage during the determination.

The literature cites the usefulness of infra-red dryers for determining the moisture content of various foods, e.g., for animal foods,[6] starch by-products,[7] sweets,[8] tomato preserves,[9] ice-cream[10a] and emulsion liquors.[10b]

The aim of the present experiments was to test the applicability of infra-red drying to the determination of moisture in different varieties of dates, to establish the best conditions for the method, and to study the importance of displacing water-vapour in order to shorten the drying time.

Experimental

Materials

The date varieties, Barhee, Halawy, Khadrawy, Maktoom and Zahidi, were available for the development of the infra-red drying method, but the last two were in insufficient quantities for proximate analyses to be carried out. The moisture contents of these different varieties at different degrees of ripeness and after various stages of processing lay between 18 and 40% approximately. Table I gives the chemical composition of date meat for three varieties as determined in this laboratory. For the varieties Maktoom and Zahidi, the figures reported by Cook & Furr[11] are reproduced.

Methods

The samples were prepared [Method 20.2 (c) of the A.O.A.C.[11a]] by passing them through a meat mincer and 5 g. were taken for drying. The results quoted in all the tables are the averages of two simultaneous determinations. Method 20.8 of the A.O.A.C.[11a] was used for purposes of comparison. In addition, the Bidwell Sterling[12] method of reflux distillation with toluene was also used as a reference method requiring a much shorter drying time than is necessary for

Table I

Chemical composition of date meats from different varieties

	(figures as % of dry matter)				
	Barhee %	Halawy %	Khadrawy %	Maktoom[11] %	Zahidi[11] %
Total sugar	83·8	78·4	81·6	77·0	78·2
Reducing sugars	83·0	78·4	81·6	77·0	70·7
Protein	1·4	2·0	1·5		
Ether extract	0·2	0·1	0·3		
Nitrogen-free extract	12·4	16·7	14·6		
Ash	2·2	2·9	2·0		

the vacuum method. Avoidance of loss of sample, in transference, by adherence to the walls of the boiling flask, was achieved by extruding the material through a stainless steel tube into the receptacle which was weighed before and after transference of the sample.

Apparatus

For simple infra-red drying, a 250-W commercial infra-red lamp, mounted in an aluminium reflector, was used. The minced date meat was spread in a thin layer on an aluminium sheet of the same diameter as the lamp.

By taking into consideration the specific energy distribution of the lamp,[13] and its diameter (124 mm.), a distance of 30 mm. between the lamp and the sample was chosen. The voltages used in the different experiments were kept constant with a variable Powerstat transformer.

For infra-red drying under forced ventilation the same lamp was used as above but in an all-glass apparatus (Fig. 1). The apparatus was connected to a vacuum pump operating at a residual pressure of 600 mm. Hg. No advantage was obtained by pre-drying the incoming air over conc. H_2SO_4. After the irradiation, forced ventilation was continued for a further minute in order to cool the aluminium sheet and sample prior to weighing.

Results

I. *Comparison between the standard vacuum drying and the reference distillation methods*

Constant weight is reached with the vacuum method only after an average drying time of 18 h., whereas the distillation method gave comparable results after 1 h. To assess the efficiency of the latter method, it was checked against the vacuum method. Table II illustrates the results with the Khadrawy variety (widely varying moisture content) and the Halawy variety (moisture content varying over a narrow range).

The coefficient of correlation between the results of vacuum drying and distillation is 0·90 which is of high statistical significance. It was therefore decided to use the distillation method alone for reference purposes.

Table II

Comparison of moisture contents (%) of date meat determined by vacuum drying and distillation

Sample	Khadrawy variety		Sample	Halawy variety	
	Vacuum Drying	Distillation		Vacuum Drying	Distillation
D 1	39·8	39·6	L 1	31·3	31·2
D 2	39·5	39·6	L 2	31·1	31·3
D 3	33·5	33·6	L 3	32·1	32·3
D 4	33·0	32·8	L 4	32·4	32·4
D 5	22·4	22·7	L 5	31·3	31·2
D 6	22·5	22·7	L 6	31·1	31·3
D 7	17·2	17·2	L 7	32·1	32·3
D 8	16·8	17·0	L 8	32·4	32·4
D 9	17·3	17·4			
D 10	17·5	17·4			

II. *Moisture determination by simple infra-red drying*

Observations in the trade literature having been noted and after a series of preliminary experiments, a wattage of 115 W was adopted for drying purposes. The relative amount of infra-red in the total emission of the 250-W lamp was thus increased. In Table III, typical results are given for the variation of moisture content in date meats with time of drying.

With a wattage of 115 W scorching appears within 15–30 min., depending on the date variety—with the Khadrawy variety, it is apparent within 15–20 min., whereas with the Halawy it becomes evident within 25–30 min. The intensive irradiation obtained with this wattage causes case-hardening in both varieties, so that the moisture from the inner portions of the date meat does not reach the surface. It follows that the cooling effect of evaporation does not operate and thus scorching occurs. Differences in sensitivity to scorching, shown by different varieties, are due to differences in colour of the date meats. Thus, the intensity and depth of absorption of infra-red radiation varies.

For subsequent experiments lower wattages were chosen in order to avoid scorching. Moreover, the wattage used at the beginning of the experiments (the first 10 min.) was lower by 10 W than that of the remainder of the drying time. Total drying time varied within 25–40 min. Results of such a trial with the Barhee variety are given in Table IV.

Simple infra-red drying at a maximum wattage of 100 W for a maximum total drying time of 40 min. determines not more than approximately 60% of the moisture as detected by the distillation method. The results reveal further that lowering of the applied wattage permits of more careful treatment of the material for drying. As against this, however, the prolonged drying time required makes the method impractical.

III. *Infra-red drying under forced ventilation*

By maintaining a partial vacuum of 160 mm. Hg and by continuous displacement of water vapour by replacement of the air within the apparatus (Fig. 1), it was found possible to increase considerably the rate of evaporation of moisture from the dates. Table V illustrates the results of such an experiment with dates of the Khadrawy variety.

Part (A) of the table shows that results comparable with those of the distillation method are obtained after a total drying time of 25 min., split into an initial 10 min. at 50 W and the remainder at 60 W. Further increase of the drying time at the higher wattage caused scorching. This was also the case when wattage combinations of 70/80 W and 60/70 W were used over a total drying time of 15 min.

Table III

Comparison of moisture contents (%) determined by simple infra-red drying and distillation

Drying time, min.	Khadrawy	Halawy
10	28·7	20·9
15	35·0	27·8
20	37·7*	32·0
25		33·6
30		34·9*
Results obtained by distillation	36·6	33·5

* Light scorching

Table IV

Influence of different wattage and drying time on the results of moisture determination by simple infra-red drying

(Barhee date meat, moisture content by distillation 21·7%)

Wattage during the first 10 min. of drying, W	Wattage during additional drying, W	% Moisture Additional drying time	
		15 min.	30 min.
90	100	11·6	12·5
80	90	10·0	10·7
70	80	6·6	7·8

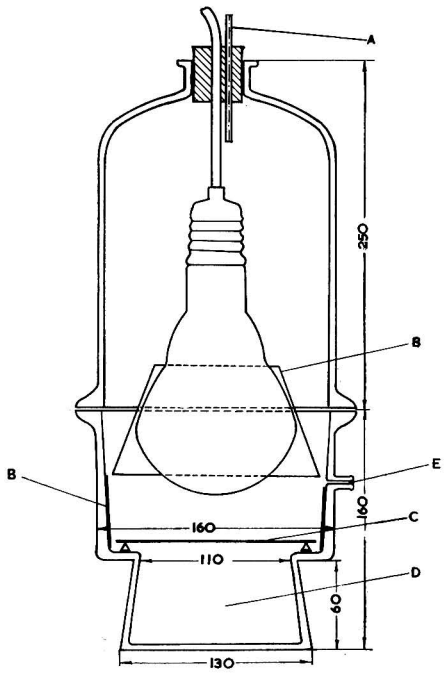

FIG. 1.—*Apparatus for forced ventilation infra-red drying*

A Capillary
B Reflector
C Aluminium sheet for sample
D Silica gel
E Connection to vacuum pump
(dimensions in mm.)

Table V

Influence of different wattage and drying time on results of moisture determination by forced ventilation infra-red drying

(Khadrawy dates, moisture content of meat 32·9% by distillation)

(A)

Total drying time, min.	Wattage during the first 10 min., W	Additional wattage, W	Drying time, min.	Moisture content, %
15	70	80	5	scorched
15	60	70	5	,,
15	50	60	5	32·0
20	50	60	10	32·5
25	50	60	15	32·8
30	50	60	20	33·5*

(B) First 5 min. at 50 W and additional drying at 60 W

Total drying time, min.	Additional drying time, min.	Moisture content, %
15	10	31·4
20	15	31·6
25	20	32·2
30	25	32·5
35	30	32·8

* Lightly scorched

Part (B) of the table illustrates that decreasing the initial drying time at 50 W from 10 to 5 min. has no advantage over the comparable wattage combination. A moisture content similar to that obtained with the reference method is reached only after a total drying time of 35 min.

The results quoted in Table VI make it evident that infra-red drying with forced ventilation has definite advantages over simple infra-red drying. In this case initial infra-red drying during 10 min. was carried out at 50 W and additional drying at 60 W.

It can be seen that forced ventilation drying at a wattage combination of 50/60 W gives results comparable with the reference method after a total drying time of only 25 min. By contrast, the simple infra-red drying method, with the same wattage combination, gives moisture contents 35–40% lower than those of the reference method, even after a total drying time of 45 min.

The reproducibility of the results obtained with the forced ventilation infra-red drying method is shown by the data in Table VII.

The maximum arithmetical difference between any figure from the drying method developed, and the corresponding one from the reference distillation method does not exceed 1%.

Table VI

Comparison of the results of moisture determination with different infra-red drying methods for three varieties of dates

Drying method	Additional drying time, min.	Moisture content, %		
		Barhee	Maktoom	Zahidi
Reference (distillation)		21·7	17·8	18·4
Simple infra-red drying	35	14·6	10·2	10·7
Forced ventliation infra-red drying	15	21·8	17·7	18·4

Table VII

Reproducibility of moisture determination results with the forced ventilation infra-red drying method for four varieties

	Barhee %	Khadrawy %	Maktoom %	Zahidi %
Reference distillation method	21·7	39·8	17·8	18·4
Forced ventilation infra-red drying (first 10 min. 50 W; additional 15 min. 60 W)	21·8	39·8	17·7	18·5
	21·6	39·8	17·7	18·4
	21·8	39·9	17·9	18·4
	21·9	39·9	17·9	18·4
	21·8	39·8	17·8	18·4
	21·6	39·4	17·7	18·4
	21·8	39·8	17·7	18·3
	21·7	39·8		
	21·9	39·8		
Average	21·8	39·8	17·7	18·4

Discussion and conclusions

Moisture determination in dates by infra-red drying with simultaneous forced ventilation, as developed in the present experiments, is based primarily on the advantages of the use of infra-red radiation for evaporating water, in contradistinction to conventional drying procedures. These advantages stem from the fact that water is a substance only slightly transparent to infra-red radiation. According to Déribéré[14] definite ranges of high infra-red absorptivity by water coincide with the emission maximum (1·0–1·5 μ) of commercial infra-red lamps. This has been verified by Lecomte[15] in experiments with thin layers of water. The latter author also established the fact that the absorptivity of water vapour is low over the range of emission of the lamp. Consequently transmission of infra-red radiation through the water-saturated atmosphere surrounding the sample is enhanced and, as a result, a higher fraction of emitted energy reaches the area of evaporation.

Ickis & Haynes[16] showed that approximately 15% of the energy emitted by an infra-red lamp penetrates through water down to a layer of 2 to 3 mm. below the surface. There is thus a considerable increase in the evaporating surface. This fact holds only for an infra-red lamp with an average temperature of 2200° K of the incandescent filament, and not for the ' dark '-irradiators suggested by Alt.[17] Thus, the appropriate range of infra-red radiation dries the inner layers, not by heating the surrounding air, but by its direct absorption into the deeper moisture layers. As opposed to conventional drying with heated air, there is no need to maintain a heat potential between the heated air and the sample.

Zimmermann[18] stressed the fact that air is even more transparent to infra-red rays than is water vapour The use of forced ventilation increases the proportion of fresh air to saturated vapour. This enables more of the total energy emitted by the lamp to reach the sample than where ventilation is not used. This fact is confirmed by the data in Table VI which show also that even with a relatively low wattage, evaporation is completed within a comparatively short time. Furthermore, the smaller absorption of heat in the air surrounding the sample means that the temperature in these air layers is lower than within the material itself. Thus, a certain cooling effect prevails and this prevents overheating. According to Willits,[19] this fact is of prime importance in materials rich in fructose where losses by decomposition may occur.

The specific ability of infra-red to cause a certain degree of drying within a mass of moisture-containing material greatly retards crust formation. Reith *et al.*[20] stress that such crust formation is largely responsible for the decreased evaporation of moisture from the inner layers of the material. Hence, avoidance of case-hardening by means of a combination of infra-red drying and forced ventilation makes possible the rapid attainment of results comparable with those obtained in greater time with conventional distillation or vacuum drying methods.

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SEPARATION OF SOME GLYCERIDES OF COCOA BUTTER BY PAPER CHROMATOGRAPHY

By E. H. STEINER and A. R. BONAR

A technique of reverse-phase paper chromatography is described by means of which the three mono-unsaturated glycerides of cocoa butter were separated and their positions on the paper located. R^F values of the glycerides are given together with their approximate proportions in cocoa butter as indicated by the chromatograms. The technique was applied successfully to ' bloom ' crystals and to illipé butter but was not found to be satisfactory in the case of butter fat or palm kernel fats.

Introduction

Methods which have been used for the determination of the glyceride structure of fats and oils have been based, to a large extent, on systematic crystallisation from solvents[1] or on countercurrent distribution.[2] These methods require extensive analytical work for the subsequent identification of the fractions obtained. For many oils where the triglyceride constitution is complex the separation achieved is generally between fractions of different degrees of saturation and the fractions obtained are not pure glycerides.

Chromatography has been used quite extensively for the investigation of lipids generally, particularly for the separation of fatty acids, sterols and mixtures of mono-, di- and tri-glycerides.[3] There appears to be less reference in the literature, however, to the resolution of a triglyceride mixture into its components by a chromatographic process. Some separation into glyceride types was obtained by Walker & Mills[4] by passing a solution of linseed oil in hexane through an alumina column and Harker,[5] also with an alumina column, found that olive oil was retained from a mixture with paraffin oil. Hamilton & Holman[6] obtained fractions from tallow varying in melting point from 19° to 60° by displacing it from carbon with 0·5% tristearin in benzene.

Priori[7] used paper treated with a 5% solution of paraffin oil in benzene, with 10% benzene and 90% methanol as mobile solvent, to resolve a fraction obtained from plant oils which had been passed through an alumina column. The paper was subsequently exposed to iodine vapour. The presence of fats of the Cruciferae order was shown by five well-defined spots while with other plant oils two spots were formed. By this means 5% of rape oil could be detected in oils such as olive, sesame and arachis. A Japanese worker[8] also claims to have separated and identified fats and oils coloured with Sudan III, by paper chromatography with a mixture of four solvents, viz., diacetone alcohol, chloroform, isoamyl alcohol and amyl acetate (3 : 1 : 1 : 1) and running for 3 h. Characteristic patterns were said to develop. Recently Kaufmann & Makus have reported the separation of some fully saturated glycerides using paper impregnated with undecane.[9]

The purpose of the work described in the present paper was to attempt the separation of the major glycerides of cocoa butter and fats of similar type by means of reverse-phase paper chromatography. Before this could be done it became necessary to develop a satisfactory procedure by which the position of the glycerides on the paper could be located.

Experimental

Location of the glycerides on the chromatogram

Some preliminary experiments were carried out with ultra-violet light as a possible means of detecting spots on the paper but this procedure was not found to be sufficiently sensitive.

According to Schlenk *et al.*[10] the position of lipid material on the paper can be shown by moistening the paper after development of the chromatogram, then hanging it in iodine vapour for 5 min. at 50°. Unsaturated fats were stated to appear as yellow or brown spots. Inouye & Noda[11] have suggested iodine dissolved in alcohol or in Wijs reagent for detecting unsaturated fatty acids on paper. Neither procedure was found to be entirely reliable in the case of cocoa butter and after various modifications had been tried the following process was found to be satisfactory.

After the chromatogram has been run, the paper is dried at room temperature and hung in a tank containing iodine vapour for approximately 30 min. at room temperature. The paper is removed from the tank and the back sprayed with 1% starch solution when clearly defined white spots appear on a blue background. When dry the outline of the spots can be traced to give a permanent record, although a distinct colour contrast remains for some weeks. The sensitivity of this method of detection was determined by spotting the paper with various amounts of cocoa butter in ether ranging from 0 to 500 μg. of fat. The limit of detection was found to be of the order of 3–4 μg. of cocoa butter per sq. cm. surface area.

Preliminary experiments

With the above technique for locating the fat, a series of experiments was carried out to investigate the effectiveness of various solvents. Whatman No. 1 chromatography-grade paper was used both with and without preliminary drying and also with and without various impregnations of non-polar solvents. Cocoa butter was spotted on to the paper from an ethereal solution by means of a calibrated capillary tube in amounts varying from 50 to 500 μg. of fat.

Mobile solvents investigated were acetone, chloroform, ethanol, methanol, light petroleum, isopropyl alcohol and furfuraldehyde, either individually or in various combinations with one another and with water, at temperatures from 0° to 30°. Furfuraldehyde was tried in view of the fact that it had been used in the partial separation of the glycerides of soya-bean oil.[12] Its use introduced a difficulty in locating the fat, however, since it interfered with the iodine treatment. This was overcome by washing the paper in water until no odour of furfuraldehyde could be detected. When dried the paper gave a satisfactory reaction to iodine vapour.

As a result of these experiments it was eventually found possible to resolve cocoa butter into three distinct areas on the paper, with either furfuraldehyde or acetone–methanol as mobile solvent and paraffin as stationary phase.

Details of the procedure finally adopted as giving the best results with cocoa butter are as follows:

Technique for the separation of cocoa butter glycerides

A strip of paper 18 in. long, cut from a 6-in. wide roll of No. 1 Whatman chromatography paper, is serrated at one end. The paper is heated at 110° for 2 h. and then passed once through a 5% w/v solution of liquid paraffin B.P. in light petroleum ether or acetone. It is subsequently hung up to dry at room temperature and finally heated for a brief period (about 2 min.) at 110°. The prepared papers may be stored in an air-tight container until required for use.

The fat is applied in ethereal solution to the paper by means of a calibrated capillary tube. In a typical case the fat was dissolved in a 5% w/v solution and the capillary tube used had a capacity equivalent to 7 μg. of fat per 1 mm. length. One application made in this way was sufficient to give about 100 μg. of fat on the paper. The paper is mounted in a descending chromatography tank with acetone–methanol (90 : 10) saturated with paraffin as the mobile solvent. The chromatogram is allowed to run for 16–20 h. and then developed as described above.

Results and discussion

In the case of cocoa butter, the application of this technique results in the appearance of three separate elongated spots on the paper. A certain amount of variation in both position and size of the pots has been observed to occur from time to time and the nature of the chromatogram was particularly affected by the quantity of fat taken. Amounts in excess of 200 μg. did not resolve in the length of paper available while, with under 50 μg. of fat, the third spot was not always detectable on development due to the limited sensitivity of the method of location.

Nature and preparation of the separated glycerides

It was expected that the separated areas appearing on the chromatogram would most probably correspond with the mono-unsaturated glycerides known to be the major components of cocoa butter. The presence of the three glycerides 2-oleodistearin, 2-oleopalmitostearin and

2-oleodipalmitin is well established,[13–15] their relative amounts being very roughly in the proportions 25%, 65%, 10% respectively.[16–18] Confirmation that the spots obtained were, in fact, due to these mono-unsaturated glycerides was given by running separate chromatograms of each of the above glycerides (prepared by synthesis in another connexion). The glycerides travelled along the paper at different rates and corresponded exactly in final position with the three spots derived from cocoa butter (Fig. 1).

R_F values for the glycerides, determined from ascending chromatograms, gave the following results (Table I).

Table I

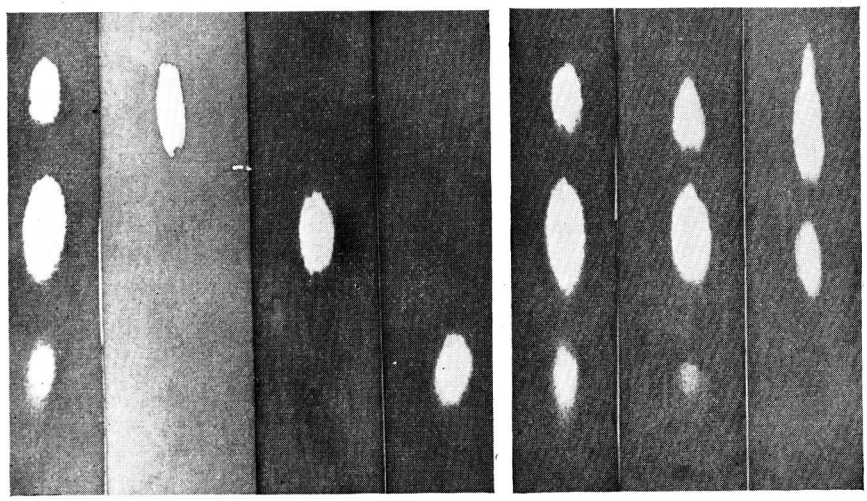
R_F values of mono-unsaturated glycerides in solvent system 90/10 acetone–methanol

Glyceride	R_F value
2-Oleodistearin	0·21
2-Oleopalmitostearin	0·27
2-Oleodipalmitin	0·33

The relative proportions of the three glycerides oleodistearin, oleopalmitostearin and oleodipalmitin estimated from the areas of the spots on the paper were found to be approximately 30%, 55% and 15% respectively. These figures are similar to those found by Scholfield[18] but differ somewhat from the values given by Hilditch & Stainsby[16] and by Meara.[17]

Results with other fats

In Fig. 2 are shown the chromatograms obtained with illipé butter (Borneo tallow) and cocoa butter 'bloom' under the same conditions as were used for cocoa butter. Crystals of 'bloom' were obtained by carefully brushing off the surface layer from samples of heavily



Chromatograms of cocoa butter and (FIG. 1, left) *synthetic glycerides and* (FIG. 2, right) *in solvent system 90/10 acetone–methanol*

A Cocoa butter; B 2-oleodistearin; C 2-oleopalmitostearin; D 2-oleodipalmitin; E Cocoa butter; F Bloom; G Illipé

bloomed plain chocolate. A number of different samples of ' bloom ' were examined, all of which showed the same three glycerides as the original cocoa butter but with a smaller proportion of oleodipalmitin. The other two glycerides appeared to be present in a proportion similar to that in cocoa butter.

Oleodipalmitin did not show up in the illipé butter, although according to Bushell & Hilditch[19] the fat contains about 8% of this glyceride. When a higher amount of fat was used for the chromatogram, however, a third spot could be obtained.

When applied to butter fat and fats of the palm kernel type, the method was not found very successful. In particular, the staining technique for locating the fat would operate only when relative high concentrations of fat were used, namely about 500 μg. of butter fat and 1000 μg. of palm kernel fats. At these concentrations cocoa butter produced one long streak stretching back to the origin. Butter fat behaved somewhat similarly but left the origin and exhibited rather more signs of breaking up. Both palm kernel stearins and hydrogenated palm kernel oil showed up as much smaller streaks with the stearin moving more rapidly than the other.

The reasons why the technique developed for cocoa butter fails to work satisfactorily with these other fats are probably two-fold. Firstly, the individual glycerides after separation must be present on the paper in sufficient concentration to stain, and secondly, there must be some unsaturation in the glycerides for the reaction with iodine to occur. Butter fat presumably did not adequately satisfy the former condition and the palm kernel fats would fail in the latter respect.

It is possible that, by modifying the mobile solvent, suitable conditions for separating these fats into their component glycerides might be realised. This would almost certainly necessitate working with smaller quantities of fat, however, for which an adequate method of locating the fat on the paper must be found.

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THE α-TOCOPHEROL CONTENT OF LEAVES AS AFFECTED BY GROWTH RATE

By V. H. BOOTH and A. HOBSON-FROHOCK*

α-Tocopherol was quickly extracted from small samples of leaf with acetone, transferred to petrol, purified by two-dimensional paper chromatography, and determined by the Emmerie-Engel test.

Fast-growing leaves of *Lepidium sativum* (cress), *Lactuca sativa* (lettuce) and other plants had tocopherol contents of about 70 p.p.m. based on dry matter (7 p.p.m. fresh weight); while evergreen and other slow-growing leaves had up to 1400 p.p.m. (450 p.p.m. fresh). In long leaves of grass, iris, narcissus and other plants the α-tocopherol content was low near the base where growth is fast and maximal near the apex where growth is slow. In young short leaves of narcissus the gradient was only slight. Tocopherol contents of leaves increased during summer and reached maxima in autumn or winter. In leaves of two conifers the maximum concentration was reached in the third winter. In evergreen leaves the values fell in spring. Dying and fallen leaves had higher contents than green leaves. In blooms of narcissus the α-tocopherol contents diminished considerably during development.

It is concluded that α-tocopherol content of leaves is inversely related to growth rate.

Introduction

Although tocopherol contents of leaves of certain plants have been reported, the data are inadequate for the formulation of patterns of distribution as a preliminary to elucidating the function of tocopherols.

We have used a method of analysis that has only recently become practicable, and have examined leaves of many species of plants at different stages of development. The most striking finding is that the α-tocopherol is present in high concentrations in slow-growing, dormant or dying leaves, and in much lower concentrations in actively-growing leaves. The evidence for this generalisation is presented in this paper.

Published observations

Green[1] observed little change in the α-tocopherol contents of wheat, pea and barley plants during the period in which they trebled their heights. He was studying the relative distributions of the several tocopherols in whole plants throughout their life cycles, and did not record values for leaves separately. Brown[2] followed the α-tocopherol content in grasses from April to September. In each of four species he found a steady fall based on dry matter and suggested that a major factor contributing to the fall with time was the decrease in ratio of leaf to stem in maturity. From neither of these reports is it possible to discover what happens to α-tocopherol in leaves during growth.

Ramanujan & Anantakrishnan,[3] in a study of the 'tocopherol' content of the leaves of the cereal Jowar over 12 weeks, observed a barely significant rise on a green matter basis and a definite fall on a dry matter basis. They also reported a small increase in tocopherol content in some forage plants from summer to winter, but no significant difference in others. Sironval & El Tannir-Lomba[4] stated that the 'vitamin E' content of leaves of *Fragaria vesca* reached a maximum in June and thereafter fell. Neither pair of authors used paper chromatography, and doubtless their 'tocopherol' and 'vitamin E' included other fat-soluble reducing substances originating both from the plant material and from the solvents.[5]

Ebele[6] found that the concentration of tocopherol in pine needles was highest in winter and early spring. Kuchina[7] found that the tocopherol content of conifers decreased during spring, increased in summer and sometimes in autumn, and increased with the age of the tree. The present observations are in agreement with the findings of these two authors.

Experimental

Fat-soluble materials including tocopherols, carotenoids and chlorophylls were extracted from small samples (about 300 mg.) of freshly-gathered leaves, with acetone and light petroleum

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(b.p. <40°) in the presence of quinol,[8a] and the acetone was removed with water in a continuous washing apparatus.[8b] The light-petroleum solution was transferred to a 50-ml. flask and concentrated to about ½ ml. under nitrogen. The whole green extract was applied by means of a Trenner pipette to a single paper treated with zinc carbonate,[9] not as the usual narrow streak, but over an area about 2 cm. × 6 cm. Two-dimensional paper chromatography and evaluation of α-tocopherol were done essentially according to the technique of the Analytical Methods Committee.[10] Many small modifications of the Committee's technique were introduced to shorten the methods, thereby enabling 6 analyses to be completed in 6 hours. For example we used Whatman No. 4 paper, 1·1% acetone in 40–60°-light petroleum in the first chromatography tank and aqueous methanol (910 vol. methanol + 90 vol. water) in the second, and papers were dried evenly on a turntable.[11]

Saponification was avoided by this technique and no heat was used for extraction. With leaves having tocopherol contents less than 80 p.p.m. on dry matter, so much extract had to be used for each chromatogram that other fat-soluble substances interfered with development of the spots. These substances were first removed by pouring the washed extract on to a chromatographic column[12] of Decalso-F 1 cm. dia. × 1 cm. high. The petrol eluate was discarded, and the tocopherols were eluted with 20 ml. of 2% acetone in 30–40°-light petroleum. [This solvent is preferred to the formerly-favoured benzene in that it can be evaporated more quickly, at lower temperature and more smoothly, and it is less toxic.] The yellow solution was concentrated and applied to paper as described above.

Tocopherol contents are expressed as p.p.m. of dry matter, and (in parentheses) as p.p.m. fresh weight. Dry matter was evaluated after heating samples at 100° for 5 h. Other tocopherols were determined, but only results on the α-form are reported.

Results

Effect of season

The α-tocopherol content of leaves was higher in autumn or winter than in spring or summer, and higher in summer than in spring. For example *Fragaria vesca* (wild strawberry) had 35 p.p.m. on dry matter (10 p.p.m. fresh weight) in May, 125 (35) in June, 250 (65) in mid-July, 330 (85) at the end of July, and 870 (230) on 2 September. Other leaves examined are listed in Table I.

An example of extreme differences with age of leaves of a deciduous plant was seen in *Rubus fruticosus* (blackberry). In April small young bright green leaves had an α-tocopherol content of 120 (30) p.p.m. Some of the previous year's leaves still adhered to the bush: these were dark green to reddish-brown and had 890 (390) p.p.m.

Table I

α-Tocopherol contents of leaves in different months, in p.p.m. dry matter (and p.p.m. fresh weight)

Medicago sativa (lucerne)	Mar. 90 (16); July 200 (45); Aug. 260 (65)
Conium maculatum (hemlock)	Apr. 60 (18); Aug. 350 (65)
Sambucus nigra (elder)	Mar. 270 (55); Nov. 1300 (250)
Urtica dioica (nettle)	Mar. 190 (35); July 680 (190); Nov. 770 (200)
Iris germanica	Mar. 115 (12); Nov. 370 (70)
Populus italica (poplar)	Apr 170 (35); July 290 (90); Oct. 350 (140)
Ligustrum vulgare (privet)	Apr. 120 (35); Feb. 1270 (280)
Urtica dioica (nettle)	Apr. 180 (35); Oct. 740 (140)
Prunus avium (cherry)	May 590 (170); Oct. 1330 (480)
Berberis vulgaris	May 140 (30); Dec. 330 (120)
Betula alba (birch)	May 100 (35); July 270 (100); Oct. 980 (400)
Taraxacum officinale (dandelion)	May 150 (20); Oct. 200 (45)
Taxus baccata (yew)	June 70 (20); July 250 (70); Jan. 1200 (400)
Solanum tuberosum (potato)	June 250 (30); Sept. 660 (120)
Hedera helix (ivy)	June 160 (45); Dec. 300 (100)
Helianthus tuberosus (artichoke)	July 65 (15); Sept. 125 (30)
Petroselinum crispum (parsley)	Aug. 55 (10); Nov. 130 (30)
Cotoneaster microphylla	Apr. 220 (65); Nov. 300 (110)
Phaseolus coccineus (runner bean)	July 170 (25); Sept. 530 (80)
Daucus carota (carrot)	Aug. 145 (25); Oct. 325 (55)

Effect of age

Leaves of plants of *Chenopodium alba* (fat hen) of different ages were analysed on the same day in July. Plants 5 cm. high had 25 (3), 7 cm. had 70 (9), 10 and 35 cm. each had 120 (15) p.p.m. Young leaves of *Achillea millefolium* (yarrow) taken in July a month after the site had been mown had 110 (18 p.p.m.). Leaves taken on the same day from other plants in bloom had 180 (35).

Leaves of very small plants of *Tropaeolum majus* (nasturtium) had 30 (5) and leaves of older plants taken concurrently in July had 75 (13) p.p.m. In September the leaves of a 7-cm. high young plant of *Cirsium arvense* (thistle) had 30 (5), and of a 60-cm. plant had 220 (40) p.p.m.

Effect of age of evergreen leaves

In certain conifers the leaves persist for many years, and growth habit is such that the age of leaves is easily determined. In *Picea pungens* young leaves in June had an α-tocopherol content of 80 (30) p.p.m. In February the one-year-old leaves had 200 (90); 2-year, 300 (130); 3-year, 350 (160); 4-year, 320 (140); 5-year, 320 (140) p.p.m. The α-tocopherol reached maximum concentration in third-year leaves.

Fig. 1 shows a third-year maximum for the α-tocopherol content of leaves of the conifer *Abies numidica* in February. The dry matter in leaves from 1 to 8 years old was 41%. In June young leaves had 120 (50) p.p.m. Third-year leaves had 480 (250) in June, i.e., less than they had in February but more than the young leaves had at either time.

Young leaves of *Taxus baccata* (yew) had 40 (10) p.p.m. in May. The value rose through 120 (30) in June, 250 (70) in July, 440 (150) in December to 1420 (470) p.p.m. in January. The value for these now 'old' leaves then fell to 820 (340) in June, 1050 (440) p.p.m. in July.

In April young leaves of lavender had 170 (25) while old leaves from the same plant had 510 (150) p.p.m. In February leaves of *Ligustrum vulgare* (privet) had 1270 (280) p.p.m. By April the content had dropped to 900 (200) at which time young leaves had only 120 (35) p.p.m. In May young leaves of *Ulex europaeus* (gorse, furze) had 85 (20) while old leaves had 120 (45) p.p.m.

Fast-growing leaves

The primary leaves of *Lepidium sativum* (garden cress), divided as to small and large size, had 60 (6) and 100 (10) p.p.m. respectively when analysed in March.

Lactuca sativa (lettuce) is another fast grower. Tom Thumb, a very small variety, and a large variety of the 'cabbage' type each had similar values, namely 80 (5) for the inner or youngest, 120 (7) for the intermediate, and 200 (12) p.p.m. for the outer, older leaves. At the flowering stage the value had risen to 150 (15).

Helianthus tuberosus (artichoke) leaves had 65 (15) p.p.m. in July while growing very fast, a value that rose to 130 (30) in September.

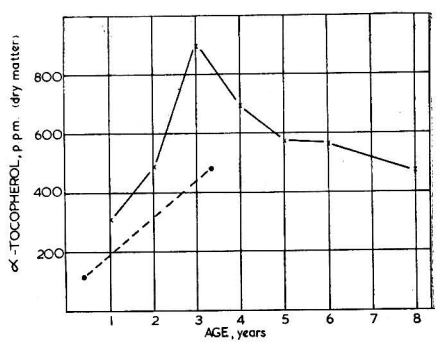

FIG. 1.—*Effect of age on the tocopherol content of leaves of the evergreen* Abies numidica

Analysed in February.
The points linked by the dotted line show values for young and third-season leaves in June

The small 'sprouts' of *Brassica oleracea* (Brussels sprouts) had 50 (6) when growing in November at a time when leaves of few species grow at all. In February when growth had almost stopped the content had risen to 170 (20).

Rhubarb produces leaves that grow fast early in the spring. The α-tocopherol content in April was 100 (10) p.p.m.

Gradient of tocopherol content along the leaf

A leaf of rhubarb, 55 cm. long × 45 cm. wide examined in June, had 220 (22) p.p.m. near its edges and near its apex, but only 130 (13) p.p.m. near the middle, with intermediate values in intermediate positions.

In July 10 healthy leaves of *Dactylis glomerata* L. (cocksfoot grass), about 40 cm. long, were analysed for α-tocopherol. Pooled samples from the base of each leaf had 30 (6) p.p.m., while samples similarly taken from the middles of the leaves had 160 (40) p.p.m. The content around 4 cm. from the apices was 190 (50), and at the apices it was 160 (45) p.p.m. A similar experiment was done with *Eryngium pandandifolium*, a long-leaved plant from Brazil, grown in Cambridge and harvested in February. The tocopherol content, shown in Fig. 2, rose to a maximum towards the apex but fell slightly at the apex whether based on fresh weight or dry weight. The tocopherol contents at 2½-cm. intervals from base to apex along 16 cm. leaves of *Plantago lanceolata* (plantain) in July were 12, 17, 25, 40, 50, 25 p.p.m. based on fresh weight. Similarly the leaves of *Iris germanica* in July had 125 (10), 200 (25), 650 (130) p.p.m. In leaves of *Allium porrum* (leek) in December, *Dactylis glomerata* in November, and *Iris germanica* in April and November, the concentrations were maximal near the apices.

Ramanujan & Anantakrishnan[3] observed an increase in fat-soluble reducing substances of about 65% from base to apex in the leaves of four grasses and a cereal.

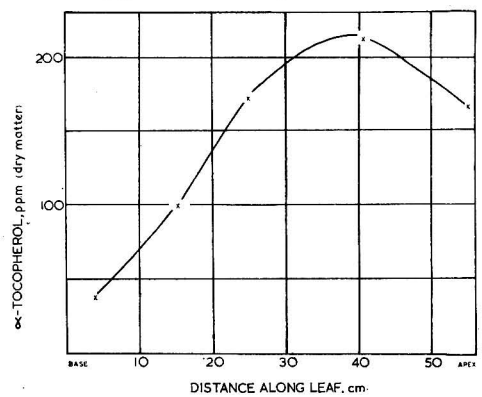

FIG. 2.—*Gradient of α-tocopherol concentration along the leaf of* Eryngium pandandifolium (dry matter was 20% near the base and 33% near the apex)

Time and topography combined

Leaves of narcissus were analysed at intervals of time during rapid growth in the spring. From each of several leaves samples were taken from near the base, from the middle and from near the apex. Fig. 3 shows that the tocopherol gradient was slight along young leaves which were growing throughout, whereas in older leaves the gradient rose sharply towards the slower-growing apex. The content near the base was a little higher in April than in May. In two other cultivars, Carbineer and Lady Diana Manners, similar changes were observed in α-tocopherol gradients. The dry matter (average of all experiments) rose from 9% near the base to 18% near the apex.

Senescence

Dying and yellowing leaves were found to have very high contents of α-tocopherol. For instance when leaves of *Daucus carota* (carrot) in different states were gathered in August,

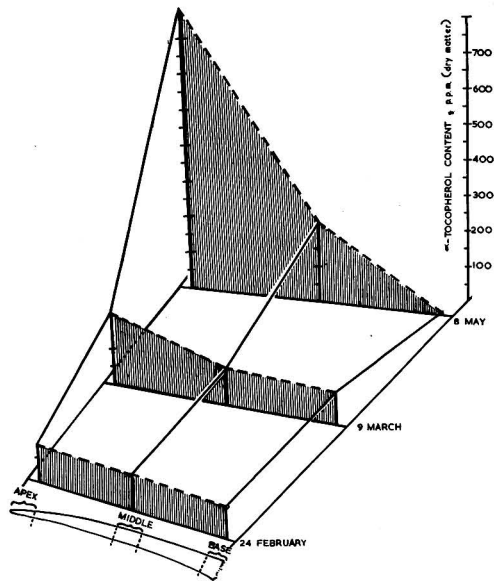

FIG. 3.—*Effect of time on the distribution of α-tocopherol along the leaves of* Narcissus *cv Fortune*
Zones analysed are indicated in the sketch. Abscissae are all on the same scale, but base lines are not to scale. Average leaf lengths were: Feb., 11 cm.; Mar., 30 cm.; May, 60 cm.

dark green leaves had 120 (26), yellow leaves had 330 (90), and reddish leaves had 540 (135) p.p.m. Other examples are given in Table II. These values are for senescent leaves that had lost their chlorophyll. They contrast with those of living variegated leaves whose yellow parts had less tocopherol than had the green parts.

The apices of long leaves usually die sooner than the lower parts. In such leaves the concentration curve no longer showed a fall at the apex as in Fig. 3; instead the α-tocopherol content was higher at the apex than in any other part. For instance green tips of *Dactylis glomerata* had 160 (45) while grey tips had 220 (90) p.p.m. The phenomenon was also observed in leaves of *Iris germanica* and *Narcissus*.

Table II

Tocopherol contents of senescent leaves in p.p.m. dry matter (and p.p.m. fresh weight)

Month	Leaf of	Green	Yellow
March	Broccoli*	250 (35)	800 (145)
July	*Achillea millefolium* (yarrow)	115 (20)	750 (100)
July	*Phaseolus coccinneus* (runner bean)	180 (25)	2150 (250)
July	*Matricaria maritima* (mayweed)	310 (52)	360 (80)
September	*Solanum tuberosum* cv Majestic	930 (165)	2000 (240)
October	*Populis italica* (Lombardy poplar)	350 (140)	
December	,, ,, †		900 (460)
July (end)	*Fragaria vesca* (strawberry)	330 (85)	480 (140)

* Average for two cultivars: purple sprouting and white
† Fallen leaf

Flowers of narcissus

Flowers of the cultivar Fortune were analysed at three stages: the unopened bud, full bloom, and bloom starting to wither. The tocopherol contents of the corolla at the three stages were 30, 20 and 15 p.p.m., and of the corona were 60, 20 and 1 p.p.m., all based on fresh weight. The time span was $3\frac{1}{2}$ weeks.

Discussion

The concentration of α-tocopherol was high in old, in dormant and in dying leaves or parts of leaves, a maximum being found in winter. The concentration was low in young and actively-growing leaves, and near the bases of long leaves. It was high in leaves of trees and bushes, low in rhubarb, cress, lettuce and other fast growers.

All the petrol-soluble substances from leaves were applied to paper and appeared on the two-dimensioned chromatograms. These included pigments, tocopherols, quinones and other substances that were visible under ultra-violet light. Doubtless some of these other substances would be included as ' vitamin E ' by less specific methods. For instance extracts of narcissus leaves contained a substance that ran so close to α-tocopherol that it would not be separated by column chromatography. α-Tocopherol was found in all green leaves examined, and was unequivocally separated from interfering substances. Other tocopherols were often present also but never in amount or circumstances to suggest that the above observations could be explained by interconversion.

Hypotheses that relate tocopherol maxima with flowering or seed formation are untenable because in some species maxima are reached long after seeding.

One hypothesis that might account for the observations is that α-tocopherol is a useless by-product that cannot be metabolised and accumulates as the leaf grows older. It might for instance be a residue from the breakdown of chlorophyll. Evidence against this hypothesis is the observation that very high values found in evergreen leaves in winter diminished in spring. Moreover the α-tocopherol contents of flowers of narcissus fell markedly during the $3\frac{1}{2}$-week period from just before the bud opened until the flower began to wither, which shows that tocopherol can be destroyed in plant tissue.

An alternative hypothesis is that α-tocopherol is produced in all leaves and is used up when the metabolic rate is high. Since chlorophyll appears to be accompanied always by α-tocopherol the obvious metabolic process in which the latter might play a part is photosynthesis.

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CITRUS ESSENTIAL OILS. I.—Evaluation of Natural and Terpeneless Lemon Oils

By C. A. SLATER

Samples of natural and terpeneless lemon oil have been examined by a combination of absorption and gas chromatography and infra-red spectroscopy. Natural lemon oil contains at least seven terpenes and nine sesquiterpenes whilst there are at least twenty-four oxygenated compounds. It is possible by examination of the oxygenated and hydrocarbon fractions to make a decision as to the authenticity and freshness of an oil, but organoleptic tests are of considerable value.

Introduction

Until quite recently the investigation of lemon oil, as of other essential oils, could only be carried out by a tedious process of fractional distillation and chemical investigation requiring many kilogrammes of material. In the case of the major oil components such methods were often fruitful and yielded much information about the composition of essential oils ;[1] classical methods however can give little information about minor constituents. With the advent of chromatography, in particular the chromatostrip technique[2] and gas chromatography,[3] the investigation of the minor components of an oil becomes possible.

One of the earliest applications of gas chromatography to the analysis of essential oils was made by Howard & Slater,[4–6] who carried out investigations of the essential oil of hops. Bernhard[7] applied the technique to lemon oils and by comparison of the peak heights of the terpene components was able to make an assessment of the authenticity of the sample. Liberti & Cartoni[8] used the method with a number of citrus oils whilst more recently Lund[9] applied it to the investigation of confectionery flavours.

In conjunction with gas-chromatographic fractionation, infra-red spectroscopy may be used for purposes of identification. Bernhard[7] and Lund[9] used the technique for the identification of the terpenes they isolated and Casu[10] used infra-red spectroscopy as a method of detecting gross adulteration of lemon oils.

The objects of the present work were three-fold. Firstly, to establish a reliable method of detecting adulteration. Secondly, to develop a technique for the isolation and examination of pure components of lemon oils prior to the determination of their chemical structure. Thirdly, it was hoped to obtain a close correlation between the flavour of oils as judged by taste panels and their chemical composition ; it is already widely accepted that a broad correlation exists between citral content and flavour of lemon oils.

Experimental

Fractionation of oils

This was carried out by chromatography on columns of silica gel as described by Howard & Slater.[6] The percentage of oxygenated compounds was determined and samples of the hydrocarbon and oxygenated fractions were analysed by gas chromatography.

Sesquiterpene fractions were obtained by separation of the hydrocarbons remaining after the terpene hydrocarbons had been removed from the oils by distillation *in vacuo*.

Gas chromatography

The apparatus used was a 4-ft. column, 3–4 mm. diameter, containing Edwards High Vacuum Silicone Grease (25% w/w) on Celite (Johns-Manville 545, size-graded and washed by the method of James & Martin[3]). The column was run at 199° for most of the chromatograms, at 100° for the terpenes (Fig. 3) and at 150° for the oxygenated compounds (Fig. 4). Nitrogen was used as the carrier gas. The detector used was the hydrogen converter described by Green[11] in conjunction with a katharometer conductivity cell at room temperature.

Infra-red spectroscopy

Infra-red spectra were recorded with a Hilger H800 double-beam infra-red spectrophotometer.

Flavour and aroma

Flavour and aroma of samples examined were assessed by an experienced panel.

Authentic lemon oil samples

(*a*) The peel was grated from Sicilian lemons (6 fruits) and extracted with light petroleum (b.p. 30–40°). The solvent was distilled off and the oil was analysed as described above.

(*b*) The peel was grated from Sicilian lemons (12 fruits) and the oil was washed off with water (1 litre). The aqueous emulsion was saturated with sodium chloride and the oil that separated on keeping was analysed in the usual way.

(*c*) Samples of commercially produced genuine oils were obtained from reputable suppliers.

Results and discussion

I. *Natural lemon oils*

(a) *Gas chromatography.*—The gas chromatograms of whole lemon oils (Fig. 1) are of little diagnostic value, only minor differences being found between known genuine and known adulterated samples or between samples of widely differing flavour. Under the conditions normally used in this laboratory the complete hydrocarbon fractions also are of little value since the large proportion of limonene tends to obscure the minor components in the gas chromatogram (cf. Fig. 2). At lower temperatures it is possible to obtain a complete resolution of the terpene fraction of the oil and this fraction may be of value in assessing the authenticity and desirability of an oil, but sufficient information is not yet available (however cf. [7]). Fig. 3 shows a typical terpene fraction, the individual components having been tentatively identified from their retention volumes (cf. [7, 8]).

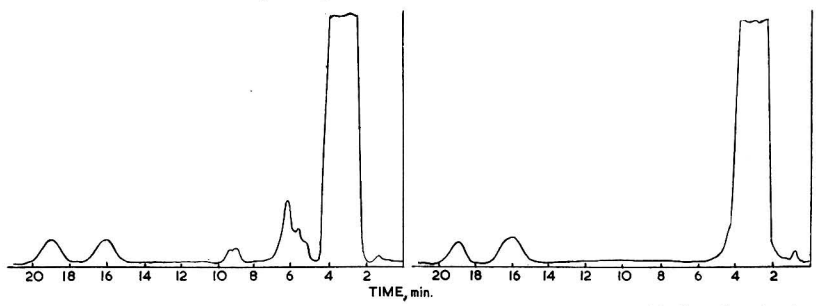

FIG. 1.—*Gas chromatogram of whole lemon oil*
Column temp. 199°

FIG. 2.—*Gas chromatogram of hydrocarbon fraction of lemon oil*
Column temp. 199°

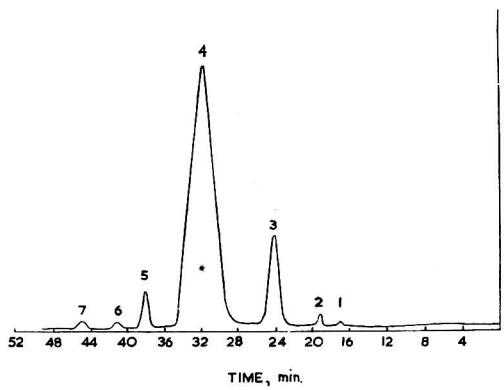

FIG. 3.—*Gas chromatogram of lemon oil terpenes*
Column temp. 100°
Tentative identification: 2 pinene, 3 pinene, 4 *d*-limonene, 5 terpinene, 6 terpinolene

By far the most useful fraction of lemon oil is the oxygenated fraction. In Fig. 4 are shown typical chromatograms of this fraction of lemon oil from various sources and it can be clearly seen that the oils show marked differences in the balance of components present. In Sicilian oils the peak numbered 20 (citral *a*) is very much higher than any of the others, whilst in Californian and Nigerian oils it is quite small in agreement with the findings of Sale.[12] The taste panel assessment of these particular oils could be closely correlated with the percentage of citral, but unfortunately this is not always the case and it is not, as yet, possible by gas chromatography alone to evaluate completely the flavour of a given oil.

In some cases it is possible to detect adulteration in natural lemon oils by examination of the gas chromatograms of the oxygenated fraction. Thus the pattern given by the adulterated oil (Fig. 5) is markedly different from that of the typical Sicilian oil (Fig. 4) with which it may be compared. On the other hand, three other adulterated oils showed no such marked difference and gas chromatography alone cannot be relied upon to show up sophistication.

(b) *Infra-red spectroscopy.*—As in the case of gas chromatography it was found that both the whole oil (apart from giving a quantitative measure of citral) and the hydrocarbon fractions gave spectra which were of little diagnostic value, the latter giving a spectrum typified by impure limonene. The oxygenated fraction on the other hand gave a spectrum of much greater value, a typical spectrum for a fraction of natural oil being shown in Fig. 6.

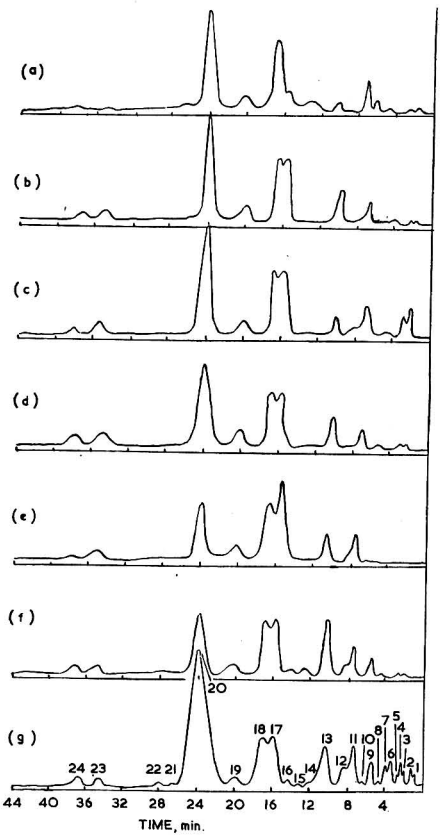

FIG. 4 (left).—*Gas chromatogram of oxygenated fractions of lemon oils from various sources, (a) Australian, (b) Greek, (c) South African, (d) Californian, (e) Nigerian, (f) Southern Rhodesian, (g) Sicilian*

Column temp. 135°
Tentative identification: 13 linalol, 14 linalyl acetate, 18 *n*-decanal, 19 citral b, 20 citral a, 21 geraniol

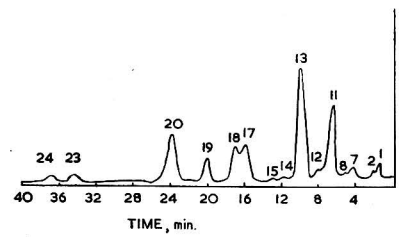

FIG. 5—*Gas chromatogram of oxygenated fraction of adulterated lemon oil* (cf. Fig. 6)

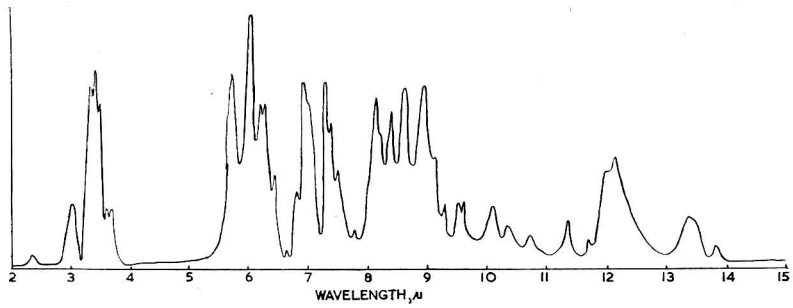

FIG. 6.—*Infra-red spectrum of oxygenated fraction of Sicilian lemon oil*

The most interesting part of the spectrum is the 8–9 μ region ; Fig. 7 shows this portion of a typical spectrum of the oxygenated fraction of a Sicilian lemon oil. Major changes in this region are indicative of a change in the balance of oxygenated compounds, particularly esters. Since these are often of great importance for flavour a correlation might be expected between the flavour of an oil and its spectrum in this region. In fact, a broad correlation does exist in that oils can be divided into three main categories by means of the spectra of their oxygenated fractions. Oils with spectra similar to that in Fig. 7*a* were invariably of good flavour and aroma ; those with the type of spectrum of Fig. 7*c* were always of a peculiar flavour and aroma and were rejected by the taste panel. In an intermediate category must be placed all those oils with the distribution of peaks shown in Fig. 7*b* for these were sometimes accepted and sometimes rejected by the taste panel. Thus, as in the case of the gas chromatogram, there is a considerable difficulty in making a direct comparison between these results and those obtained by organoleptic testing. This is undoubtedly due in part to the fact that the chemical senses, taste and smell, are very much more sensitive than any of the physical methods as yet developed for the examination of flavours.

The 8–9 μ region of the spectrum of the oxygenated fraction is also useful in detecting adulteration. The oils whose spectra are shown in Figs. 7*a*, *b* and *c* were all known genuine lemon oils. In Fig. 7*d* is shown the 8–9 μ region of the spectrum of the oxygenated fraction (Fig. 5) from an oil which had been adulterated. Whereas genuine oils always give strong bands at 8·13, 8·40, 8·70 and 8·91 μ, all these bands are missing in the case of this adulterated oil and have been replaced by a single strong band at 8·25 μ. Oils of this type were invariably rejected by the taste panel.

Useful as the 8–9 μ region of the spectrum is, it is not always possible to decide upon possible sophistication of an oil from examination of this region alone ; the whole spectrum must be examined. In Fig. 8 are shown the spectra of the oxygenated fractions of two lemon oils. The top spectrum shows a peculiar distribution of peaks in the 8–9 μ region (cf. Fig. 7*c*), but from an examination of this part of the spectrum alone the oil might be regarded as genuine.

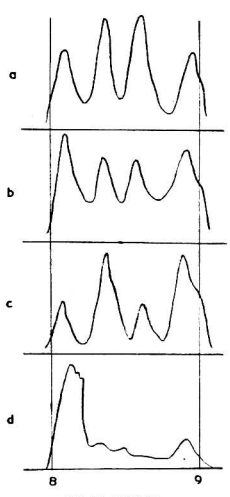

FIG. 7.—*Infra-red spectra, 8–9 μ region, of oxygenated fractions from various lemon oils, (a) acceptable oils, (b) variable oils, (c) unacceptable oils, (d) adulterated oils*

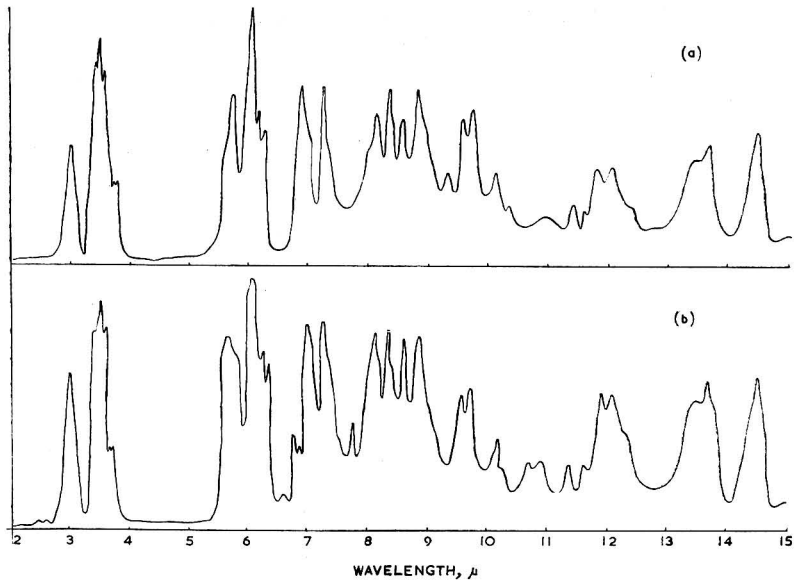

FIG. 8.—*Infra-red spectra of oxygenated fractions from two lemon oils having a different type of adulteration from that of (d)*

However if the remainder of the spectrum is examined (cf. Fig. 6) a number of strong bands in the 13–15 μ region can be seen and it is clear that this oil has a spectrum quite unlike that of a typical lemon oil. This is also true of the spectrum of the other oil. It can be assumed therefore that both the oils whose spectra are shown in Fig. 8 have been adulterated in some way.

In addition to the authenticity of an oil, the infra-red spectrum of the oxygenated fraction of an oil can provide information about the freshness of the oil. When an oil deteriorates, alcoholic compounds are produced as a result of oxidation and citral is lost. Thus, if the heights of the bands at 2·95 μ (OH) and 5·95 μ (C=O aldehyde) are compared, the ratio of the two band heights should give a measure of the deterioration of the oil. It is found that, for a good fresh oil, this ' deterioration index ' is never greater than 0·30 and is usually between 0·15 and 0·20, whilst for a deteriorated oil the value may be greatly in excess of 0·40. It is of interest that the adulterated oils (Fig. 8) would have been rejected by virtue of high deterioration indexes, the oil (*a*) having a value of 0·49 and the other of 0·64. In the same way the adulterated oil with the spectrum shown in Fig. 7*d*, had a deterioration index of 0·42. The high values in these cases are probably due to the nature of the adulterant and, in the samples so far examined, the deterioration index has proved a useful indication of sophistication.

(c) *Percentage of oxygenated materials.*—Most of the lemon oils examined were commercial samples of cold-pressed oil and usually contained 8·5–10% of oxygenated compounds. Two samples of oil were prepared in the laboratory from Sicilian lemons. In one case the peel was grated and the oil was extracted with light petroleum. This oil had a fine strong aroma and contained 18·9% of oxygenated materials. The second oil was expressed and washed out with water. In this case the oil, although of excellent aroma, contained only 12·5% of oxygenated compounds. The actual balance of the constituents of the oxygenated fractions was similar in both cases and similar also to that of genuine commercial samples of oil. This result emphasises strongly the importance of avoiding too great a contact of the oil with water. The volume of water used to wash the oil from the fruit should always be kept to an absolute minimum.

(d) *Sesquiterpene fraction.*—The sesquiterpene fractions (Fig. 9) were separated by chromatography on silica gel of two commercial samples of terpeneless lemon oil. Although this fraction is of importance for the assessment of the value of a terpeneless lemon oil, it appears to be of little value in the case of natural oils. It is, however, undoubtedly of importance in the type of evaluation made by Casu[10] who examined, by the infra-red method, the residual oil obtained by distilling out the terpenes.

II. *Terpeneless lemon oils*

(a) *Percentage of oxygenated compounds.*—The percentages of oxygenated compounds in the 15 terpeneless oils examined fall in the range 70–90%. With flavour and bouquet as the main criteria in assessing the acceptability or otherwise of an oil, it has been found that only very rarely does a good oil contain more than 80% of oxygenated compounds, the best oils containing about this figure. Furthermore, no oil containing 90% of oxygenated compounds was found to be acceptable, whilst those containing only 70% were always acceptable but usually described as having a 'thin' flavour and note. The oils with very high percentages (90%) of oxygenated compounds had unnatural flavours and aromas which were probably produced by excessive or prolonged heating.

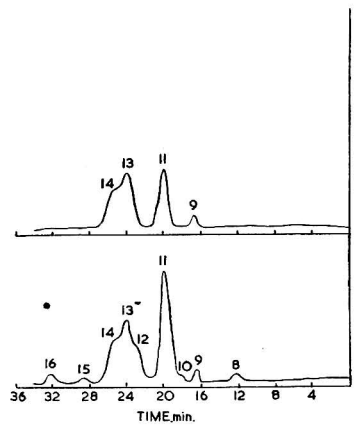

FIG. 9.—*Gas chromatogram of lemon oil sesquiterpenes*
Column temp. 100°

(b) *Infra-red spectrum and deterioration index.*—The infra-red spectrum of the oxygenated fraction, as in the case of natural oils, was of value in deciding upon the authenticity of an oil. In Fig. 10 the spectrum of an adulterated oil (Fig. 10*a*) is compared with that of a typical authentic oil (Fig. 10*b*). Unfortunately only one adulterated terpeneless lemon oil has so far become available but the infra-red spectrum leaves no doubt that it has been sophisticated. For general purposes, as with natural lemon oils, the 8–9 μ region of the spectrum provides a useful pointer to the acceptability of an oil. All acceptable oils have patterns like that shown in Fig. 10*b* whilst a different balance of bands in this region invariably indicates an unacceptable oil.

For a good terpeneless oil the deterioration index should not exceed 0·30 (cf. [13]) but in an unacceptable oil this figure may increase very markedly (Table I). A high index is most probably due to careless distillation (over-heating) or prolonged distillation carried out in an attempt to produce a better terpeneless oil. Fresh oils of good character, containing about 80% of oxygenated compounds, usually have a deterioration index of between 0·15 and 0·20 and one supplier actually obtained an oil with 82·3% of oxygenated materials and a deterioration index of 0·14.

(c) *Gas chromatography.*—The gas chromatograms of the oxygenated fractions are also of use in the detection of adulteration. In Fig. 11 a typical pattern of an acceptable oil (Fig. 11*a*) is compared with that of an adulterated oil (Fig. 11*b*). It is of interest to note that the adulterated oil contains a very large quantity of linalol which is not present in genuine oils.

The chromatograms of the hydrocarbon fractions, on the other hand, are useful in the determination of acceptability of the oil for use in flavouring. In Fig. 12 the gas chromatogram

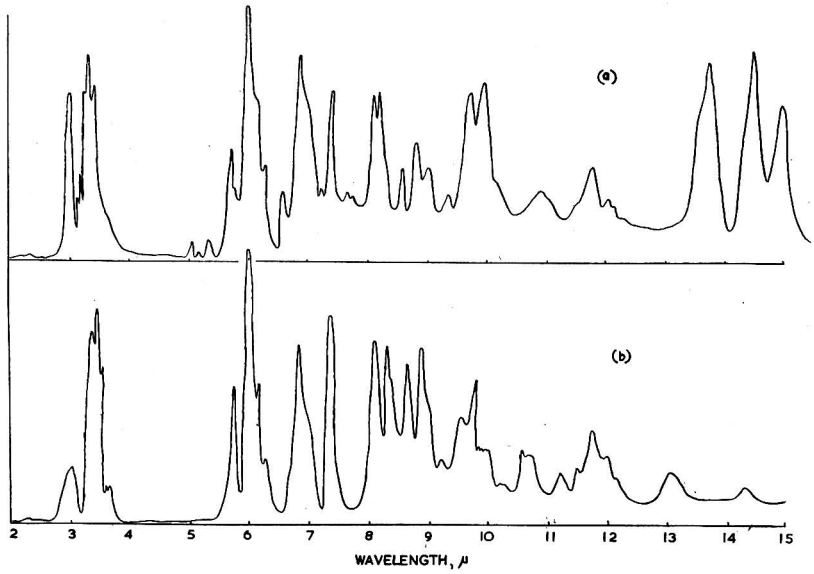

FIG. 10.—*Infra-red spectra of oxygenated fractions of (a) adulterated and (b) authentic terpeneless lemon oils*

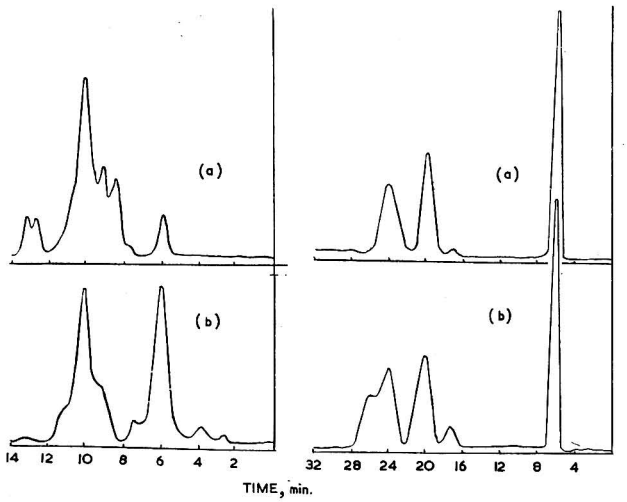

FIG. 11.—*Gas chromatograms of oxygenated fractions of (a) authentic and (b) adulterated terpeneless lemon oils*

Column 25% w/w silicone grease on Celite 545; carrier gas: 30 ml. per min. N_2. Temp. 199°

FIG. 12.—*Gas chromatograms of the hydrocarbon fractions of (a) acceptable and (b) rejected terpeneless lemon oils*

Conditions as in Fig. 2

Table I

Analysis of terpeneless lemon oils

Sample no.	% of oxygenated compounds	Index of deterioration	Whether acceptable as judged by taste panel	Sample no.	% of oxygenated compounds	Index of deterioration	Whether acceptable as judged by taste panel
1	89·1	0·76	No (adulterated)	9	82·7	0·14	Yes (outstanding)
2	90·5	0·39	No	10	74·1	0·23	Yes
3	89·3	0·47	No	11	80·7	0·21	Yes
4	88·2	0·63	No	12	82·7	0·21	Yes
5	80·7	0·39	Doubtful	13	72·9	0·25	Yes
6	71·5	0·28	Yes	14	71·9	0·22	Yes
7	74·5	0·30	Yes	15	75·3	0·21	Yes
8	80·0	0·32	Yes				

of the hydrocarbon from an acceptable oil (Fig. 12*a*) is compared with that of a rejected oil (Fig. 12*b*). It will be seen that both oils contain a high proportion of *d*-limonene together with some sesquiterpenes. In the case of the acceptable oil there are two major sesquiterpenes, whilst in the case of the rejected oil a much more complex pattern is obtained, there being four or five sesquiterpenes. It seems reasonable to suppose that the added complexity of the patterns from unacceptable oil is due to the formation of sesquiterpenes as artefacts during distillation.

Conclusions

From the results obtained it may be concluded that any given sample of natural or terpeneless lemon oil can be evaluated by physical means. The authenticity and degree of deterioration of an oil may be assessed by infra-red and gas chromatographic examination and, although the method does not supersede organoleptic testing, it is of undoubted value as an addition to the classical methods of oil examination.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

MARCH, 1961

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

MARCH, 1961

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Morphology and genesis of some soils containing fragipans in Northern Michigan. N. J. Yassoglou and E. P. Whiteside (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 396—407).—Characteristics of the soils are presented and modes of genesis discussed. A. H. CORNFIELD.

Mineralogy of clay minerals of districts of Altiplano and Palude. G. Gattorta (*Agrochemica*, 1960, **4**, 255—260).—Chemical and physical data and mineralogical classifications are given for the clay fractions of 42 soils. P. S. ARUP.

Clay fractions of a series of Italian soils. O. T. Rotini and G. Lotti (*Agrochimica*, 1960, **4**, 185—198).—Chemical and physical data are given for 70 soils. P. S. ARUP.

Some podsol soils of Alberta. S. Pawluk (*Canad. J. Soil Sci.*, 1960, **40**, 1—14).—The genesis of these soils differs from that usually ascribed to podsols, although they resemble podsols morphologically. Both pH and base saturation are higher. The clay content is lower than that of the parent rock in the A but higher in the B horizon. Illite, montmorillonite–illite, montomorillonite and kaolinite are the main clay minerals in the A and C horizons, whilst in the B horizon chlorite predominates. M. LONG.

Influence of forest stand and ground cover vegetation on soil formation. J. Låg (*Agrochimica*, 1959, **4**, 72—77).—Soil formation in Norwegian forests is more closely correlated with ground cover than with the forest stand. Without prejudice as to cause and effect, it appears that brown earths occur more abundantly than podsols under grasses and herbs than under *Vaccinium myrtilis*. Podsols occur most frequently under *Vaccinium* spp., *Calluna vulgaris*, lichens or Sphagnum moss. P. S. ARUP.

Clays of soils of Guadalquivir valley. S. G. Garcia (*Agrochimica*, 1959, **4**, 49—71).—A soil survey is presented with detailed analyses of the clay fractions of 33 profiles. Values for the exchange capacity, glycol absorption, and K content furnish an index for the relative proportions of illite, montmorillonite and kaolinite which agrees well with results obtained by chemical, *X*-ray and differential thermal analysis. (32 references.) P. S. ARUP.

Clays of trachytic formation of Tuscany. L. Carloni and G. Lotti (*Agrochimica*, 1960, **4**, 110—118).—Analyses of the soils (11) are tabulated. Differential thermal analysis and *X*-ray spectrography of the clay fractions reveal a vermiculitic material as the chief constituent of the trachytic rocks of the " selagitic " type, and illite and kaolinite as the chief transformation products of the " tuscanites." P. S. ARUP.

Intersalation as technique for differentiation of kaolinite from chloritic minerals by X-ray diffraction. R. W. Andrew, M. L. Jackson and K. Wada (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 422—424).—The method is described. A. H. CORNFIELD.

[A] Field studies and [B] laboratory studies and genesis of a clay-enriched horizon in the lowest part of the solum of some Brunizem and Gray-Brown Podsolic soils in Illinois. L. J. Bartelli and R. T. Odell (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 388—390, 390—395).—[A] Macromorphological, catenary and drift-texture relationships indicated that the β horizon (clay-enriched layer) occurring below the B_2 horizon were more strongly developed in Gray-Brown Podsolic profiles than in associate Brunizems.

[B] Chemical and physical data indicated that the clay-enriched horizon is a zone into which fine clay and org. matter, primarily from the horizons above, are moving and being deposited in an oriented pattern. Clay migration and differential moisture flow at the boundary of the coarser-textured substrata and finer-textured subsoil are primarily responsible for the development of this horizon. A. H. CORNFIELD.

Janert's apparatus for the measurement of air permeability in the field. U. Babel (*Z. PflErnähr. Düng.*, 1960, **91**, 14—21).—The apparatus proposed by Janert (*Bodenkundliches Praktikum*, Berlin, 1953) fails for soils of low permeability, but substitution of smaller jets removes the lack of sensitivity. The rate of airflow (given as time for 1 l. to pass through the soil) is proposed in place of the original value. M. LONG.

Formation and breakdown of ^{60}Co-tagged water-stable soil aggregates in a Norton silt loam soil. S. J. Toth and R. B. Alderfer (*Soil Sci.*, 1960, **90**, 232—238).—Water-stable aggregates $\geq$2—1 mm. isolated from a surface soil were tagged with ^{60}Co and incubated at 28°. At 4-week intervals an aggregate and ^{60}Co analyses were made. Greenhouse tests with the same soil mixed with tagged soil were made with bluegrass. During the first 4 weeks of incubation there was a breakdown of 2—1 mm. aggregates (8% loss) and smaller losses in the 1—0·5 mm. fractions; the <0·5 mm. fraction increased. After 4 weeks there was a gradual build up of aggregates>1·0 mm. and 1·0—0·5 mm. with corresponding decreases in the smaller sizes, but the initial concn. of the 2—1·0 mm. sizes was not regained. After 4 weeks only 40% of the ^{60}Co activity remained in the aggregates >1 mm. and at the end of the period fragments of the initially tagged aggregates could be found in the aggregates of all sizes. The percent of ^{60}Co removed by bluegrass from the various treatments ranged between 0·05 and 0·06. Isotope retained by the smaller aggregates was less available to the plant than that of the larger aggregates. T. G. MORRIS.

Approximate equations for fluid flow into buried drains. B. S. Vimoke and G. S. Taylor (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 418—419).—Approx. equations were derived to calculate the rates of fluid flow into buried drains which are equally spaced over an impermeable layer. A. H. CORNFIELD.

Soil and water losses and infiltration rates on Ida silt loam as influenced by cropping systems, tillage practices and rainfall characteristics. W. C. Moldenhauer and W. H. Wischmeier (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 409—413).—Maize planted up- and down-slope on a coarse loess soil with 12% slope (maize–oats rotation with sweet clover catch crop) resulted in an average annual loss of 25 tons of soil per acre over 11 years. Contour planting reduced the loss to 10 tons and listing on the contour to 5 tons per acre per annum. Extent of run-off was reduced to a smaller extent than were soil losses by the latter two treatments. A ton of soil per acre per annum was lost from maize contour listed in a maize–oats–meadow–meadow rotation. Contour planting was not effective in reducing soil losses with storms of high erosion intensity. Soil aggregate stability was less under oats than under maize. Initial infiltration rates with contour listing were much higher than with the other cultivation systems, but all rates tended towards a similar value after some time. A. H. CORNFIELD.

Moisture storage on fallowed wheatland in the Great Plains. O. R. Mathews and T. J. Army (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 414—418).—Long-term data from 25 locations throughout the Great Plains showed that the average quantity of water stored during the fallow period of a crop–fallow sequence was 3·96 in. (23·6% of the rainfall). On annually cropped soil, 2·02 in. (23·6% of the fall) was stored during the fallow period between crops. In general, storage efficiency decreased from the northern to the southern Great Plains. A. H. CORNFIELD.

Effect of irrigation interval on peak flow requirements in sprinkler irrigated orchards. J. C. Wilcox (*Canad. J. Soil Sci.*, 1960, **40**, 99—104).—Water wastage is greater on coarse- than on fine-textured soils, since growers tend to apply more water than the soil can hold. M. LONG.

Rate of soil drainage following an irrigation. II. Effects on determination of rate of consumptive use. J. C. Wilcox (*Canad. J. Soil Sci.*, 1960, **40**, 15—27).—Water consumption cannot be distinguished from drainage immediately after an irrigation, at which stage water taken up by plants would normally be lost by drainage in an uncropped plot. After this period rate of consumption decreases, progressively so with depth. The difference between moisture losses from an uncropped plot and from a cropped plot gives too low a measure of consumption, whereas the difference in moisture contents gives too high a measure. M. LONG.

Soil compaction integrator. D. N. Ram and P. J. Zwerman (*Agron. J.*, 1960, **52**, 484).—A convenient method of measuring soil roughness due to compaction is described. A. H. CORNFIELD.

Influence of management systems and cover crops on soil physical conditions. D. N. Ram and P. J. Zwerman (*Agron. J.*, 1960, **52**, 473—476).—A rye-grass cover crop increased water-stable aggregation and soil-moisture content and reduced bulk density on a cropped silt loam over 5 years. Wood chips, ploughed under or top-dressed,

in addition to the cover crop, further increased aggregation and water content and reduced bulk density. Seasonal variation of the measured factors was less where cover crops and wood chips had been used than on control plots. Heavy inorg. fertilisation tended to decrease the water-stability of the large aggregates and increase the proportion of stable micro-aggregates. A. H. CORNFIELD.

Characteristics of the ploughed layer of flooded rice fields. S. Aomine and Y. Shiga (*Soil & Plant Fd*, 1959, **5**, 64—73).—Structural layers in flooded ploughed paddy soils are described. Relationships with mechanical composition, porosity and redox potential are examined. A. G. POLLARD.

Conservation of soil moisture from autumn and winter precipitation. W. J. Staple, J. J. Lehane and A. Wenhardt (*Canad. J. Soil Sci.*, 1960, **40**, 80—88).—Fields in stubble conserve winter precipitation better than those in fallow. Disking to remove weeds causes a reduction in conservation due to smaller accumulation of snow. Each inch of soil moisture present in the autumn reduces conservation by approx. 2 in. M. LONG.

Evaluation of winter cover crops for the control of splash erosion. D. N. Ram, M. T. Vittum and P. J. Zwerman (*Agron. J.*, 1960, **52**, 479—482).—Domestic rye-grass (*Lolium* spp.) as a winter cover crop was more effective than lucerne, rye or brome grass in controlling splash erosion. The effectiveness of the materials was related to the product of plant height and density of stand. A. H. CORNFIELD.

Sediment ratio studies in Mississippi. I. Preliminary report. J. A. Spraberry, R. Woodburn and J. R. McHenry (*Agron. J.*, 1960, **52**, 434—436).—An evaluation of the erosion susceptibility of watersheds in the loessial-capped hills of northern Mississippi was made using two equations for the computation of gross erosion. The two equations gave similar results although the computed gross erosion varied greatly among the watersheds. The sediment delivery % computed for the watersheds also varied greatly, but the differences were not correlated with size or location of the watersheds. A. H. CORNFIELD.

Soil drying and soil fertility. H. F. Birch (*Trop. Agriculture, Trin.*, 1960, **37**, 3—10).—A review and discussion of published data. (15 references.) P. S. ARUP.

Effects on different cropping systems and of a soil conditioner (VAMA) on some soil physical properties and on growth of tomatoes. J. J. Doyle and F. G. Hamlyn (*Canad. J. Soil Sci.*, 1960, **40**, 89—98).—Continuous potato cropping reduced the % of water-stable aggregates, porosity, soil org. matter content, available water capacity and cation-exchange capacity. VAMA increased the first-named two in cultivated soils but not in permanent grassland, increased cation-exchange capacity, decreased available water capacity but increased tomato yields. M. LONG.

Relative magnitude of evaporation and transpiration. D. B. Peters (*Agron. J.*, 1960, **52**, 536—538).—In areas where frequent summer showers occur, up to 50% of the total water loss during the reason was accounted for by evaporation from the soil surface. In sow crops sufficient energy reaches the ground surface to account for the observed soil-water evaporation. Transpiration from a particular crop varied within fairly narrow limits. A. H. CORNFIELD.

Apparatus for controlling soil temperatures. D. J. Cooper, K. F. Neilson, J. W. White and W. Kalbfleisch (*Canad. J. Soil Sci.*, 1960, **40**, 105—107).—Apparatus is described by which soil temp. is controlled independently of foliage temp. ; it consists of pots of soil held in a specially constructed waterbath. M. LONG.

Effect of plastic mulch on the micro-climate and plant development. L. J. Fritschen and R. H. Shaw (*Iowa St. J. Sci.*, 1960, **35**, 59—71).—At the depths measured the soil temp. in plastic covered plots were higher and less subject to fluctuation than those of the controls ; greatest temp. differences occurred at night. Maize grown in plastic covered plots produced 3—4 rings of brace roots while that in natural plots produced 1—2 rings, but a better root system both horizontally and vertically. Maize in natural plots reached a greater height but growth was less rapid. (12 references.) E. G. BRICKELL.

Effects of electrolyte imbibition upon cation-exchange behaviour of soils. G. W. Thomas (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 329—332).—Effluent curves from Ca-saturated soils were studied. Marked deviations from ideal chromatographic behaviour was attributed to hold-up of anions by soils. The effects on cation-exchange reactions are discussed. A. H. CORNFIELD.

Cation-exchange hysteresis in clay minerals. A. A. Tabikh, I. Barshad and R. Overstreet (*Soil Sci.*, 1960, **90**, 219—226).—From suspensions of three fully hydrated bentonites 1μ particles were obtained by sedimentation and saturated with H^+ by passage through an ion-exchange column. Homionic suspensions of different cations were prepared by adding hydroxides to the H^+ suspensions. Samples were dried either at 60°, partially at 60° and then at room temp., at 115°, or in a vac. at 15°. Exchange experiments were made by shaking the suspension with the chloride of the appropriate cation and after 48 h. determining the concn. of the cation in the supernatant. In the undried homionic suspensions the exchange reactions were completely reversible, i.e., there was no hysteresis. With clays previously dried and rehydrated for 48 h. before use it was impossible to obtain repeatable results. Monovalent–divalent reactions were particularly non-uniform. If a clay after being dried was recycled through the H^+ column and then resaturated with the same cation, exchange reactions showed no hysteresis. T. G. MORRIS.

Unexpected reaction between aluminium-clay or aluminium-soil and calcium chloride. N. T. Coleman, J. L. Ragland and D. Craig (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 419—420).—When acid soils or clays containing exchangeable Al were treated with $CaCl_2$ and subjected to alternate wetting and drying or to continuous heating (80°) Cl was lost from the system (probably as HCl) exchangeable Al was displaced by Ca and the Al was probably converted to Al_2O_3,yH_2O. A. H. CORNFIELD.

Some characteristics of a model Donnan system. K. L. Babcock (*Soil Sci.*, 1960, **90**, 245—252).—The Donnan theory is discussed in relation to the behaviour of model suspension systems. Its applicability to soil systems is considered. T. G. MORRIS.

Nitrogen movements and transformations in soils as evaluated by a lysimeter study utilising isotopic nitrogen. L. D. Owens (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 372—376).—Studies with cropped lysimeters and eight soil types were carried out to determine the losses of N from winter application of ^{15}N-labelled $(NH_4)_2SO_4$ (120 lb. of N per acre). After 2 years an average of 33 ± 6% of the applied N could not be accounted for in the leachates, soils or crops and was probably lost by denitrification (since the soils were acid, losses by volatisation as NH_3 were probably negligible). Varying moisture application rate (12—24 in.) during Feb.–May had no effect on the loss of N. Losses of N by leaching increased with level of water applied. After 2 years an average of 38% of the applied fertiliser-N was still present in the soils. A. H. CORNFIELD.

Nitrate-supplying power of eight grassland soils of the Kamploops, B.C., area and correlation with range forage production. J. D. Beaton, R. A. J. Warren and W. A. Hubbard (*Canad. J. Soil Sci.*, 1960, **40**, 63—70).—Incubation tests, as a measure of NO_3^- supplying power of soil, indicate that these soils produce insufficient NO_3^- for optimum growth. Correlations of accumulative total of NO_3^- with C/N ratio, total N or org. matter are not highly significant, whilst that with forage is poor. M. LONG.

Determination of the mineralisable nitrogen in the soil. C. Nigro (*Ann. Staz. chim.-agr., Roma*, 1959, iii, 154, 13 pp.).—Preliminary trials are reported of a proposed method of evaluating org. N fertilisers. Soils were incubated for 84 days at 30° without org. additions and NO_3^--N was determined at weekly intervals. Three of the soil samples showed more or less regular increases in NO_3^--N throughout the period, but a fourth gave irregular results. Only soils giving approx. rectilinear development of NO_3^--N in preliminary trials should be used for the incubation test of org. fertilisers. (43 references.) E. C. APLING.

Potassium-supplying power of some British soils. P. W. Arnold (*Nature, Lond.*, 1960, **187**, 436—437).—British soils with similar K content vary greatly in their ability to release non-exchangeable K. A study was made of soils from 19 locations and these were listed in order of ability to release K. A description of the substratum is given in each case. C. A. SLATER.

Forms of soil-potassium with special reference to exchangeable, fixed and fixable potassium. A. Malquori and L. Radaelli (*Agrochimica*, 1959, **4**, 25—48).—Relevant analyses are given for 18 Italian soils of different types ; the effects of the constituent minerals on K-availability are examined. Increases of 3·5—163% in the exchangeable K are observed for the majority of the soils after heating at 80° during 8 days. (14 references.) P. S. ARUP.

Effect of drying on exchangeable potassium of Ontario soils and the relation of exchangeable potassium to crop yield. B. C. Matthews and C. G. Sherrell (*Canad. J. Soil Sci.*, 1960, **40**, 35—41).—Exchangeable K extracted by 0·1N-NH_4 acetate decreases on drying the soil if the % K saturation in the cation-exchange capacity exceeds 1·11 ± 0·12 and *vice versa*. The degree of correlation of exchangeable K at different moisture contents with yields of maize is unaffected by drying, but with potatoes the correlation coeff. is highest for oven-dried soils. M. LONG.

Apparent mobility of potassium and chloride ions in soil and clay pastes. J. Letey and A. Klute (*Soil Sci.*, 1960, **90**, 259—265).—

The apparent mobilities of K^+ and Cl^- were determined at various concn. by calculation from transference no. (*idem., ibid.*, 1960, **90,** 121) and specific conductance (Low, *Proc. Soil Sci. Soc. Amer.*, 1958, **22,** 395). A method for measuring ionic mobilities is described, a plug of soil being confined between two compartments in each of which aq. KCl is placed and that on one side is tagged with ^{36}Cl. Data obtained showed that with increasing salt concn. the apparent mobility of K^+ increased and that of Cl^- decreased. T. G. MORRIS.

Phosphorus fractions in a soil sampled at different depths and the effect of lime and fertiliser on oats and clover in a greenhouse test. H. A. Hamilton and J. R. Lessard (*Canad. J. Soil Sci.*, 1960, **40,** 71—79).—The subsoil is more saturated with bases than is the top. The inorg. contribution to the total P increases and that of org. P decreases with soil depth. Oats grown in pots give higher yields on the top layers; clover yields were highest on lower layers. Overliming may reduce clover yields when P is not applied, whilst oat yields are unaffected. M. LONG.

Modifications in Chang and Jackson's procedure for fractionating soil phosphorus. Aung Khin and G. W. Lepper (*Agrochimica*, 1960, **4,** 246—254).—The procedure is critically examined, and several improvements are suggested. There remain, however, difficulties in the satisfactory separation of the phosphates of Al and Fe, and of the Ca phosphates from the resistant (" reductant sol.") Fe phosphate fraction. Analyses are given of the phosphates of three Australian soils. P. S. ARUP.

Influence of temperature and moisture on soil phosphorus. I. Effect on soil phosphorus fractions. A. R. Mack and S. A. Barber (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 381—385).—Two silt loam soils (pH ~6·5) incubated at −20·5° for 9 months released more P on leaching with water than when incubated at 2·7° prior to leaching. More P was released at a leaching temp. of 32° than that of 16°. The amount of P released in successive aliquots of leachate was correlated with a decrease in acid-sol. P and an increase in alkali-sol. P (" Fe phosphate ") in the soil after leaching. A. H. CORNFIELD.

Influence of neutral salts on the phosphate ion concentration in soil solution. J. S. Clark and M. Peech (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 346—348).—The constancy of the ion activity products involving Ca^{2+}, H^+, K^+ and $H_2PO_4^-$, observed in soil and clay suspensions upon increasing the electrolyte concn. in the suspension, may be explained either by assuming that $H_2PO_4^-$ is co-adsorbed with exchangeable cations on the surface of soil particles or by postulating the existence in the soil of any cryst. PO_4^{3-} compound having a definite solubility product. A. H. CORNFIELD.

Movement of phosphate ions in soil particles; use of ^{32}P. I. Eynard and C. Tendille (*Agrochimica*, 1960, **4,** 156—166).—Diffusion of $^{32}PO_4^{3-}$ into the interior of soil particles is probably slower than that of $^{31}PO_4^{3-}$. Evidence is advanced that native soil PO_4^{3-} is gradually transformed into an exchangeable form and that this is favoured by alternate wetting and drying. A. G. POLLARD.

Effect of exchangeable calcium on the retention of phosphorus by clay fractions of soils of the Memphis catena. D. V. Calvert, H. F. Massey and W. A. Seay (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 333—336).—The effect of Ca saturation on the P-retaining properties of the (initially) acid-washed clay fraction of five soils of a catena varying in efficiency of drainage was studied. The amount of P retained, against extraction with water, of all the freshly washed clays increased with extent of Ca-saturation. When stored after acid-washing P retention decreased with extent of Ca-saturation. Storage of the base-free clays also resulted in increased P retention. This was attributed to release of Al during storage. A. H. CORNFIELD.

Fixation of phosphorus in soil. I. Penetrability of phosphates in relation to typical fertilising chemicals. II. Influence of some salts on penetrability of phosphate ions of superphosphate. U. Pallotta and C. Antoniani (*Agrochimica*, 1960, **4,** 167—184, 236—245).—I. The penetrabilities of several chemicals and commercial fertilisers are compared in laboratory experiments with three soils of different textures. The penetrability of $Ca_3H_2(PO_4)_2$ is much lower than that of $(NH_4)_2HPO_4$; that of the K^+ ion is greater than that of PO_4^{3-}. The penetrability of NH_4NO_3 is very high in light soil, and decreases somewhat with the increasing clay content of the soil. (40 references.)

II. In experiments similar to those in Part I, the addition of various salts (10) in comparatively high concn. has no great effect on the percolation of the phosphate ions. Small differences are noted in the effect of the various anions and cations in this respect. (15 references.) P. S. ARUP.

Phosphate adsorption by charcoal. J. D. Beaton, H. B. Peterson and N. Bauer (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 340—346).—Adsorption isotherm data at 25°, 35° and 45° for aq. PO_4^{3-} equilibrated with synthetic and natural charcoals were interpreted thermodynamically, indicating the adsorption mechanism probably involves dehydration of ions and/or sites. Probably the presence of charcoals on burned-over soils may have an adverse effect on PO_4^{3-} availability, but only under extreme conditions. A. H. CORNFIELD.

Comparison of phosphate sources for lucerne on a calcareous soil. C. O. Stanberry, W. H. Fuller and N. R. Crawford (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 364—366).—Yields of and P uptake by lucerne over 3 years on a calcareous fine sand showed that $CaHPO_4$ (**I**) was as effective a source of P as were concentrated superphosphate and H_3PO_4. Uptake of applied P was particularly high where **I** was mixed with the soil. Application of **I** as fine particles was more effective than as coarser particles in the first year, but yields over the 3 years were similar with both grades. Application of the whole of fertiliser initially was as effective as splitting the dressings over 3 years. A. H. CORNFIELD.

Soil phosphorus fractions as affected by annual application of bonemeal and their influence on yield of paddy. S. Digar and A. K. Mandal (*Soil Sci.*, 1960, **90,** 227—231).—Paddy was grown for 10 years on a lowland clay treated annually with 0—60 lb. of P_2O_5 per acre. At the 10th harvest P content of the plants was determined and the P components of the soil were fractionated (0·5N acetic acid, then 0·25N-NaOH and finally 2N-H_2SO_4). After 10 years' annual treatment at all P levels the acetic acid- and alkali sol.-org. P did not accumulate in the soil; the inorg. alkali sol.- and the H_2SO_4-sol. phosphates did accumulate. A balance sheet of phosphate indicated that some may have been lost from the top 6 in. of soil, or become insol. in all the extractants. T. G. MORRIS.

Antagonism between phosphorus and zinc in presence of excessive phosphate manuring, and probable effect of sodium. S. Fortini and V. Morani (*Agrochimica*, 1960, **4,** 209—215).—The presence of >330 p.p.m. of soil-P_2O_5 (as added mono-Ca phosphate) depresses Zn-uptake by tomato plants and (especially) by spinach growing in soils with low Zn content. Increases in soil-Zn (by ~20 p.p.m.) restore Zn-uptake to normal levels and also cause very marked increases in P-uptake, especially at the higher levels of P-manuring. The additional presence in the soil of Na in excess (1330 p.p.m.) has no effect on the Zn-uptake, but decreases the effect of Zn on the P-uptake. P. S. ARUP.

Effect of phosphorus concentration on the absorption of arsenate by oats from nutrient solutions. C. B. Rumburg, R. E. Engel and W. E. Meggit (*Agron. J.*, 1960, **52,** 452—453).—The uptake of As (from Na_2HAsO_4) by oats from nutrient solutions decreased with increasing P concn. of the solutions (PO_4^{3-}-P 1—62 p.p.m.). A. H. CORNFIELD.

Soil solution extractions at tenth-bar moisture percentages. F. M. Eaton, R. B. Harding and T. J. Ganje (*Soil Sci.*, 1960, **90,** 253—258). —Soil is wetted to tenth-bar moisture with water and after equilibration the soil solution is displaced by CO_2 under pressure (apparatus described). Corresponding saturation extracts of the soils were also obtained for comparison, Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+, HCO_3^-, SO_4^{2-} and NO_3^- being determined. In all the soils the ion content of the saturation extract was less (30—100%) than that of the tenth-bar solutions except in the case of NH_4^+, the divergence being wider than that between moisture percentages. T. G. MORRIS.

Determining free iron [oxide] in paddy soils. T. Asami and K. Kumada (*Soil & Plant Fd*, 1959, **5,** 141—146).—The hydrosulphite method is unsatisfactory owing to the possible formation of ppt. of FeS during the extraction. In a modified procedure formation of FeS is prevented by addition of EDTA to the hydrosulphite solution. Appropriate details of procedure are presented. A. G. POLLARD.

Estimation of free iron oxides in soils. V. J. Kilmer (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 420—421).—Free Fe oxides are extracted by shaking 4 g. of soil with 4 g. of $Na_2S_2O_4$ + 75 ml. water for 16 h. The pH of the mixture is then adjusted to 3·5—4·0 with HCl. An aliquot of the solution is separated by filtration or centrifugation and after appropriate preliminary treatment (details given) the Fe is determined volumetrically with standard $K_2Cr_2O_7$. A. H. CORNFIELD.

Release of iron oxide in red-brown soil formation from the weathering of limestone. II. Rôle of grass extract. D. H. Khan (*J. Sci. Fd Agric.*, 1960, **11,** 632—635; cf. J.S.F.A. Abstr., 1960, i, 4).—An aq. grass extract in contact with powdered limestone rock under controlled laboratory conditions at room temp. mobilised Fe from the limestone in amount ~100 times that released by carbonic acid leaching. The release of Fe_2O_3 resulting in the formation of red-brown soil is probably a function of aq. extracts of grass and other plant materials with rain water plus carbonic acid leaching due to appreciable rainfall over limestone districts. E. M. J.

Gley formation in soils. I. Mechanism of formation of active ferrous iron in soils. K. Yamanaka and S. Motomura (*Soil & Plant Fd*, 1959, **5**, 134—140).—Incubation of water-logged soils with glucose lowered the pH rapidly to 3—4, accompanied by the production of Fe^{2+} and the formation of a bluish-grey colour. In presence of inhibitory substances (NaN_3, $HgCl_2$, iodoacetic acid) no Fe^{2+} or blue colour was formed. Gley formation is dependent on the presence of Fe-reducing bacteria. A. G. POLLARD.

Determination of the complexing capacity of aqueous plant extracts for copper and iron. S. Fortini and M. Tarantola (*Ann. Staz. chim.-agr., Roma*, 1959, iii, 155, 17 pp.).—The method measures the effect of the extracts in catalysing the oxidation of ascorbic acid by Cu and Fe. Extracts prepared from the leaves of *Vicia faba minor, Trifolium incarnatum, Hedysarum coronarium, Brassica napus* and *Zea mays* all reduced the catalytic effect of Fe and Cu. Max. reduction was found at pH 5·3, but complexing power is still evident between pH 7 and 8, and may explain the beneficial effects of green manure in calcareous and alkaline soils. (12 references.) E. C. APLING.

States of copper in various soils and their effects on the cultures. I. Serial determination of active copper in soil. A. Baroccio and A. Saponaro (*Ann. Staz. chim.-agr., Roma*, 1959, iii, 157, 15 pp.).—In the proposed method Cu is extracted by shaking the soil sample with 0·05M-NH_4-EDTA. The extract is evaporated to dryness, ashed, dissolved in AcOH and the Cu determined colorimetrically with rubeanic acid. Results obtained are compared with those of the *Aspergillus niger* method and the AcOH method. The method is claimed to be speedy and reproducible. (20 references.) E. C. APLING.

Chemical extraction and crop removal of manganese from air-dried and moist soils. J. K. Hammes and K. C. Berger (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 361—364).—The uptake of Mn by oats from 20 different soils (kept moist prior to use) in pot tests was significantly correlated ($r = 0{\cdot}848$) with Mn extracted from moist soils by 0·1N-H_3PO_4. Significant correlations were also obtained by extracting air-dried soils with 0·1N-H_3PO_4, N-HNO_3 or 0·2% quinol in N-NH_4OAc. Air-drying soils prior to cropping with oats generally increased the uptake of Mn compared with soils previously kept moist. Fractionation of soil-Mn into weakly acid-sol., organically complexed and easily reducible oxides of Mn indicated that the release of Mn on air-drying probably stems from oxidation of organically complexed Mn. A. H. CORNFIELD.

Manganese deficiency in oats and correlation of plant manganese with various soils tests. J. K. Hammes and K. C. Berger (*Soil Sci.*, 1960, **90**, 239—244).—Field trials of Mn foliar sprays and soil treatments showed that 30 p.p.m. or more of Mn in oats at the boot stage is sufficient for normal growth, whilst less than 15 p.p.m. causes severe deficiency. On air-dry samples of soil four methods of extraction tested gave highly significant correlations with plant-Mn content, the highest being with the 0·05M-EDTA procedure. Use of moist soils gave lower correlation coeff. In general 60—100% more Mn was extracted from air-dry than from moist soils. Subsoils were deficient in Mn. The deficiency was corrected with one foliar spray treatment with $MnSO_4$. Broadcast treatment of the soil was not efficient but if $MnSO_4$ was applied with a fertiliser carrier the deficiency was corrected. T. G. MORRIS.

Effect of soil type on mobility of zinc in the soil and on its availability from zinc sulphate to tung. H. L. Barrows, M. S. Neff and N. Gammon, jun. (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 367—372).—The rate of movement of Zn in soils from surface-applied $ZnSO_4$ was not related to any one chemical or physical property. The greatest movement occurred in a fine sand low in org. matter, extractable PO_4^{3-} and clay and was sufficiently high to be toxic to 1-year-old tung trees. Zn movement was less in fine sandy loams and even less in a loamy fine sand. With the last soil little Zn was absorbed by the trees from surface application of $ZnSO_4$. This was probably due to the high Zn-fixing capacity of the degraded vermiculite present in this soil. A. H. CORNFIELD.

Boron status of "ferrettised" soils of Agro-Cromonese-Grandisco (West Friuli). R. Candussio (*Agrochimica*, 1959, **4**, 78—96).—Coeff. of correlation between the available B in these soils (70% of which are B-deficient) and the following data are: for the B content of lucerne leaves grown on the soil 0·998, for org. matter 0·55, for total soil-B 0·35 and for clay content of soil −0·3. The ratio Ca/B reaches 8—9 in lucerne leaves grown on highly deficient soils. P. S. ARUP.

Availability of sulphur to rice plants in submerged and upland soil. D. C. Nearpass and F. E. Clark (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 385—387).—Yields of and uptake of S by rice were less when the plants were grown under submerged than under non-submerged (upland) conditions in pot tests with six soils. The effect of submergence in reducing availability of S to the plant occurred with both native S and applied SO_4^{2-}. Addition of org. matter (ground rice plant tissue) to flooded cultures depressed the growth of rice plants. This growth depression was overcome by addition of SO_4^{2-} or by submerging the org. matter-treated soil for some time prior to planting. A. H. CORNFIELD.

Determination of available sulphur in soils. V. J. Kilmer and D. C. Nearpass (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 337—340).—"Available" soil-S was extracted by 0·5M-$NaHCO_3$ (pH 8·5) (10 g. of soil + 40 ml. of extractant; 1 h. shaking) and determined (SO_4^{2-} + org. S) in the extract by the Johnson–Nishita method (*Analyt. Chem.*, 1952, **24**, 736). The amounts of S extracted from 30 surface soils by this reagent were highly correlated with S uptake by plants in a greenhouse test using the isotopic dilution technique. A. H. CORNFIELD.

Determination of carbon in soils. J. Kosaka, C. Honda and A. Iseki (*Soil & Plant Fd*, 1959, **5**, 77—83).—Apparatus is described for carrying out the wet combustion ($K_2Cr_2O_7$–H_3PO_4) method, CO_2 being absorbed in soda-lime. Carbonate-CO_2 is removed by preliminary boiling with 5% H_2SO_4 + 5% $FeSO_4$. Cl_2 from soil chlorides is trapped in solid KI. Org. S compounds and sulphides did not greatly affect the results obtained. (36 references.) A. G. POLLARD.

Chemical, physical and biological properties of soil organic matter. H. H. Johnston (*Dissert. Abstr.*, 1960, **20**, 4523).—Assimilation of N by soil micro-organisms during the early stages of decomposition of org. material was reflected in reduced uptake of N by wheat, increased microbial no. and CO_2 output, and decreased mineralisation of N. Acid-hydrolysable amino-N increased. Subsequent mineralisation of microbial N was reflected in increased uptake of N by wheat, declining microbial no. and CO_2 output, and narrowing C/N ratio in the soil. Release of microbial N occurred earlier with more readily decomposable residues, such as maize and wheat straws, than with sawdust. As decomposition progressed, there was an increase in non-acid-hydrolysable N, apparently consisting of oxidative complexes of N compounds with lignins. Soils with high levels of non-acid-hydrolysable N released N to wheat faster than did soils with low levels. M. D. ANDERSON.

Components o soil humic acids. VII. Determination of distribution of components. T. Hayashi and T. Nagai (*Soil & Plant Fd*, 1960, **5**, 153—160).—Fractionation of humic acid of A and B types (*idem, J. Sci. Soil Manure, Japan*, 1953, **24**, 212) by adsorption on active Al_2O_3 followed by paper electrophoresis is described. Data for various soil types are presented. A. G. POLLARD.

Preparation and purification of tritium-labelled grey and brown humic acid preparations and tritium purpurogallin. H. W. Scharpenseel (*Z. PflErnähr. Düng.*, 1960, **91**, 131—146).—Details of the processes are given. The two forms are interconvertible under suitable conditions favouring one or the other. Purpurogallin appears as an end-product in humic acid decomposition as well as being a precursor in humic acid synthesis. M. LONG.

Complex formation between soil humus and polyvalent cations. K. Kawaguchi and K. Kyuma (*Soil & Plant Fd*, 1959, **5**, 54—63).—Both humic (**I**) and fulvic (**II**) acids form complexes (probably chelates) with Al^{3+} and Fe^{3+} but not with Ca^{2+}. The chelates of **I** are generally precipitated and are partly or completely hydrolysed in alkaline media. The chelates of **II** are generally water-sol. The bearing of these observations on certain processes of soil formation is discussed. A. G. POLLARD.

Organo-mineral colloidal complexes of paddy soil. IV. Humus associated with G1 colloidal complexes in high-yielding paddy soils. H. Hashimoto and T. Harada. **V. Clay minerals contained in the G1 colloidal complexes of the high-yielding paddy soils.** H. Hashimoto, T. Harada and T. Yumoto (*Soil & Plant Fd*, 1959, **5**, 49—53, 114—119).—IV. Adjacent high- and low-yielding paddy soils showed no consistent differences in the total humus content of the G1 complex, in the readily-sol. humus or in the non-readily sol. brown or grey humic acids.

V. No outstanding differences in clay minerals between high- and low-yielding soils were apparent. A. G. POLLARD.

Influence of organic matter content and plant roots on the leaching of cations from paddy soils. T. Kiuchi and S. Omukai (*Soil & Plant Fd*, 1959, **5**, 108—113).—Leaching of NH_4^+ and K^+ was greater in soils having the higher org. matter contents. Losses of Ca and Mg were mainly influenced by the total amounts of these elements present in the soil and to smaller extents by crop growth and by drying-out of the soil. Leaching of Fe and Mn was increased by the growth of plants. A. G. POLLARD.

Significance of non-metallic oxides in organic reaction-cycle of soil, with special reference to catalytic influence of silicic acid on synthesis and decomposition of humic acid. F. Scheffer and W. Kroll (*Agro-*

chimica, 1960, **4**, 97—109).—The recent literature of the subject is reviewed. The synthesis of humic acid from quinol in a suspension of ground quartz can be greatly accelerated by rapid stirring, giving increased access to atm. O_2 and friction amongst the quartz particles; the pH decreases from 6 to 3 within 24 h. Decomposition of humic acid, involving decarboxylation and oxidation, occurs comparatively slowly under similar conditions; this process can be accelerated by adding H_2O_2 (0·5%) to the suspension of quinol and quartz or silicic acid; a loss of 50—60% of the material can be demonstrated; the process can be followed by spectrophotometric measurements. The probable mechanism of the surface activity of the silicic acid is considered. (35 references.) P. S. ARUP.

Influence of stubble mulching on organic matter and nitrogen content of the soil. F. A. Norstadt and T. M. McCalla (*Agron. J.*, 1960, **52**, 477—479).—Total org. C, easily oxidisable org. matter (modified Walkley–Black method), and total N were 5—10% higher on a stubble-mulched than on a ploughed silty clay loam after 14–19 years' differential treatment. The differences occurred mainly in the top 1 in. soil layer and there were no measurable differences in the 1—6 and 6—12 in. soil layers. A. H. CORNFIELD.

Conception of farming on sub-tropical soils with use of crop by-product, trash, as a means to improve yields. C. H. O. Pearson (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 579—588).—The first application of trash can act as a mulch for the first ratoon crop; its fertilising value as an org. layer becomes apparent during the following season when it has been protected by a second layer of trash, which, in its turn, is protected by the shade of the second ratoon crop. The effects are not carried over through green-manure or fallow rotations. P. S. ARUP.

Comparison of microbial respiration in soil after a 20-year period of subtilling or ploughing. G. W. Olson and T. M. McCalla (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 349—352).—Respiration rates (Warburg method) of samples from the 0—1 in. layer of a silty clay loam were greater where the soil had been subtilled (stubble mulched) than where it had been ploughed for the previous 20 years. There were no differences in respiration rates between the two cultural methods for the lower soil depths. Respiration rates decreased with increasing soil depth. Addition of org. materials (dextrose, mannitol, straw) and herbicides (2,4-D, dalapon, carbamate) usually increased the respiration rate of soil from the 0—1 in. layer. Addition of KCN decreased the respiration rate. The extent of the effect of the additives on respiration was similar with soil from either cultural treatment. A. H. CORNFIELD.

Areal contrasts in the abundance of hydrocarbon-oxidising microbes in soils. J. B. Davis, R. L. Raymond and J. P. Stanley (*Appl. Microbiol.*, 1959, **7**, 156—165).—The occurrence and distribution of organisms, notably *Mycobacterium paraffinicum*, capable of oxidising ethane and other hydrocarbons are examined in soils exposed to petroliferous emanation, e.g., oil-fields. Methods of enumeration are compared. A. G. POLLARD.

Nitrogen fixation by algae in Arizona soils. R. E. Cameron and W. H. Fuller (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 353—356).—Data for C and N of algal and lichen crusts and subsurface samples from arid and semi-arid soils of Arizona are presented. Soil crusts containing mixed algae or algae and lichens fixed atm. N_2 when incubated in the laboratory. Species of algae belonging to the genera *Nostoc*, Vauch., *Scytonema*, Ag., and *Anabaena* fixed atm. N_2 in pure cultures. Algae probably make an appreciable contribution to the C and N status of these arid soils. A. H. CORNFIELD.

The cellulose test for determining cellulolytic activity of soils in field trials. H. Unger (*Z. PflErnähr. Düng.*, 1960, **91**, 44—52).—The amount of pure cellulose wadding decomposed when buried in the soil is used as a measure of cellulolytic activity. Visual observations are also used. The period of decomposition depends on climate and is found by burying a control and inspecting this periodically for the amount of decomposition between successive samplings. M. LONG.

Ion-exchange separations in the determination of some polyvalent metal ions in plant tissue. A. H. Hunter and N. T. Coleman (*Soil Sci.*, 1960, **90**, 214—218).—Methods for the determination of Al, Mn, Co, Cu, Fe, Mo and Zn are described. Plant tissue is dried, ground and wet-ashed with HNO_3–$HClO_4$ or dry-ashed at 500° for 6 h. The ash is dissolved in 9M-HCl, the ions are separated on a Dowex column and eluted with HCl of decreasing molarity and volume. Good separation of all ions except Fe and Mo is achieved under the conditions given. Final analysis is made colorimetrically in all cases. Precision in terms of coeff. of variation was from 1·5 to 7%. T. G. MORRIS.

Comparison of soil investigation techniques (Egnér-Riehm, Neubauer, Lettuce, Mitscherlich and Dirks-Scheffer). K. Ehrendorfer (*Z. PflErnähr. Düng.*, 1960, **91**, 97—114).—Factors to bring values for P and K, obtained by the techniques investigated, into comparable terms of available nutrient/100 g. of soil, are proposed. Plant-available nutrient per hectare is given by the product of the following: mg. of nutrient per 100 g. soil, appropriate correction factor, vol.-wt. of dry soil in topsoil and depth of soil in which the supply of the nutrient is adequate. M. LONG.

Nutrition studies by foliar diagnosis in 157 vineyards of French Switzerland. E. Bovay (*Landw. Jb. Schweiz.*, 1959, **60**, 605—620).—Mean reference standards of nutrition are given as results of foliar diagnosis carried out at three stages of development during the season. On clay soils, values for leaf-K or -P do not always reach the values that might be expected from soil analyses. The need for periodic analyses of the subsoil is pointed out. Standards for economic manuring deduced from the data obtained are compared with similar data obtained in French vineyards. P. S. ARUP.

Optimum ratios of nutrient elements for agricultural crops. Z. Zurbicki (*Agrochimica*, 1960, **4**, 119—134).—Mineral nutrients required by crops are assessed on the basis of optimum ratios of nutrients determined experimentally. The system takes into consideration changes in optimum ratios during the growth of the plant. A. G. POLLARD.

Determination of free phosphoric acid in superphosphate. F. Yamazoe (*Soil & Plant Fd*, 1960, **5**, 160—166).—The method is based on the extraction of H_3PO_4 from the sieved (0·5 mm.) sample by ether–acetone (1 : 1-vol.), evaporation of the filtered extract to dryness, dissolution in water and titration with 0·1N-NaOH using dimethyl yellow to the first end-point (NaH_2PO_4) and then adding phenolphthalein to obtain a clear final end-point (Na_2HPO_4). A. G. POLLARD.

Nutrient application using plastic encased granules. J. Jung (*Z. PflErnähr. Düng.*, 1960, **91**, 122—130).—Encasing Nitrophoska granules (10—15 mm.) with Plastopal (a urea–formaldehyde resin) results in a slightly longer delayed action than encasing in Diofan (a vinylidene chloride copolymer). Acronal (a polyacrylic ester) and Lutofan (a vinyl chloride copolymer) have no effect. No delaying action with small "Kalkammonsalpeter" is found with any plastic coating. A very slow release of nutrients results on treating finely ground Nitrophoska with Diofan. M. LONG.

Chilean nitrate of soda. Nitrate Corp. of Chile Ltd. (*Chilean Nitrate agric. Serv. Inform.*, 1960, April, 14 pp.).—A description of the value of commercial $NaNO_3$ as a fertiliser. An appendix gives a list of crops which have poor tolerance to soil acidity which may be rectified by application of $NaNO_3$. H. S. R.

Urea–formaldehyde condensation. I. Conditions for the formation of monomethylurea. II. Some properties of monomethylurea. T. Hayase (*Soil & Plant Fd*, 1959, **5**, 120—126, 174—178).—I. Conditions favouring high yields of the compound (mol. ratio, temp., pH, catalysts, period of reaction and other factors) are examined. II. Monomethylurea is stable under ordinary conditions but admixture with other fertilisers [$(NH_4)_2SO_4$, superphosphate, KCl] causes changes affecting its water-sol. N content. A. G. POLLARD.

Influence of composted city garbage on yield and on some important aspects of soil condition. W. Seiberth (*Z. PflErnähr. Düng.*, 1960, **91**, 53—62).—Composted refuse does not give improved yields as the N it contains is too slowly available and no appreciable amounts of P or K are present. Effects on soil structure can be assessed only on long-term trials—no improvement of sorption capacity was found after 15 years. M. LONG.

Fertilisers. Scottish Agricultural Industries Ltd., J. B. Bookey and R. Graham (B.P. 828,297, 6.1.56).—The (phosphate) fertiliser particles are given a coating of oiled, water-sol. alkaline powder, e.g., Na_2CO_3, Na_2HPO_4, K_3PO_4, MgO or $MgCO_3$, to reduce their corrosiveness to ferrous metals. J. M. JACOBS.

Controlled availability fertiliser compositions. Esso Research & Engng Co. (B.P. 828,400, 22.4.58. U.S., 23.7.57).—A fertiliser product (containing N, P and K) is dipped in a molten, normally solid petroleum hydrocarbon (wax, asphalt or resin), to give a coated fertiliser (containing 0·1—25 wt.-% of hydrocarbon) from which the fertiliser components are released (in the soil) at a controlled rate. F. R. BASFORD.

Phosphatic fertilisers. Lummus Co. (B.P. 828,891, 13.5.56. U.S., 29.3.55).—A phosphatic fertiliser of controlled P_2O_5 content and satisfactory physical properties is produced by blending solid inorg. fertiliser (viz., phosphate rock, dolomite or limestone) with 0·1—20 pt. of Ca, K or Na metaphosphate in granular form, then adding acid, e.g., HCl, HNO_3, H_3PO_4 or (when using K or Na metaphosphate) H_2SO_4 (>3 wt.-% but not more than the stoicheiometric

amount to effect acidulation) and water (to hydrolyse the metaphosphate). The resulting slurry may be continuously mixed for 0·5—3 min., then tumbled in a zone at 75—150°, to give a granular product. In an example, KPO_3 (33) is blended with phosphate rock (33), then water (6) and 78% aq. H_2SO_4 (33 pt.) are added at 52°. After 1 min. (temp. rising to 112°) the slurry is poured into a cardboard container and allowed to cool therein, and after 4 days there is obtained a product analysing: water 7·5, total P_2O_5 31·5, citrate insol. P_2O_5 0·8, available P_2O_5 30·7, K_2CO_3 13 and H_2SO_4 2·5%. F. R. BASFORD.

Fertilisers. Facerform Co. (Inventor: [C] J. C. Arnold) (B.P. 828,881—3, 28.12.55. [B,C] divided out of [A]).—Phosphate fertiliser of enhanced crop-producing capacity per unit of P_2O_5 is obtained by mixing an inorg. acid (H_2SO_4 and/or H_3PO_4), or [B] acidic charge, e.g., from a treated phosphate rock etc., with phosphate rock, or [C] phosphatic ore with or without ground dolomitic limestone, and keeping the mass until it has set. [A] The mass is broken up, with further hardening and curing taking place, then finally cured, so that there is no appreciable loss of water during cooling. The product is a dry, free-flowing material. [B] The primary mixture is allowed to set in the form of discrete particles which are then agglomerated. Optionally after addition of other fertiliser, the particles are coated with an absorptive material (which may be reactive with the particles and may be ground phosphate rock, etc.) to give dry, free-flowing pellets. F. R. BASFORD.

Mixed fertiliser containing ammonium nitrate and calcium carbonate. Commercial Solvents Corp. (B.P. 828,675, 8.1.58. U.S., 16.1.57).—$CaCO_3$ is admixed with molten NH_4NO_3 in presence of $\leq$1% of $(NH_4)HPO_4$ or K_2HPO_4, then the mixture is solidified as a thin sheet, which is then granulated, to provide a solid, stable mixed fertiliser. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Influence of variety, temperature and stage of growth on the response of spring barley to photoperiod. A. A. Guitard (*Canad. J. Plant Sci.*, 1960, **40**, 65—80).—Increases in photoperiod during the stage of seeding to internode elongation reduced the no. of days for that change, leaf no. and height, wt. of stem and head, stem length, head length, no. of florets and kernels per head, and fertility for the first culm but increased no. of days from heading to maturity. Increases in photoperiod between internode elongation and heading reduced the no. of days for that change but generally increased leaf, stem and head development, fertility for the first culm and no. of fertile tillers. Increases in photoperiod from heading to maturity reduced the no. of days for that change and increased the no. of fertile tillers. Increase in temp. (55—75°F) reduced the duration and extent of development of the first culm, delayed commencement and reduced the duration of tiller initiation and reduced the no. and extent of development of tillers. Two varieties differed in duration of leaf initiation and no. of leaves associated with the first culm, in kernel wt. and in relative time of initiation of tillering. E. G. BRICKELL.

Water content of seeds. I. Moisture relations of seed peas, etc. O. F. Lubatti and G. Bunday (*J. Sci. Fd Agric.*, 1960, **11**, 685—690).—A method of determining the hygroscopic equilibrium curve of seed peas is described and is compared with the equilibrium curve for broad beans and lucerne seed. With viable seeds, over the range 30—85% of R.H., wet basis, Smith's equation (*J. Amer. chem. Soc.*, 1947, **69**, 464) appears to be adequate. (12 references.) E. M. J.

Determination of components of plant cuticles. J. L. Martin (*J. Sci. Fd Agric.*, 1960, **11**, 635—640).—Quant. methods are described. Waxy materials on the surface of leaves or fruits are removed by immersion in $CHCl_3$ at room temp.; those embedded in the cuticle, e.g., of fruits, are extracted from disks of skin after removal of surface waxes. Acidic materials are removed from the extract and the wax and oil fractions are obtained by partition between n-heptane and methanol. The cuticular membranes are set free by refluxing disks from the leaves with NH_4 oxalate–oxalic acid solution and the cutin is determined by saponification. Leaves, e.g., tomato, show low levels of waxiness and cutin (<50 μg./sq. cm.) whereas the developed fruit skin contains ~1000 μg/sq. cm. of cutin. (20 references.) E. M. J.

Inhibitory action of glyoxylic acid on oxidation of citric acid in spinach leaves. T. Eschena (*Agrochimica*, 1960, **4**, 142—155).—The citric acid content of spinach extracts incubated at 38° and pH 6·8 remains fairly constant in presence of oxaloacetic and glyoxylic acid (6—8 μM), whereas it is totally oxidised within 1 h. in presence of either of the acids alone, or in the blank experiment. The inhibition due to the two acids in combination can be nullified by the presence of Fe^{2+} Chemical and polarographic experiments suggest that the oxaloacetic acid promotes the oxidation of the Fe^{2+} to Fe^{3+} which then binds the glyoxylic acid as a complex. (18 references.) P. S. ARUP.

Use of enzymes in isolation and analysis of polysaccharides. E. T. Reese and M. Mandells (*Appl. Microbiol.*, 1959, **7**, 378—387).—Processes based on enzymic hydrolysis in combination with chromatographic separation are discussed. (39 references.) A. G. POLLARD.

Sulphur loss from and sulphur exchange in wheat roots. H. Marschner and G. Michael (*Z. PflErnähr. Düng.*, 1960, **91**, 29—44).—Loss of S from the roots, slight under normal conditions, occurs to an extent of up to 4% of total plant-S when CO_2 is passed into the nutrient solution, this loss being at the expense of inorg. S. After 28 days exchange between nutrient solution and plant-S amounts to less than 5% of total plant-S. In spite of large uptakes of inorg. S from nutrient solutions, the $^{35}SO_4^{2+}$ content of the roots varies little, indicating that SO_4^{2+}-S is mainly located in the vacuoles. M. LONG.

Absorption of sulphur from organic and inorganic sources by bush beans. C. E. Bardsley (*Agron. J.*, 1960, **52**, 485—486).—A solution culture trial with bush beans grown for 24 days showed that cystine, cysteine and methionine were as effective sources of S as was $CaSO_4$, as measured by dry matter yields and S uptake. The org. compounds were probably absorbed without first being mineralised. Sulphanilic acid was ineffective as a source of S. A. H. CORNFIELD.

Dependence of root formation of sunflowers on boron. W. Bussler (*Z. PflErnähr. Düng.*, 1960, **91**, 1—14).—In the complete absence of B no root formation occurs with sunflower cuttings, whilst with insufficient B the following symptoms appear: death of the growing point, proliferation of the cambium, premature hardening of the central cylinder, enlargement of the cortical tissue and abnormal bending. M. LONG.

Strontium in higher plants. I. Uptake of strontium by peas and oats and its action on plant growth. G. Michael and G. Schilling (*Z. PflErnähr. Düng.*, 1960, **91**, 147—158).—The Ca/Sr ratio in plant tissue is the same as that in the soil. Both Ca and Sr contents are reduced by K applications. Root-CO_2 tends to reduce the Sr more than the Ca content, due to the lower solubility of $SrCO_3$. M. LONG.

Synthetic chelating agents in plant nutrition and some of their effects on carboxylating enzymes in plants. A. Wallace (*Ann. N.Y. Acad. Sci.*, 1960, **88**, 361—377).—A review of recent work. (29 references.) C. V.

Basic values in plant analysis, with special reference to seasonal variations in apple and pear leaves. A. Bussmann and H. Gerber (*Landw. Jb. Schweiz*, 1959, **60**, 21—23).—Variations in values for N, P, K, Ca, Mg and Fe calculated on dry matter or fresh wt. follow a more consistent pattern than those based on the crude fibre, ash, N or leaf-surface values. Values based on leaf-surface are definitely not suitable for this purpose. (15 references.) P. S. ARUP.

Estimation and distribution of phosphorus fractions in leaves of plants. E. J. Hewitt and B. A. Notton (*J. Sci. Fd Agric.*, 1960, **11**, 653—658).—Serial extractions with 85% ethanol, 0·2N-HCl, N-$HClO_4$ at 4°, 0·5N-$HClO_4$ at 70° and 2N-NaOH remove 99·8% of total P from tomato or cauliflower leaves. Single extractions by ethanol or 0·2N-HCl remove ~75% of the respective P compounds. Org. P remaining after exhaustive extraction with ethanol is largely removed by 0·2N-HCl. Use of labelled inorg. P shows that ribonucleic acids (RNA) are stable in N-$HClO_4$ at 4° for 18 h. but residual nucleic acid is decomposed significantly by 0·5N-$HClO_4$ at 70° in 20 min. More acid-labile P (**I**) was obtained in 0·2N-HCl than in the ethanol extracts. **I** comprised 2—4% of ethanol sol. and 8—12% or more of 0·2N-HCl-sol. org. P. The amounts and proportions of org.-P fractions in plants varied widely between different species and between the same species on different occasions. E. M. J.

Control of growth of hypocotyl in *Lactuca sativa* L. seedlings by visible radiation. H. Mohr and M. Wehrung (*Planta*, 1960, **55**, 438—450).—The action-spectrum for inhibition of lengthening shows practically no effect in the range 500—700 mμ, and peaks in the ranges 400—700 and 700—750 mμ. Published opinions concerning the "high energy reaction" are critically examined. (24 references.) P. S. ARUP.

Determination of cellulose, starch and protein in plant tissue on a semi-micro scale with formic acid. E. Lehmann and H. Schanze (*Z. PflErnähr. Düng.*, 1960, **91**, 115—122).—Fatty matter in a 0·1-g. sample is removed by Soxhlet extraction, and the residue is treated with formic acid. Cellulose remains undissolved and is removed for determination. Starch is precipitated from the formic acid solution by the addition of acetic acid. Protein is finally precipitated from the solution by the addition of ether, light petroleum and benzene. All three fractions are deformylated by heating with aq. piperidine

and weighed. Cellulose values for fibres are high because insol. pectin, hexosan and cell membrane remain with the cellulose fraction. Sol. pectin and pentosans appear with the starch fraction.
M. LONG.

Spectrophotometric determination of macro-nutrients in biological material. K. Scharrer and G. K. Judel (*Agrochimica*, 1960, **4**, 135—144).—The determination of Al, Ca, Cu, Fe, K, Mg, Mn and Si is carried out by intimately grinding the ash of the sample with powdered spectrographic graphite and Li_2CO_3, pressing the mixture into the hollow of a graphite electrode, and volatilisation in a direct current at 110 V and 11 A. The accuracy is of the order ±10%. (11 references.) P. S. ARUP.

Determination of pH in colorimetric [carbon dioxide-]assimilation measurements. J. Catsky (*Planta*, 1960, **55**, 381—389).—For visual estimations of pH in $NaHCO_3$ solutions in the Friedland apparatus, the substitution of a mixed indicator containing Thymol-blue and cresol-red (2 : 1) for cresol red alone, gives greatly increased accuracy in the range pH 7·8—8·4. The Lange formula for calculating the concn. of CO_2 in air from the pH of the solution holds good for the mixed indicator; differences between results thus obtained and those given by the URAS method are not important. A bubbling device is described for use in photocolorimetric determinations of CO_2 with the Friedland apparatus, without interruption of the air-current. (11 references.) P. S. ARUP.

Cell-division factor from peas. J. A. Zwar (*Dissert. Abstr.*, 1960, **21**, 33).—Kinin activity, assayed by growth of explants of tobacco callus, was detected in aq. solution of an enzymic digest of soya-bean meal, and in detoxified aq. extracts of tobacco leaves and roots, and of pea seedlings. The kinin in the pea seedling extract was concentrated 370 times by pptn. with basic Pb acetate and adsorption on Dowex-50-H^+. The substance was partially sol. in acetone, less so in abs. methanol, ethanol and butanol; unlike kinetin, it was insol. in ether at alkaline pH, and was not precipitated by $AgNO_3$ at acid pH. It was further concentrated (1160 times) by adsorption on Norite and elution by pyridine. Activity was enhanced by the presence of myo-inositol, scyllo-inositol or sorbitol, which compounds do not affect kinetin. M. D. ANDERSON.

Influence of the extract of some crops and soil residues on germination and growth. K. F. Nielsen, T. F. Caddy and W. B. Woods (*Canad. J. Plant Sci.*, 1960, **40**, 188—197).—Lucerne extract caused the greatest reduction in shoot and root length as well as in % germination of the plant species investigated. Timothy extract was not quite as harmful as that of lucerne; extracts of oats, maize and potatoes were still less harmful, potato extract causing the least effect. Plant species showed marked differences in tolerance to the extracts, lucerne being the most and timothy the least resistant.
E. G. BRICKELL.

Growth, development and mineral uptake in tomato plants, as affected by maleic hydrazide and gibberellin. P. C. Bose (*Dissert. Abstr.*, 1960, **21**, 13—14).—Growth of tomato plants in sand culture was inhibited by foliar applications of maleic hydrazide, root length being affected more than shoot length. The contents of minerals in the plants were also lessened. Foliar applications of gibberellin stimulated growth, elongation of plant parts being related to concn. of gibberellin. Uptake of water and K was increased; size of largest leaf and no. of flowers were not affected; fruits were fewer in no., smaller and malformed. There was chlorosis of lower leaves, perhaps because of lower uptake of Fe or Mn.
M. D. ANDERSON.

Effect of temperature and gibberellin on growth of tomato fruits. L. Rappaport (*Naturwissenschaften*, 1960, **47**, 285).—Gibberellin had no significant effect on fruit wt. or dia. at night temp. of 11° or 23° (with day temp. 23°), but it markedly reduced wt. and dia. at the night temp. (17°) that gave highest yields from untreated plants. Previous work has been mainly at night temp. near the optimal. (12 references.) (In English.) M. LAPIDOT.

Effects of gibberellin treatments on germination of various species of weed seeds. W. G. Corns (*Canad. J. Plant Sci.*, 1960, **40**, 47—51).—Treatments of up to 500 p.p.m. in the medium, or of up to 2000 p.p.m. as a 24-h. pre-soaking, were without appreciable effect on most of the dormant species among 19 tested, but the chemical has a significant effect in overcoming dormancy of some, but not all, samples of wild oats. Dormant seeds of wild mustard and stinkweed were very sensitive to gibberellin treatment, while hemp nettle and blue bur showed a smaller response. E. G. BRICKELL.

Growth and flowering of strawberry plants in relation to endogenous growth substances, applications of gibberellic acid, and environmental factors. I. C. Porlingis (*Dissert. Abstr.*, 1960, **20**, 4502—4503).—One main ether-extractable auxin was found in the apices and leaves of three varieties of strawberry; this was not indolylacetic acid, indolylacetonitrile or ethyl indolylacetate. Concn. was higher in younger leaves. Chilling strawberry plants for 1—2 months at 35—42°F stimulated vegetative growth on return to warm temp. Long days caused both chilled and unchilled plants to be more vegetative than did short days. Gibberellic acid sprays stimulated the vegetative growth of terminal axes, the development of previously differentiated inflorescences, and stolon production by plants growing with short-day illumination; larger doses of gibberellic acid were needed to induce stolon production by plants growing with long-day illumination. M. D. ANDERSON.

Effects of gibberellic acid on sugar cane. R. E. Coleman, E. H. Todd, I. E. Stokes and O. H. Coleman (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 588—603).—Effects obtained by various methods of application resemble those observed for other crops, and have no practical value. The chief responses are reduction of germination by application to seed-pieces, and (temporary) stalk-elongation at the expense of stalk-diameter. P. S. ARUP.

Gibberellin stimulates growth of sugar cane plants affected with Fiji disease. G. O. Ocfemia and F. L. Nuque (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 1045—1046).—Shoots from diseased plants sprayed fortnightly with gibberellin (50—75 p.p.m.) produce lighter coloured and much better grown leaves than do untreated shoots. Galls occur both on treated and untreated plants.
P. S. ARUP.

Crops and Cropping

Seed treatment and sowing methods for cover crops. Anon. (*R.R.I. Plant. Bull.*, 1960, 94—98).—A review. E. G. BRICKELL.

Abrasive action of windblown soil on plant seedlings. L. Lyles and N. P. Woodruff (*Agron. J.*, 1960, **52**, 533—536).—Seedlings of El Reno sideoats grama were more tolerant to windblown soil than were Blackwell switchgrass, sand lovegrass and Indian grass. Buffalo lucerne was the least resistant of all the species tested.
A. H. CORNFIELD.

Asparagine test in relation to nitrogen nutrition status of crop plants. II. Barley. III. Rice. M. Singh, K. Kumazawa and S. Mitsui (*Soil & Plant Fd*, 1959, **5**, 101—107, 167—173).—II. Prolongation of the dark period for barley cultures increased the asparagine (**I**) and decreased the NO_3^- content of the leaves; with heavy applications of $(NH_4)_2SO_4$ the increase in **I** is intensified and the decline in NO_3^- is restricted. In young leaves the NO_3^- levels are generally lower and the **I** levels higher than in older leaves or stems. In plants placed in complete darkness with limited supplies of N, leaf-NO_3^- increased and -**I** diminished. The bearing of these observations on N manuring practice for barley is noted.

III. Nitrate was present in roots and stems of rice in the early stages of growth and appeared in nearly all leaves 10 days after the application of $NaNO_3$ or NH_4NO_3. Panicle formation was associated with the localised appearance of **I** in leaves, especially in plants having high-N supplies. The formation of **I** was influenced by the N source in the order NH_4^+ > urea > NO_3^-. **I** is probably translocated from ageing to younger leaves as the plant develops.
A. G. POLLARD.

Fertilisers and the nutritive value of wheat grown on a sulphur-deficient Grey Wooded soil. C. F. Bentley, J. A. Casson and J. P. Bowland (*Canad. J. Plant Sci.*, 1960, **40**, 146—155).—In general grain was of substantially superior nutritive value when grown following legumes and these differences tended to be greater with fertiliser treatments which increased yields. Animal growth and food efficiency are closely related to the protein content of the foods.
E. G. BRICKELL.

Cold hardiness of sprouting wheat as affected by duration of hardening and hardening temperature. J. E. Andrews (*Canad. J. Plant Sci.*, 1960, **40**, 94—103).—Sprouting winter wheat will harden to cold in the dark. The ultimate level of cold hardiness attained depends on the hardening temp., the duration of hardening, and the stage of development of the seedling. E. G. BRICKELL.

Temporary appearance of visible magnesium-deficiency symptoms in oats. K. Scharrer and K. Mengel (*Agrochimica*, 1959, **4**, 3—24).—Periodic mineral analyses of pot-grown oats and spring barley in Mg-deficient and -adequate soil show the symptoms to be due to temporary Mg-shortage caused by high consumption during early vigorous growth. The symptoms do not appear during the (slower) preliminary growth of winter barley. (22 references.)
P. S. ARUP.

Accumulation of nitrates in several oat varieties at various stages of growth. A. Gul and B. J. Kolp (*Agron. J.*, 1960, **52**, 504—506).—The NO_3^- content of the above-ground portion of 12 varieties of oats was highest at the 25% flower stage and decreased to the 50% dough stage. There were significant differences in NO_3^- content due to variety and location of growth, but the variety × growth stage and variety × location interactions were not significant. NO_3^- contents were higher at all growth stages at the location where

N was applied and irrigation was practised than at the location where no N was applied and no irrigation practised. There was poor correlation between NO_3^- content and either plant height or forage yield. A. H. CORNFIELD.

Distribution of ^{15}N applied late in matured barley plants with special reference to grain protein. G. Michael, H. Faust and B. Blume (*Z. PflErnähr. Düng.*, 1960, **91,** 158—169).—N applied late is found mainly in the secondary growth and stalk in the vegetative parts of the plant and in the sol. N fraction in the grain. The stalk acts as an N reservoir. Part of the late N-application is found in the grain but this later declines. Glutelin and prolamine in the grain are the first to benefit from applied N and so provide the best indication of N status at any instant. M. LONG.

Rôle of silicon in rice nutrition. S. Yoshida, Y. Ohnishi and K. Kitagishi (*Soil & Plant Fd*, 1959, **5,** 127—133).—Silicon has comparatively little effect on the early growth of rice plants but becomes important during flowering and grain formation. Si-deficient plants had relatively high mineral contents (notably Mn), this being associated with excessive transpiration and also enhanced susceptibility to disease and insect attack. Such conditions are also associated with the disorder known as "Akiochi." (20 references.)
A. G. POLLARD.

Nutrient composition of hybrid maize as influenced by fertilisation. I. Nitrogen percentage of leaf and grain. J. M. Fulton and W. I. Findlay (*Canad. J. Soil Sci.*, 1960, **40,** 42—48).—The % of N in the ear leaf shoot (**I**) and in the grain (**II**) and also grain yields increased with increasing rates of N fertilisation, but the correlation of yield with **I** was greater than with **II**. Only when P is deficient does luxury consumption of N occur. M. LONG.

Factors influencing the development of hollow heart in Irish Cobbler potatoes (*Solanum tuberosum*, L.). D. H. Dinkel (*Dissert. Abstr.*, 1960, **21,** 14—15).—High incidence of hollow heart in potatoes was associated with high rate of tuber growth shortly after tuber initiation, high moisture supply (leading also to high yield of tubers), smaller no. of stalks and tubers per plant and high incidence of internal browning. Nutrients, e.g., ^{32}P, may be withdrawn rapidly from tubers to support the growth of other tissues. A period of smooth transition from top growth to tuber initiation and enlargement, without flushes of excessive top growth, prevent the development of hollow heart. M. D. ANDERSON.

Absorption of fertiliser phosphorus by sugar beet, as influenced by placement of phosphorus and nitrogen. C. H. E. Werkhoven and M. H. Miller (*Canad. J. Soil Sci.*, 1960, **40,** 49—58).—Placement of N is of greater importance than that of P (as superphosphate). When N–P mixtures were banded the uptake of P was greater than when the mixture of N alone was mixed with the top 2 in. of soil. When N was present deep band placement was the most effective, but when N was mixed with the soil or absent, shallow banding or mixing with the soil was the most effective. M. LONG.

Heavy nitrogen fertilisation of cool-season turf grasses. N. R. Goetze (*Dissert Abstr.*, 1960, **21,** 2—3).—The ability of rye-grass turf to support a constant wt. and quality were improved more markedly by applications of N in natural org. materials than by comparable amounts of N in sol. or urea–formaldehyde fertilisers. The density of the turf increased with increasing N, irrespective of source. The dry wt. of clippings also increased more markedly with N from org. materials; the N content of successive clippings decreased after an early peak, of height related to N supply. N-deficiency symptoms appeared when the N content of the rye-grass fell below 3%. The N recovered in clippings varied from 33% of that supplied in sol. form to 17% of that in urea–formaldehyde; 5 weeks' leaching removed 19% of N in sol. fertiliser, but only 2·2% of urea–formaldehyde-N. The quality and density of Merion blue-grass turf, and the yield of clippings, were not affected by source or amount of N applied.
M. D. ANDERSON.

Effects of temperature and light on growth and flowering of tall fescue (*Festuca arundinacea*, Schreb.). W. C. Templeton, jun. (*Dissert. Abstr.*, 1960, **21,** 20).—Tillering of tall fescue grass was increased by low temp. and short photoperiod. Rate of appearance of leaves was increased by short photoperiod, but decreased by exposure to low temp. during a part of each day. Induction of flowering was hastened by exposure to cold, though some plants flowered without a period of growth at low temp. Flowering behaviour after spring sowing did not indicate the earliness of seed production characteristic of other plants after exposure to winter temp. It would be difficult to develop leafy strains of tall fescue with low seed production for growth in areas with cold winters without sacrificing early spring growth. M. D. ANDERSON.

Effects of varying nutrient supply on the contents of lysine, arginine, aspartic acid and other organic constituents of bromegrass. L. W. Reed, V. L. Sheldon and W. A. Albrecht (*Agron. J.*, 1960, **52,** 523—526).—The effect of varying levels of N, P, K, Ca and S on the contents of lysine, arginine, aspartic acid, reducing and non-reducing sugars, starch and hemicellulose in bromegrass grown in clay–sand cultures, are reported. A. H. CORNFIELD.

Response of Pensacola bahia grass to nitrogen fertilisation. E. R. Beaty, R. A. McCreery and J. D. Powell (*Agron. J.*, 1960, **52,** 453—455).—Application of N (25—240 lb. per acre) to established bahia grass on a loamy sand increased forage yield approx. in proportion to the amount of N applied, but had little effect on the seasonal distribution of yields. Of the total forage 64% was produced in June–July, 24% in Aug.–Sept. and 12% in April–May. N % in the forage was increased only when ⩽100 lb. of N per acre was applied.
A. H. CORNFIELD.

Botanical composition changes in annual grassland as affected by fertilisation and grazing. M. B. Jones and R. A. Evans (*Agron. J.*, 1960, **52,** 459—461).—Changes in botanical composition due to application of N and P, with and without sheep grazing, on the annual range types of California were studied at two locations. The % of short chess, *Bromus mollis*, on natural range was increased by N on grazed plots for 2—3 years, but only in the first year on ungrazed plots. Soft chess on re-seeded range was increased in both years by N when grazed but decreased when ungrazed. The % of ripgut, *Bromus rigidus*, on native range was increased by P, to extents smaller on grazed than on ungrazed plots. On the seeded range, where available P was adequate, ripgut was increased by N. Grazing reduced ripgut on both types of range. In general the % of clovers was decreased by N and increased by P.
A. H. CORNFIELD.

Nitrate nitrogen content of herbage. I. Observations on herbage species. G. ap Griffith and T. D. Johnston. **II. Effect of different levels of application of sulphate of ammonia on the nitrate content of herbage.** G. ap Griffith (*J. Sci. Fd Agric.*, 1960, **11,** 622—626, 626—629).—I. Nitrate accumulation in grass following heavy dressings of N varies with species and variety, notably with S.23 and S.24 perennial rye-grass and S.143 cocksfoot. With rape, NO_3^- accumulation occurred after moderate dressings of N and persisted up to 8 weeks.

II. Various levels of N as $(NH_4)_2SO_4$ were applied in April to three grass mixtures which were cut in June. The NO_3^--N content of the herbage was detectable at the 4-cwt./acre level and considerable at the 12-cwt. level. A close connexion was observed between NO_3^--N content and crude protein value (**I**) especially when **I** was >18%.
E. M. J.

Response of legumes to molybdenum and lime fertilisation on Mardin silt loam soil. W. M. Kliewer and W. K. Kennedy (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 377—380).—Application of Mo (5—8 oz. per acre as Na_2MoO_4) to a silt loam (pH 4·9) in pot tests increased the dry matter yields, N %, and Mo % of birdsfoot trefoil tops and roots. Liming the soil increased all these values to an even greater extent and there were no responses to Mo application when sufficient lime was added to raise soil pH to 5·6 or greater. Mo treatment increased the size but reduced the no. of individual nodules on the plant. Yields and N % of lucerne and medium red clover were increased only slightly by Mo, whilst those of ladino clover were increased somewhat more. Liming was again much more effective than Mo in increasing yields of all species and there were no responses to Mo at the higher lime levels. A. H. CORNFIELD.

Effects of fertility level, autumn management and stage of cutting, on forage and seed yield of red clover (*Trifolium pratense* L.). H. N. Markham (*Dissert. Abstr.*, 1960, **21,** 16—17).—Application of fertiliser to red clover increased infestation by annual grassy weeds, and reduced clover stands in both seedling and harvest years; forage yield was not affected but the larger dressings reduced seed yield. Clipping in the autumn of the seedling year diminished the competitive effect of weeds, and gave denser stands of clover, and greater yields of forage in the first but not in the second crop, and of seed in the second crop. Clipping in early spring to simulate grazing did not delay flowering, but reduced the first-crop yield; the second-crop forage yield was increased, but that of seed decreased. Yield of the first crop increased as cutting was delayed, and yield of second crop was proportionally decreased. The highest annual yield was obtained when the first cutting was made near full bloom. Seed yield was proportional to stand and decreased with increase of grassy weeds. M. D. ANDERSON.

Growth of some species of *Trifolium* following γ-irradiation of the seeds. I. Jones and G. L. Plummer (*Agron. J.*, 1960, **52,** 462—464).—Irradiating the seed of six species of *Trifolium* with 7100 r of γ-irradiation prior to sowing resulted in somewhat increased height growth and no. of leaves in most cases, but had little effect on leaflet size. A. H. CORNFIELD.

Lucerne response to irrigation frequencies in the presence of a water table. R. E. Campbell, W. E. Larson, T. S. Aasheim and P. L.

Brown (*Agron. J.*, 1960, **52**, 437—441).—Application of irrigation water six times per season over 4 years to lucerne growing on a clay loam having a water table at 5—9 ft. below the surface increased average yields only slightly in comparison with non-irrigated plots. Salinity was higher, particularly in the 3—7 ft. soil depth, in the non-irrigated than in the irrigated soil. Irrigation had no effect on P (%) in the plant. A. H. CORNFIELD.

Forage production of Vernal lucerne under differential cutting and phosphorus fertilisation. J. L. Parsons and R. R. Davis (*Agron. J.*, 1960, **52**, 441—443).—Cutting lucerne five times during the season produced forage of the highest % of protein, but caused low yields and injured the stand. Cutting three times during the season gave the max. yield of dry matter with the lowest % of protein, but maintained a satisfactory stand. Total protein production per acre per annum was similar with three or four cuttings per annum. P fertilisation improved dry matter production, % of protein and stands. A. H. CORNFIELD.

Variety, fertiliser and management interactions in lucerne. B. E. Twamley (*Canad. J. Plant Sci.*, 1960, **40**, 130—138).—In the second crop year hardiness was the main factor in determining both yields and stands; bacterial wilt was of less importance. In the third crop year, wilt became the main factor. P helped the winter-hardy varieties more than the non-hardy whereas K was of greater benefit to the winter susceptible varieties. Sept. clipping was detrimental to all varieties and particularly to the less hardy ones, DuPuits and Ranger. Only with Vernal and Grimm was it feasible to compensate by the addition of fertilisers for the mismanagement involved in untimely cutting. E. G. BRICKELL.

Phosphate and sulphate fertilising experiments on lucerne. A. Baroccio, G. Pisano and V. Morani (*Ann. Staz. chim.-agr., Roma*, 1959, iii, 156, 17 pp.).—Field trials with lucerne using top-dressed or incorporated superphosphate and gypsum on three differently structured neutral soils are reported. Gypsum had no effect on the fertilising efficiency of top-dressed superphosphate even in SO_4^{2-}-deficient soils, and improved lucerne growth only when large amounts were applied before sowing in soils having deficient structure. Surface application of gypsum generally reduced yields, slightly reduced the P_2O_5 content and increased the K content, of first cuttings. (27 references.) E. C. APLING.

Development of carbohydrate reserves by lucerne during the initial season of establishment. A. G. Matches (*Dissert. Abstr.*, 1960, **21**, 17).—Partial shading of lucerne plants diminished the dry wt. and content of available carbohydrate in the roots. Increasing supplies of K increased plant height, root length and dry wt. of the roots and their content of available carbohydrate (**I**). Dry wt. and **I** contents of roots were closely associated. The **I** content increased steadily until the plants were 10 weeks old, and then remained constant. Reserves of carbohydrate in the roots appeared to be adequate by the stage of 1/10 bloom to enable the plant to withstand bad conditions. M. D. ANDERSON.

Effectiveness of inorganic nitrogen as a replacement for legumes grown in association with forage grasses. L. P. Carter (*Dissert. Abstr.*, 1960, **20**, 4480—4481).—Response of pure grass stands to N fertilisers had not reached a max. at 240 lb./acre. The N content showed the greatest increase in the first cutting, and by the fourth cutting was the same for grass from plots receiving 0 and 240 lb. of N/acre. With 240 lb. of N/acre, there was little carry-over of N to the next year. Yields of dry matter and N were about the same from pure grass stands receiving 150—200 lb. of N/acre, and from an unfertilised mixture of grass with lucerne or clover. Application of N to grass–legume mixtures increased yields of dry matter and N, usually through increased growth of the grass, accompanied by decreased growth of the legume. Small amounts of NO_3^--N were found in most plants; NO_3^--N in forage grasses began to accumulate in large amounts when 120—240 lb. of N/acre was supplied. M. D. ANDERSON.

Oat–pea or oat–vetch mixtures for forage or seed. R. G. Robinson (*Agron. J.*, 1960, **52**, 546—549).—Oat–legume mixtures were superior to oats alone as forage crops; both protein % and protein yields per acre were greater with the former. As feed-grain crops, oat–pea mixture was superior to oats alone or oats–vetch in protein yield per acre. On a sandy soil oat–legume mixtures produced more forage and oat–pea mixtures produced more seed than did oats alone. On heavier soils, seed yields of the mixtures were less than those of oats alone. A. H. CORNFIELD.

Effects of long-term surface applications of limestone and fertilisers to permanent hayland. L. B. MacLeod, R. F. Bishop, L. P. Jackson and E. T. Goring (*Canad. J. Soil Sci.*, 1960, **40**, 28—34).—The pH of plots receiving limestone increased at all depths sampled. The adsorbed, easily acid-sol. P tended to remain in the top 3 in., whilst exchangeable K was not increased by the K top dressings. Plots receiving complete NPK and limestone treatments gave the highest yields, P being particularly effective. $(NH_4)_2SO_4$ was slightly superior to $NaNO_3$ or NH_4NO_3. K as KCl and limestone applied separately had no effect. Yields decreased over the 27-year period of the trial, due to inadequate fertility for maintenance of required biological species. M. LONG.

Effect of deficiencies of major nutrients on growth and leaf analysis of banana. D. B. Murray (*Trop. Agriculture, Trin.*, 1960, **37**, 97—106).—In continuation of sand-culture experiments (cf. *ibid.*, 1959, **36**, 100), it is found that growth can be sustained during a few months from the resources of the corm with respect to all the major nutrients except N. Data are given showing the levels at which deficiencies are indicated by leaf analysis. Visual deficiency symptoms occur at levels much below those necessary for optimum growth. P. S. ARUP.

Fertiliser experiments with Gros Michel banana. A. F. Butler (*Trop. Agriculture, Trin.*, 1960, **37**, 31—50).—In long-term experiments in Jamaica and Honduras, significant economic responses have resulted from N-manuring, but not from P-, K- or PK-manuring alone, or together with N. Applications of the minor elements Mn, Fe, B or Zn have proved unnecessary or undesirable. P. S. ARUP.

Distribution of normal and toxic amounts of boron in leaves of rough lemon. J. J. Oertli (*Agron. J.*, 1960, **52**, 530—532).—Normal leaves from 1-year-old rough lemon seedlings showed a fair variation in B concn. over the leaf area; the highest concn. occurred near the leaf edges. When the seedlings were treated with water containing toxic quantities of B, leaf toxicity symptoms were accompanied by the accumulation of very high concn. of B; the tip areas contained ~5000 p.p.m. (fresh basis) and were necrotic. The extent of toxicity symptoms was correlated with B concn. of the particular portion. A. H. CORNFIELD.

Effect of graded applications of potassium as KCl and K_2SO_4 on the yield and water, N, K, Cl and S contents of spinach grown in pots. K. Schmalfuss and I. Reinicke (*Z. PflErnähr. Düng.*, 1960, **91**, 21—29).—High concn. of Cl^- increase yields but this is not a micronutrient. The water content of the tissue increases with increasing $[Cl^-]$ as also does the protein fraction of the total N. The uptake and conversion of S proceeds more slowly. From the S balance of plants and soils some absorption of volatile S compounds from the air is indicated. M. LONG.

[A] **Soil as factor in varietal yield decline [of sugar cane].** R. P. Humbert. [B] **Possible rôle of insects in varietal yield decline of sugar cane.** C. E. Pemberton (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 51—59, 59—61).—[A] A review covering the following topics: the causes of soil-compaction and its harmful effects on the root-system. The repeated use of $(NH_4)_2SO_4$ as a cause of increasing soil-acidity and depletion of soil-Ca, -Mg, -K and -P. The occurrence of harmful fungi, viruses and nematodes. Favourable results obtained by crop-rotation with lucerne or pineapple. (14 references.)

[B] The possibility is examined of the (almost symptomless) decline being due to a virus with the mealybug, *Saccharococcus sacchari*, (Ckll) as vector. P. S. ARUP.

Six years' studies on nitrogen utilisation by sugar cane plant using ^{15}N as tracer. D. T. Takahashi (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 377—390).—The ^{15}N fed to cane plants by way of nutrient solution is assimilated within a few days to protein, peptides, amides and amino-acids. The first amide to be formed is asparagine, which is mainly synthesised in the root tissues; proteins are synthesised in the leaves, meristem and roots. In normal plants, most of the ^{15}N, whether fed through the roots or the leaves, is translocated to the young actively growing leaves and the upper nodes. In deficient plants, which show reduced metabolic activity (including reduced protein synthesis), the basal parts are preferentially enriched. (24 references.) P. S. ARUP.

Relationship between growth of sugar cane and yield of cane at harvest. A. de Sornay and O. Davidsen (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 390—398).—Coeff. of correlation between actual total cane yields and data from small (10-ft. lengths of lines) random plots are, on total stalk length at the end of June 0·896, on wt. of cane 0·984 and on visual estimates 0·867. Further statistical analysis confirms the wt. of cane as being the most reliable index. Stalk-length is the most important vegetative factor for correlation with total yields. P. S. ARUP.

Recent advances in nutrition of sugar cane in S. Africa. J. L. du Toit (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 432—441).—An account is given of responses obtained by N-, P- and K-manuring, based on field trials and leaf- and soil-analysis. P. S. ARUP.

Potash manuring of sugar cane. R. F. Innes (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 441—450).—An account of observations in Jamaica on the value of field experiments, and soil and tissue analyses in relation to yield responses. Foliar diagnosis has given the most satisfactory indications of requirements. (24 references.) P. S. ARUP.

Growth of sugar cane as influenced by nitrogen fertilisation. T. Tanimoto and G. Burr (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 450—462).—In comparative experiments in which the amounts of N applied were 0—400 lb. per acre, and times of application were from 1 month until the 13th month, growth and yields depended on the total N applied more than on the timing. Full use of heavy early applications cannot be made during the first 11 months. With delayed applications, lower yields from primary and secondary stalks are compensated for by higher yields from subsequent sucker stalks. The crowding of stalks due to heavy applications does not increase the proportion of dead stalks. With low or zero N, the basal parts of the primaries show the greatest deterioration. P. S. ARUP.

Growth of sugar cane as influenced by phosphorus. [A] Critical range of soil phosphorus. A. S. Ayres. **[B] Critical range of plant phosphorus.** C. E. Hartt (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 462—467, 467—473).—[A] The advantages of soil analysis are pointed out, and analytical methods are discussed. Results of 126 experiments on the relationship between soil-P and response to additional P show that the critical range for soil-P in which a few of the soils responded to additional P, is 45—75 lb. of P per acre-foot. A form for reporting on soil-P status is shown.

[B] The 8—10 internodes from plants 6—12 months old form the best indicator-tissue for assessing P requirements. Using the % of total P in the dry matter as an index: values $>0{\cdot}038\%$ indicate that additional P is not needed, 0·032—0·040 or 0·045% that additional P may possibly be needed, and $<0{\cdot}032\%$ that additional P is needed. P. S. ARUP.

Elements other than nitrogen, potassium and phosphorus in mineral nutrition of sugar cane. H. Evans (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 473—508).—A review of published data concerning deficiency and/or toxic symptoms associated with Ca, Mg, Fe, Mn, Cu, Zn, B, Mo, Al, sulphides, and excesses of sol. salts. (83 references.) P. S. ARUP.

Relative merits of various methods of foliar diagnosis for sugar cane. G. Samuels (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 529—537).—A review of the merits and limitations of five methods. (10 references.) P. S. ARUP.

Influence of age of sugar cane on its leaf-nutrient (N–P–K) content. G. Samuels (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 508—514).—In non-irrigated cane, leaf-N decreases fairly steadily to a low level during 8 months; irrigation retards the rate of decrease. Decreases in P and K are interrupted by temporary minor increases; overall rates are retarded by irrigation. Values are not affected by any seasonal factor. Suggested correction factors for age are tabulated for the leaf-nutrients in irrigated and non-irrigated plant and ratoon cane. Average standard values are given for six degrees of fertility status in Puerto Rico. P. S. ARUP.

Determination of nitrogenous fertiliser requirement of sugar cane crops by foliar diagnosis. P. Halais (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 515—521).—Examples are given of the economic aspects of N-manuring by foliar diagnosis with respect to variety of cane and its capacity for yield-response, and to suitability of location. P. S. ARUP.

[A] Evaluation of rainfall in sugar cane irrigation. R. K. Rogers. **[B] Evapotranspiration of sugar cane in Hawaii as measured by in-field lysimeters in relation to climate.** R. B. Campbell, Jen-Hu Chang and D. C. Cox (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 630—636, 637—649).—[A] Improvement is needed in the uniformity of distribution of irrigation-water and its content of dissolved fertiliser. Tentative rules are suggested on the basis of observations mentioned in the following abstract.

[B] A preliminary report of data on moisture requirements during the growing season, and the extent to which these can be met by rainfall. The ratio of lysimeter water consumption to evaporation, as determined by Weather Bureau pans, increases with the age of the plant from ~0·4 to ~1·0 with a standard deviation of ±0·2 on a large no. of observations. (15 references.) P. S. ARUP.

Drying and maturity of grain sorghum as affected by water loss from plant parts. I. Wikner and R. E. Atkins (*Iowa St. J. Sci.*, 1960, **35**, 25—40).—Loss of moisture from the grain, pedicle, rachis, peduncle, leaves and stalk of four grain sorghum varieties and two hybrids are reported for a period of ten successive harvests. Results were comparatively uniform and followed a linear pattern through the first seven harvests and then tended to level off at 12—13%. A consistent association of rate of moisture loss from the grain with that from other plant parts was not established nor could any consistent relationship between compactness of the head and moisture loss be shown. (13 references.) E. G. BRICKELL.

Acid chlorosis and iron uptake by sorghum Double Dwarf-38 in solution culture. E. B. Kurtz, jun., and R. H. Maier (*Agron. J.*, 1960, **52**, 486—487).—The sorghum variety Double Dwarf-38, widely grown on alkaline soils of Arizona, was grown for 5 weeks with nutrient solutions ranging in pH from 3 to 10. Plants grown at pH 3—6·5 made poor growth and showed severe chlorosis and low uptake of Fe. Plants grown at pH 7 or above made satisfactory growth, showed little or no chlorosis and had a much higher uptake of Fe. The extent of total Fe uptake increased with pH from 7 to 10. A. H. CORNFIELD.

Effects of nitrogen and phosphorus fertilisers on yield of irrigated grain sorghum in southwestern Kansas. G. H. Herron and A. B. Erhart (*Agron. J.*, 1960, **52**, 499—501).—Trials at 19 locations with soils of pH 7·2—8·2 showed that optimum yields of grain sorghum under irrigation occurred with the application of ~90 lb. of N per acre. There were no responses to P applications. The org. matter content of the soils was a poor indicator of the response of sorghum to N fertilisation. A. H. CORNFIELD.

Effect of row spacing, fertiliser and planting rate on the yield and water use of irrigated grain sorghum. K. B. Porter, M. E. Jensen and W. H. Sletten (*Agron. J.*, 1960, **52**, 431—434).—Sorghum grain yields were significantly higher with 12- and 20-in. row spacings than with 30- and 40-in. spacings on a silty clay loam. Planting rate (4—18 lb. seed per acre) had little effect on grain yields, although the high rates produced the heaviest yields of forage. Row spacing and planting rate had little effect on total water use, although water use early in the season was somewhat greater with the narrower spacings. Water used per lb. of grain produced increased with spacing. The effects of the treatments on other plant characteristics are discussed. A. H. CORNFIELD.

Relationship of nitrogen and grain sorghum yields under three moisture regimes. A. C. Mathers, F. G. Viets, jun., M. E. Jensen and W. H. Sletten (*Agron. J.*, 1960, **52**, 443—446).—Sorghum grain yields on a silty clay loam were significantly correlated with initial soil NO_3^--N + fertiliser-N only where fair levels of irrigation water were applied. The relationship held for soil samples taken every ft. down to 6 ft. in the case of the heaviest irrigation treatment. Poor correlations were obtained between grain yields and initial NO_3^--N + nitrifiable N (NO_3-produced during 14 days' incubation at 35°). A. H. CORNFIELD.

Effect of time of application of nitrogen fertiliser on yield of hops. S. N. Brooks and K. R. Keller (*Agron. J.*, 1960, **52**, 516—518).—The application of NH_4NO_3 (100 lb. per acre) at any time from April to July either in one or split application resulted in similar yield increases of hops over 4 years. Application in Aug. or split application in July–Aug. did not increase yields. The treatments had no effect on the α-acid or β-fraction contents of the strobiles. A. H. CORNFIELD.

Chemical composition of irrigation waters in the South Carolina coastal plain and effects of chloride in irrigation water on the quality of flue-cured tobacco. T. C. Peele, H. J. Webb and J. F. Bullock (*Agron. J.*, 1960, **52**, 464—467).—Data for Cl^- and other ions in the water from many ponds, wells and streams are presented. The % of Cl and of reducing sugars in tobacco leaf grown on a loamy fine sand increased with level of Cl^- in the irrigation water (2—225 p.p.m.). Nicotine (%) was not affected by the Cl^- level of the irrigation water, but was in general lower in leaf from irrigated than that from non-irrigated plots. The commercial value of the leaf was not affected by the Cl^- content of the irrigation water, although tobacco grown with water containing Cl^- 225 p.p.m. had the poorest smoking quality. A. H. CORNFIELD.

Soil reaction and rubber cultivation. Anon. (*R.R.I. Plant. Bull.*, 1960, 98—103).—Soil pH may be taken as a measure of the base saturation of the clay fraction and is associated particularly with the levels of the exchangeable bases Ca, Mg and K in the soil. For *Hevea* cultivation the more acid soils are preferable provided they contain satisfactory levels of K and Mg. Soils of higher pH limit the supply of Fe and the availability of rock phosphate. Urea is preferable to $(NH_4)_2SO_4$ when applied as a single N fertiliser. E. G. BRICKELL.

Effects of fertiliser applications on two generations of slash pine. F. Mergen and G. K. Voigt (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 407—409).—Application of 3–12–6 N–P_2O_5–K_2O (5—15 lb. per tree) to 8-year-old slash pines significantly increased N %, but not K %

and P %, in the needles and twigs after 20 months. Fertiliser treatment of 22-year-old trees increased seed wt. This seed produced larger seedlings with higher N % than did seed from control trees. A. H. CORNFIELD.

Pest Control

Promising new fungicide for control of *Pythium* root rot of sugar cane seedlings in flats. C. A. Wismer (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 1133—1137).—The Bayer fungicide no. 22555 (*p*-dimethylaminobenzenediazo-Na sulphonate as a 50% wettable powder) gives adequate protection when applied as a drench at 500 p.p.m. of active material. P. S. ARUP.

Alkoxyphenyl *N*-methylcarbamates as insecticides. R. L. Metcalf, T. R. Fukuto and M. Y. Winton (*J. econ. Ent.*, 1960, **53,** 828—832).—The physical constants of 24 related compounds are given together with their toxicity to housefly larvae, mosquito larvae, *Estigmene acrea* and *Panonychus citri*. The *o*- and *m*-ethoxy- *o*-isopropoxy-, *m*-propoxy-, 3,4-methylenedioxy-, 2,5-dimethoxy- and 3,5-dimethoxyphenyl *N*-methylcarbamates were most active. Piperonyl butoxide was synergistic to all compounds. (21 references.) C. M. HARDWICK.

New techniques for screening cockroach repellants. L. D. Goodhue (*J. econ. Ent.*, 1960, **53,** 805—810).—The slanting card method involves the counting of the no. of cockroaches which climb up impregnated cards placed in a slanting position amongst the colony. The other method uses three interconnected ground glass cylinders in which different treated surfaces are placed. The movement of the roaches between the cylinders is recorded. Typical results are given. C. M. HARDWICK.

Screening soil insecticides against white grubs. G. Wilson (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 978—982).—BHC gives good control (in some cases 100%) of *Lepidiota* sp. nov. (Mossman grub), *L. consobrina*, *L. frenchi*, *Pseudholophylla furfuracea* (childers grub) and *Dermolepida albohirtum* (greyback grub); the lethal effect increases with the concn. (2—32 p.p.m. on dry soil), and the time of exposure (8—49 days). Aldrin and dieldrin are effective against *D. albohirtum*, but not against the other grubs; endrin, chlordane, DDT and thiodan are practically ineffective against any of the grubs. P. S. ARUP.

Loss of activity of chlorinated hydrocarbon insecticides in soil as measured against the Eye gnat, *Hippelates collusor*. M. S. Mulla (*J. econ. Ent.*, 1960, **53,** 785—787).—The biological activity of laboratory-treated soil was measured at 3-monthly intervals. DDT (26 lb./acre) and endrin (4·8 lb./acre) still gave 90% reductions of emerging gnats after 9 months. Lindane at 32 lb./acre gave poor results after 3 months. Dieldrin retained its activity for a year. The effectiveness of aldrin and SD-4402 (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) was greatly reduced initially and this level remained static after 9 months. C. M. HARDWICK.

Chemical structure and insecticidal activity of compounds with a chlorinated cyclopropane ring. H. Komrsová and J. Farkaš (*Coll. Trav. chim. Tchécosl.*, 1960, **25,** 1977—1980).—Chlorinated cyclopropane deriv. (**I**) were prepared from eight styrene deriv., *p*-chlorophenyl vinyl ether (**II**) and two hydrocarbons with two double bonds, by reaction with chlorocarben (CCl_2, from $CHCl_3$ and KOBu^t). Only **II** and a tricyclodecadiene gave **I** of appreciable insecticidal activity. (14 references.) (In German.) M. LAPIDOT.

Assay for the detection of nematode repellants. D. Davis and J. E. Deak (*Plant Dis. Reptr*, 1960, **44,** 622—624).—The assay method described is based on the observation that *Panagrellus redivivus* preferentially collects on areas of a Petri dish harbouring colonies of *Fusarium oxysporium* f. *lycopersici*. A. H. CORNFIELD.

Method of evaluating fungicides in the soil under controlled conditions. J. H. Reinhart (*Plant Dis. Reptr*, 1960, **44,** 648—652).—A method for testing chemicals as soil fungicides is described using *Fusarium oxysporium* f. *cucumerinum* and the cucumber plant as the host–disease complex. A. H. CORNFIELD.

Effect of soil fungicides on soil-borne plant pathogenic bacteria and soil nitrogen. D. E. Munnecke and J. Ferguson (*Plant Dis. Reptr*, 1960, **44,** 552—555).—Application of chloropicrin and methyl bromide to soil in amounts normally used for control of fungi and nematodes was also effective in controlling *Xanthomonas pelargonii*, *Corynebacterium michiganense* and *Agrobacterium tumefaciens*. Na *N*-methyldithiocarbamate was very effective against the last-named organism, but only moderately effective against the first two. The treatments reduced considerably nitrification during incubation of the soil, but reduced ammonification to only a small extent. A. H. CORNFIELD.

Persistence of certain pesticides in the soil and their effect on crop yields. A. W. MacPhee, D. Chisholm and C. R. MacEachern (*Canad. J. Soil Sci.*, 1960, **40,** 59—62).—Arsenic, DDT, BHC and chlordane persist in the soil in this decreasing order. As, DDT and S depress yields, and lime does not correct this except in the case of S. DDT, BHC and parathion are translocated to plant roots and As appears in plants. M. LONG.

Microbiological activity of certain saturated and unsaturated fatty acid salts of tetradecylamine and related compounds. P. M. Borick, M. Bratt, A. G. Wilson, L. Weintraub and M. Kuna (*Appl. Microbiol.*, 1959, **7,** 248—251).—Tetradecylamine (**I**) salts of saturated acids (C_2—C_{18}) showed considerable microbiological toxicity; undecylenates of **I** and of lauryltrimethyl- and *NN*-dimethyloctadecyl-ammonium were effective fungicides and bactericides. A. G. POLLARD.

Toxicity of pentachloronitrobenzene to *Magnolia fuscata*. N. Zummo and A. G. Plakidas (*Plant Dis. Reptr*, 1960, **44,** 559).—A foliar spray of Terraclor (75% pentachloronitrobenzene, 10 g. per gal). or a soil drench (200 lb./acre) caused striking leaf symptoms and defoliation of *Magnolia fuscata*, thus confirming reports of spray drift injury. The foliage-sprayed plants later developed further foliage, which appeared normal. A. H. CORNFIELD.

Effect of soil type and moisture on germination and growth of wheat from seed treated with Phorate. R. E. Kirk and M. C. Wilson (*J. econ. Ent.*, 1960, **53,** 771—774).—Variations in germination in six different soil types indicates that soils with high org. content adsorb the Phorate. Seed viability was reduced by excess moisture in all soils while min. moisture conditions reduced subsequent growth. The interaction of soil type and moisture content is discussed. (11 references.) C. M. HARDWICK.

Experiments with calcium cyanamide against eye-spot lodging of wheat. W. H. Fuchs and F. Grossmann (*Agrochimica*, 1960, **4,** 216—235).—The use of $CaCN_2$ (especially if repeated on two consecutive occasions) greatly reduces infestation by *Cercosporella herpotrichoides*; other N-fertilisers tend to promote the incidence of the disease. P. S. ARUP.

Infection of wheat with *Helminthosporium sativum* in relation to nitrogen content of the plant tissues. P. M. Simmonds (*Canad. J. Plant Sci.*, 1960, **40,** 139—145).—Invasion and colonisation of one-half of a leaf produced variable degrees of chlorosis in the uninvaded half. N was retained and accumulated in the colonised portion of the leaf which also retained its green colour. Leaching readily removed N from the affected tissues. Inoculation of the culms resulted in increased production of low-N kernels. E. G. BRICKELL.

Influence of maternal tissue on loose smut infection of barley kernels. R. Loiselle and R. G. Shands (*Canad. J. Bot.*, 1960, **38,** 741—746).—Depending on the stage of development of the kernel, scanty to abundant mycelium was observed in the crease region, integument, aleurone, endosperm and embryo of susceptible (**I**) and in the chalazal region and parenchyma associated with the vascular bundle in resistant varieties (**II**) after artificial inoculation of *Ustilago nuda*. After floret inoculation, the maternal tissues of F, hybrid kernels of reciprocal crosses between **I** and **II** had mycelial development (**III**) similar to that in the female parental varieties. Hybrid tissues of Odessa (**I**) × Anoidium possessed scanty **III** whereas those of the reciprocal were free. Hybrid tissues of the reciprocal crosses between Odessa and Trebi had scanty **III**. Maternal tissues influenced infection of the hybrid embryo to some extent, but this was secondary to that of the genotype of the embryo itself; 24 photomicrographs. (18 references.) E. M. J.

Sugar beet yellows disease in the U.S. C. W. Bennett (*U.S. Dep. Agric., agric. Res. Serv.*, 1960, *Tech. Bull.* 1218, 63 pp.).—The original and geographical distribution, host range, symptoms of the disease, economic importance, transmission, properties, strains, relation to various vectors, relation to plants, and control measures are described. (61 references.) E. G. BRICKELL.

Control of sugar beet yellows by treatment with systemic insecticides. J. Münster and E. Joseph (*Landw. Jb. Schweiz*, 1959, **60,** 579—595).—Reductions in the no. of infected plants by 85—90% have been achieved by spraying with Thiometon (75 gal./acre in concn. of 0·06%) on the first appearance of winged aphids (*Myzus persicae*, Sulz), and by one repetition at half the above rate, 6—12 days (according to the aphid population) later. Parathion is less persistent in effect. (10 references.) P. S. ARUP.

Effect of topical applications of granulated systemic insecticides and of conventional applications of other insecticides on control of insects and spider mites on sugar beet plants. H. T. Reynolds, T. R. Fukuto and G. D. Peterson, jun. (*J. econ. Ent.*, 1960, **53,** 725—729).—Of the ten compounds tested, granulated Phorate and Disyston topically

applied to the beet plants gave good reductions of *Myzus persicae*, *Empoasca solana* and *Tetranychus cinnabarinus*. No residues were found in large roots but residues in foliage were 0·07 p.p.m. after 29 days. C. M. HARDWICK.

Insecticide row treatments for the control of wireworms in potatoes. R. H. Burrage (*Canad. J. Plant Sci.*, 1960, **40,** 178—182).—Treatments with aldrin or heptachlor dusts, granules, or impregnated fertilisers, at 1 or 3 lb./acre during planting, reduced damage enough to bring the crop to top table stock grade, without culling, although only where potential damage was light. None of the treatments reduced no. of wireworms more than 75% or increased the yield of tubers. E. G. BRICKELL.

Soil insecticides for control of the tuber flea beetle, *Epetrix tuberis*, (Gent.), in the interior of British Columbia. F. L. Banham (*Canad. J. Plant Sci.*, 1960, **40,** 165—171).—Single treatments of aldrin, chlordane, dieldrin or heptachlor applied before or soon after planting at 4·0, 7·5, 1·5 and 3·0 lb. of toxicant per acre, respectively, and immediately incorporated into the soil by disking or harrowing were highly effective. E. G. BRICKELL.

Sprinkler irrigation as a means of applying soil insecticides for the control of the tuber flea beetle, *Epitrix tuberis*, (Gent.), in the interior of British Columbia. F. L. Banham (*Canad. J. Plant Sci.*, 1960, **40,** 172—177).—Dieldrin emulsifiable concentrate applied through sprinkler irrigation systems at the rate of 2 or 2·5 lb./acre and incorporated into the soil by disking or harrowing was highly effective but similar treatment with aldrin emulsifiable concentrate at 4 lb./acre was much less so. Mechanical incorporation is necessary as no appreciable amount of toxicant penetrates below the top inch of soil even when a large amount of irrigation water is applied. E. G. BRICKELL.

Control of stripe smut, *Ustilago striiformis*, (West.) Niessi, on Kentucky bluegrass. R. J. Lukens and E. M. Stoddard (*Plant Dis. Reptr*, 1960, **44,** 672).—Application of 0·05% nabam (1 pint/sq. ft.) in Oct. or May to Kentucky bluegrass infected with stripe smut controlled the disease. Oxyquinoline (0·05%) sulphate was ineffective. A. H. CORNFIELD.

Mercury residues on apple fruit and foliage. R. G. Ross and D. K. R. Stewart (*Canad. J. Plant Sci.*, 1960, **40,** 117—122).—Apple foliage and fruit sprayed with org.-Hg fungicides was investigated. Total Hg on the foliage decreased rapidly in the first 2 weeks after spraying and more gradually in the succeeding 2 months. Pre-cover sprays resulted in negligible fruit residues; the average harvest residue from an early cover spray was 0·05 p.p.m., of which peel, pulp and seeds contained about 41, 57 and 2% respectively. E. G. BRICKELL.

Influence of insecticide–fungicide spray programmes on growth of apple nursery trees. F. L. Gambrell and R. M. Gilmer (*J. econ. Ent.*, 1960, **53,** 717—719).—The greatest reduction in severe infestations of *Eriosoma lanigerum* were given by demeton; lindane was nearly as effective but malathion and DDT were ineffective. 100% reduction of *Empoasca fabae* within 24 h. was given by DDT alone or with malathion, lindane or demeton. After 28 days demeton + DDT with a 70% reduction was best. Trees which received a treatment containing S to control powdery mildew were graded as most vigorous. C. M. HARDWICK.

Three plans for using acaricides to control mites on apple. D. Asquith (*J. econ. Ent.*, 1960, **53,** 735—737).—In trials with Aramite, Kelthane, Tedion and Dimethoate, mixtures of two acaricides at half the standard concn. gave better control of *Panonychus ulmi* and *Tetranychus telarius* than did alternate applications of standard-concn. sprays. C. M. HARDWICK.

Chemical control of the raspberry root borer, *Bembecia marginata*, (Harr.) on loganberry in British Columbia. J. Raine and H. Andison (*Canad. J. Plant Sci.*, 1960, **40,** 160—164).—A single spray of 25% diazinon emulsifiable concentrate at 2 pints per 100 gal. and 0·5 pint of drench per plant (43 gal./acre) in March, April or Oct. reduced a severe infestation (77% or more) to 4% or less of the crowns. The following emulsible concentrates reduced infestation to between 0 and 10%: at 1 pint of drench per crown applied in Oct., lindane at 5 pints, Thimet at 1 pint, or 12008 at 1 pint per 100 gal.; at 2 pints of drench per crown applied in April, Sevin at 8 pints, NC 262 at 0·5 pint, or Phosdrin at 1 pint per 100 gal. E. G. BRICKELL.

Effects of Sevin on phytophagous mites and predators in an Ontario peach orchard. W. L. Putman and D. C. Herne (*Canad. J. Plant Sci.*, 1960, **40,** 198—201).—European red mite (*Penonychus ulmi*, Koch) increased but the brown mite (*Bryobia arborea*, Morgan and Anderson) and the silver mite (*Vasates cornutus*, Banks) decreased after sprays of Sevin. Sevin also practically eliminated the predacious mite *Typhlodromus rhenanus*, Oudms., and larvae and pupae of *Stethorus punctillum*, Weise, and greatly reduced the no. of adults of *S. punctillum*, chrysopid larvae, and spiders. E. G. BRICKELL.

Control of oriental peach moth (*Laspeyresia molesta*, Busck) in Italian Switzerland from 1953 to 1958). P. Bolzani and M. Baggiolini (*Landw. Jb. Schweiz*, 1959, **60,** 621—644).—The only completely effective treatment consists in five to six sprayings with diazinon (at 20 g. of active material per hectolitre) carried out during June–Aug. (12 references.) P. S. ARUP.

Phaltan for the control of grape diseases. E. F. Taschenberg and A. J. Braun (*Plant Dis. Reptr*, 1960, **44,** 560—565).—Excellent control of powdery mildew, downy mildew and black rot of grapes was obtained with 2—3 post-blossom applications of Phaltan (50% *N*-trichloromethylthiophthalimide, 2 lb. per 100 gal.). Foliage injury occurred in only one of three years. Phaltan deposits on grapes were lost rather rapidly by weathering. Phaltan residues on grapes 14 days after application were 8·6 p.p.m., whilst juice from the grapes contained <0·2 p.p.m. A. H. CORNFIELD.

Hexachlorocyclopentadiene, a promising new insecticide for the control of the root form of the grape phylloxera. J. A. Cox, J. Van Geluwe and D. Lawatsch (*J. econ. Ent.*, 1960, **53,** 788—791).—In field tests, with injection rates of >100 lb./acre, hexachlorocyclopentadiene reduced the no. of *Phylloxera vitifoliae* considerably 6 weeks after treatment. At 200 lb. dosage, it was effective 7—10 in. from the point of treatment. Good control was also given by a soil drench. C. M. HARDWICK.

Control of parasitic eelworms in bananas. D. Price (*Trop. Agriculture, Trin.*, 1960, **37,** 107—109).—Preliminary experiments in the Southern Cameroons show significantly improved growth resulting from the watering-on of (diluted) Nemagon 75EC under the slashed-back ground-cover. The economic aspects of different methods of application (of which the above is the most promising) are considered. P. S. ARUP.

Insecticide residues on forage under sprayed pecan trees. M. R. Osburn, L. H. Dawsey and D. W. Woodham (*J. econ. Ent.*, 1960, **53,** 719—721).—Analysis of grass cuttings after spraying during three seasons showed that parathion accumulated and persisted less than either Guthion or EPN. Both decreased by >90% in 13 days. After 13 days parathion residues of <1 p.p.m. are within the tolerance limit for grazing cattle. Toxaphene residues remained at >23 p.p.m. for 70 days. C. M. HARDWICK.

New insecticides for lygus bug control on vegetable seed crops. E. C. Carlson (*J. econ. Ent.*, 1960, **53,** 767—771).—Of nine sprays or dusts, DDT + Dylox gave lowest initial counts of *Lygus hesperus* adults and nymphs and the longest residual control; Thiodan + Dylox was almost as effective. The effect on *Orius tristicolor*, *Hippodamia* spp. and aphids is also given. DDT + Dylox gave the greatest increase in amount and viability of seed while all treatments gave some increase. DDT with Dylox or Trithion and Thiodan alone reduced both bugs on carrot seed plants when dusted from aircraft. The effect on seed quantity and viability is also discussed. C. M. HARDWICK.

Field experiments on the insecticidal control of insects attacking peas, snap and lima beans. R. H. Ratcliffe, L. P. Ditman and J. R. Young (*J. econ. Ent.*, 1960, **53,** 818—820).—Methyl-trithion and Thiodan sprays gave better control of *Macrosiphum pisi* than did Dimethoate or Phosphamidon and did not cause off-flavours. Dimethoate-treated early snap beans had the lowest *Emporasca fabae* counts although all five P compounds gave low counts. Sevin and DDT gave highest percentage of pods free from *Heliothis zea* on late snap and lima beans. C. M. HARDWICK.

Residues in vegetable crops following soil applications of insecticides. R. P. Muns, M. W. Stone and F. Foley (*J. econ. Ent.*, 1960, **53,** 832—834).—Twelve of 15 crops had residues below the 0·3 p.p.m. tolerance after the application and disking-in of chlordane spray (10 lb./acre). DDT at 20 lb./acre gave no residues in 9 of 12 crops and the two others were below tolerance level. Potatoes had 0·5 p.p.m. Injected ethylene dibromide gave 9—11 p.p.m. of inorg. Br in three root crops. Aldrin (4 lb./acre) gave 0·24 p.p.m. residues of dieldrin in radishes while in dieldrin-treated soil, radishes had no residues. Residues from 3 lb./acre of toxaphene in four crops were below 0·4 p.p.m. C. M. HARDWICK.

Control of the onion maggot, *Hylemya antiqua*, (Meig) (Diptera: Anthomyiidae), with insecticides in organic soils of S.W. Quebec. J. P. Perron and J. Lafrance (*Canad. J. Plant Sci.*, 1960, **40,** 156—159).—Dieldrin, heptachlor and endrin wettable powders at 1 oz. per lb. of seed were highly effective; the heptachlor treatment appeared to stimulate plant growth. Toxaphene as seed treatment was poor; Di-syston was effective but reduced germination. Chlordane dust at 4·5 lb./acre gave a fair control where seed had

not been treated. Combinations of dieldrin or DDT seed treatments with chlordane or aldrin soil surface treatments when plants averaged 2 in. high were not more effective than a seed treatment alone. E. G. BRICKELL.

Costa Rican cacao insects: their rôle in cherelle wilt and effect on yield. M. J. Stelzer (*Dissert. Abstr.*, 1960, **21**, 4).—Sucking insects greatly increased the incidence of cherelle wilt on cacao pods. A small Membracid, *Amastris obtegens*, was especially detrimental to pod development. In one area, treatment with dieldrin almost doubled the average no. of pods harvested per tree, and appeared to reduce the incidence of cherelle wilt. In other areas, DDT or dieldrin, alone or with Bordeaux mixture, did not affect the no. of pods harvested, or the extent of infection with *Phytophthora* (black pod). M. D. ANDERSON.

Field experiments with several insecticidal sprays against the boll weevil and bollworm. T. R. Pfrimmer, E. P. Lloyd, M. E. Merkl and R. E. Furr (*J. econ. Ent.*, 1960, **53**, 711—714).—In two seasons, general control of *Anthonomus grandis* and *Heliothis zea*, and increased yields followed spraying with Guthion + DDT, Monsanto CP 7769 [hexaethyl (ethylthiomethylidene) triphosphonate] Sevin, methyl parathion + DDT, toxaphene + malathion or DDT and malathion + DDT or endrin. C. M. HARDWICK.

Field experiments against several late-season cotton insects in 1959. C. B. Cowan, jun., J. W. Davis and C. R. Parencia, jun. (*J. econ. Ent.*, 1960, **53**, 747—749).—The effects of DDT with toxaphene, Strobane, methyl parathion, Guthion and malathion, of four proprietary compounds and of Sevin alone as dusts and sprays against bollweevils and bollworms, *Aphis gossypii*, *Tetranychus dessertorum* and *Alabama argillacea* are recorded. C. M. HARDWICK.

American, Egyptian and Indian cotton-wilt *Fusaria*. G. M. Armstrong and J. K. Armstrong (*U.S. Dep. Agric., agric. Res. Serv.*, 1960, *Tech. Bull.* 1219, 19 pp.).—The pathogenicity of the U.S., Egyptian and Indian cotton-wilt *Fusaria* in cotton and other plants; the reaction of American upland, Egyptian, Indian and several wild cottons to the wilt *Fusaria* from other plants; and attempts to find isolates from the U.S. cotton-wilt *Fusarium* that might show differential pathogenicity for varieties of cotton are discussed together with certain aspects of the wilt–nematode complex. (29 references.) E. G. BRICKELL.

Control of sugar cane borer with insecticides. W. H. Long, E. J. Concienne, L. D. Newsom, S. D. Hensley and W. J. McCormick (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 947—954).—In comparison with seven other insecticides, the best increases in sugar and cane per acre are obtained in Louisiana with endrin granules (in four fortnightly aerial applications at 12 lb per acre). Endrin gives much better results in small granules (made with attapulgite) than when applied as dust. The use of DDT increases borer-damage. P. S. ARUP.

Effect of soil fumigation on growth of sugar cane. H. T. Chu and T. K. Tsai (*Rep. Taiwan Sug. Exp. Sta.*, 1959, No. 20, 73—89).—Improved growth was obtained after soil fumigation with Ortho-fume 85, D–D mixture, formalin or Chlorofin 22. Ortho-fume 85 gave best results, especially on sandy or sandy loam soils, with spring plantings. Nematode population in the soil and root stocks was significantly reduced by fumigation (except with formalin). (13 references.) (From English summary.) E. C. APLING.

A five-year report of observations in Japanese beetle control area at Sheldon, Illinois. W. H. Lukmann and G. C. Decker (*J. econ. Ent.*, 1960, **53**, 821—827).—Large-scale treatment with granulated dieldrin (2—3 lb./acre) eliminated *Popillia japonica* larvae. The effect on >40 species of insects is given. Pastures treated with 20—30 lb./acre of 10% granulated dieldrin did not affect livestock but spray drift from 3 lb./acre treatments was toxic. The residues present on forage varied with its condition. C. M. HARDWICK.

Effects of low doses of ethylene dibromide on some stages of confused flour beetle, *Tribolium confusum*. S. R. Loschiavo (*J. econ. Ent.*, 1960, **53**, 762—767).—The mortality of treated adults was negligible until the sixth day. Total mortality was higher for females. Treated females laid few eggs and many of these did not hatch. The treatment of males did not reduce fecundity. Doses of <1 mg./l. did not affect the hatchability of one-day-old eggs but 4-day-old eggs were susceptible to 0·5 mg./l. Most 8-day-old pupae treated with 2 mg./l. failed to metamorphose but 2-day-old pupae were little affected. Resistance in the field is discussed. (17 references.) C. M. HARDWICK.

Sampling lucerne for the determination of insecticide residues. E. W. Huddleston (*Dissert. Abstr.*, 1960, **20**, 4471—4472).—Analyses of methoxychlor residues on lucerne foliage showed a great reduction in variation as the no. of samples was increased from 2 to 7 per plot, a smaller effect with increase to 14 per plot and a still smaller effect with increase to 21 per plot. A sampling scheme was devised to incorporate the advantages of stratified, systematic and random sampling. Variance component analysis techniques were used to study the optimum allocation of material from different locations. M. D. ANDERSON.

Simple method for estimating oil-mist deposit on banana leaves. L. Calpouzos and T. Theis (*Trop. Agriculture, Trin.*, 1960, **37**, 51—52).—The oil-indicator card method of Davis and Elliott (cf. *J. econ. Ent.*, 1953, **46**, 696) for evaluating aerial spray deposits on plants of oil or oil-based fungicides is described. P. S. ARUP.

I. Colorimetric methods for the determination of 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine (Simazine) and related compounds. II. Degradation of ^{14}C-labelled Simazine by plants and soil micro-organisms. M. T. H. Ragab (*Dissert Abstr.*, 1960, **20**, 4507—4508).—I. Simazine (**I**) with pyridine in presence of alkali gives a yellow colour, which may be measured at 436·5 mμ, fading rapidly. Immediate addition of ethyl cyanoacetate to the yellow colour gives a more stable pink colour, measurable at 550 mμ. Destroying the yellow colour by AcOH and adding barbituric acid gives a reddish-violet colour measured at 582 mμ; 2-thiobarbituric acid gives a blue colour measured at 625 mμ. Saturation of the pyridine with glycine enhanced colour in all four procedures.

II. Sweet maize and cucumber seedlings were treated with **I** labelled with ^{14}C in the triazine ring, and expired $^{14}CO_2$ was determined. Radioactivity from cucumber (susceptible to **I**) was initially much higher than that from sweet maize (resistant). Analysis of extracts of parts from both spp. showed that both adsorb, translocate and metabolise **I**, more activity being found in roots than in leaves or stems, and more in cucumber than in sweet maize. Collection of CO_2 from sterile and non-sterile soils treated with **I** showed that soil micro-organisms decompose **I**. M. D. ANDERSON.

Chemical weed control in Hawaii. N. S. Hanson (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 538—549).—A review including methods of testing toxicities of herbicides to weeds and crops, and results obtained. P. S. ARUP.

Evaluating herbicides in sugar cane cultivation. E. Rochecouste (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 549—555).—A review and discussion of methods used in Mauritius, covering the lay-out of plots and assessment of weed-populations and -eradication, of injury to cane, and of effects on yields of cane and sucrose. P. S. ARUP.

Effects of arboricides on East African trees and shrubs. IV. *Tarchonathus camphoratus*, L. and *Euclea divinorum*, Hiern. G. W. Ivens (*Trop. Agriculture, Trin.*, 1960, **37**, 143—152).—*T. camphoratus*, if not previously cut, can be killed by applications of 2,4,5-T (ester, 1·5%) in diesel oil to basal ring-barked surfaces, the treatment being preceded and followed by frilling with 2,4-D (ester, 2·5%) in diesel oil. Under similar treatment, *E. divinorum* cannot be prevented from regenerating. Regenerative growth of the trees cannot be permanently checked. P. S. ARUP.

Effect of Silvex (2,4,5-T-P) as chemical herbicide on sugar cane in Louisiana. E. R. Stamper and S. J. P. Chilton (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 537—538).—As judged by yields of cane, Silvex (at 1 lb./acre) as a pre- and post-emergence spray appears to have a consistently small, but not significant, advantage over dalapon and over 2,4-D + TCA mixture. Silvex (and aminotriazole) give good control of "wild lettuce" (*Lactuca* spp.). P. S. ARUP.

Factors affecting the herbicidal toxicity of ethyl di-n-propylthiol-carbamate. A. K. Ghosh (*Dissert. Abstr.*, 1960, **20**, 4481—4482).—Pre-emergence treatments of maize and soya-beans with ethyl di-n-propylthiolcarbamate (EPTC) controlled weeds less effectively, but caused less damage to crops, than incorporating the herbicide with the soil. Weed control and crop damage were related linearly to dose of EPTC. EPTC incorporated with dry soil did not injure oat seeds left in the soil for 6 weeks and then germinated in fresh soil. EPTC at 100 p.p.m. was toxic to germinating maize seeds; length of shoots was decreased with lower concn. EPTC was highly volatile from a free surface, and did not move in wet or dry soil, probably because of adsorption. EPTC incorporated with soil at 4 lb./acre showed residual activity after 11 weeks, greater in dry than in moist soil. Weather affected EPTC incorporated with soil much less than it did EPTC used for pre-emergence treatments. M. D. ANDERSON.

Effect of chemicals as herbicides alone and in combination for weed and grass control in Louisiana sugar cane. E. R. Stamper and S. J. P. Chilton (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 556).—For control of weeds and Johnson grass in plant cane by pre-emergence and later applications, the effect of

trichloroacetic acid (TCA) alone as shown by increased sugar yields is somewhat, but not significantly improved by combination with dalapon and 2,4-D. For heavy infestations of the grass in stubble cane, early applications of TCA (11 lb./acre) followed by dalapon (4—5 lb.) give the best control with increased sugar yields. Decreased yields follow the use of >5 lb. of dalapon per acre. P. S. ARUP.

Reactions of tobacco plants to some herbicides. W. Wurgler (*Landw. Jb. Schweiz*, 1959, **60**, 597—604).—Herbicides based on phenoxyalkylcarboxylic acids (2,4-D, MCPA, MCPB) or triazine even in low concn., cause various distortions and abnormalities in the plants. P. S. ARUP.

Effects of 2,4-dichlorophenoxyacetic acid on the growth and respiratory metabolism of tissue from the red kidney bean plant, and of the antagonistic effects of various uncoupling agents toward the growth-promoting effects of the auxin substance. G. K. Burlingham (*Dissert. Abstr.*, 1960, **21**, 30).—Decotyledonised cultured kidney-bean embryos, and sections of bean hypocotyl tissue, when treated with 2,4-D, were insensitive to 2,4-DNP, which increased the O_2 consumption of similar untreated tissues (but not of untreated first internode or bud tissues). Several uncoupling agents, including 2,4-DNP, inhibited the abnormal growth of hypocotyl sections induced by 2,4-D. Abnormalities in bean plants induced by spraying with 2,4-D were prevented by simultaneous spraying with 2,4-DNP. The supply of ATP within the mitochondria apparently decreased in presence of 2,4-D, which seems to divert metabolic energy into abnormal growth reactions that are especially sensitive to the effects of uncoupling agents. M. D. ANDERSON.

Growth of *Sorghum halepense* under controlled conditions and physiological responses of plants to 2,2-dichloropropionic acid. M. Ingle (*Dissert. Abstr.*, 1960, **21**, 15—16).—The vegetative growth of seedlings of *S. halepense* increased with the photoperiod at 27° and 32° but was little affected at 21°. Lower temp. in the dark period reduced vegetative growth. Flowering was more abundant with shorter photoperiod. Of herbicides tested, 2,2-dichloropropionic acid (**I**) was the most toxic to buds; it was translocated to the rhizomes, and retained the ability to inhibit sprouting for at least 4 weeks. Germination of grass seeds was inhibited by 5×10^{-3}M-**I**, and of dicotyledons by 10^{-2} M; 10^{-3}M inhibited the elongation of maize and cucumber roots. Concn. inhibiting growth inhibited phosphate esterification by about 25%, and also inhibited uptake of P by carrot slices, and the activity of glutamic acid decarboxylase. Inhibition was not reversed by pantothenic acid, L-pantoic acid, or β-alanine. M. D. ANDERSON.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 828,671, 13.12.57. Ger., 21.12.56).—Compounds $(OR)_2PX{\cdot}S{\cdot}[CH_2]_n{\cdot}NR'R''R'''{\cdot}Br$ are obtained by interaction of $NR'R''R'''$ with $(OR_2)PX{\cdot}S{\cdot}[CH_2]_n{\cdot}Br$ in an inert solvent (R is alkyl of 1—4 C; R′—R‴ are hydrocarbon groups; X is O or S; *n* is 2—3). They are useful as insecticides (e.g., against spider mites and caterpillars) and compositions containing them (0·00001—1%) for use as such are claimed. The prep. is described of OO-*Et*$_2$ S-2-(*trimethylammonium*)*ethyl thiophosphate bromide*, which is hygroscopic and is readily sol. in water; at 0·001% concn. in water it is 100% lethal to aphids. F. R. BASFORD.

Herbicidal compositions. Shell Research Ltd. (Inventors: J. T. Hackmann and P. ten Haken) (B.P. 828,871, 12,2,57).—Compositions for use in the control of the growth of plants comprise a liquid or solid carrier, a surface-active agent, and (as herbicidal component) a compound $Y{\cdot}CO_2{\cdot}CHClR$ (also claimed) (Y is O·CHCl·R, OR′ or $NR'R'''$; R is hydrocarbon radical of 1—4 C or Ph optionally substituted by halogen; R′ is hydrocarbon radical of 1—8 C; R″ and R‴ are H, aliphatic group of 1—4 C, or together with N comprise a heterocyclic radical). Compounds specifically claimed include: 1,2,2,2-*tetrachloroethyl allylcarbamate* and *diallylcarbamate*. F. R. BASFORD.

Substituted halogenated bicycloheptenes. Velsicol Chemical Corp. (B.P. 827,435, 3.1.58. U.S., 8.1.57).—Treatment of 1,2,3,4,7,7-hexachlorobicyclo(2,2,1)hepta-2,5-diene with O_2 at 75—150°/1—200 p.s.i. in presence of Cl_2 as catalyst gives *inter alia* 1,2,3,4,7,7-hexachloro-6-hydroxy-5-oxobicyclo(2,2,1)hept-2-ene and 3,4,5,6,8,8-hexachloro-3,6-methano-2-oxo-1-oxacyclohept-4-ene useful as herbicides and insecticides. H. S. R.

Antifungal compositions and procedure for protecting articles therewith. Établissements Sergent Laboratoires Prolac (Inventor: J. Ploquin) (B.P. 827,434, 31.12.57).—An improved, synergistic antifungal composition, suitable for incorporating into emulsions, creams, paints and especially useful for protecting early fruits or vegetables, tobacco leaves, cheese rinds, etc. against attack by moulds, comprises an oxygenated acid and anhydride B derivative (or an ester or salt thereof), e.g., B_2O_3, HBO_2, H_3BO_3 or $H_2B_4O_7$ (3—15 wt.-%); an org. compound containing two similar or different radicals selected from OH, NH_2 and NH, e.g., tartaric acid, lactic acid, salicylic acid, halogenated salicylic acid, a glycol, glycerol or an *o*-diphenol (0·2—5 mol. per mol. of B compound); and a base (hydroxide of Cu, Zn, K, Na, Ca or NH_4, ethanolamine, cyclohexylamine or morpholine), to give pH ≮9. A typical composition contains boric acid, lactic acid and triethanolamine. F. R. BASFORD.

Halonitrostilbenes. Dow Chemical Co. (Inventor: D. N. Robertson) (B.P. 827,357, 20.6.58).—α-Nitrostilbenes with one or both of the phenyl groups having a chloro (halogen) substituent and optionally other groups are prepared for use *inter alia* as agricultural chemicals and insecticides. A description is given of the prep. of *2-chloro-α′-nitrostilbene*, m.p. 90·2—91·2°. H. S. R.

Aryloxyaliphatic compounds. May & Baker Ltd. (Inventors: B. J. Heywood and W. G. Leeds) (B.P. 827,372, 2.3.56).—Compounds $4,6,3,1\text{-}Cl{\cdot}C_6H_2XY{\cdot}O{\cdot}R{\cdot}OH$ and herbicidal compositions containing them are claimed (X is Cl and Y is H or Cl, or X is Me or H and Y is H; R is hydrocarbon chain of 4 C, optionally substituted by Me on the C adjacent to phenoxy radical and optionally containing a double bond). They may be made by the usual methods employed in the synthesis of phenoxyalkanols, or by reduction of the corresponding aryloxybutyric acids or esters, e.g., with $LiAlH_4$. In an example, the prep. of 4-(*4′-chloro-2′-methylphenoxy*)*butyl bromide*, b.p. 193—197°/16 mm., is detailed. F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 828,636, 12.3.57. Ger., 12.3.56).—Compounds, useful as insecticides and plant-protectants, comprise thiophosphoric acid esters, viz., $(OR)_2PX{\cdot}R'{\cdot}YR''$ [R is alkyl of 1—4 C; X is O or S; R′ is alkylene of 2—4 C; Y is COS, CS_2 or $SO_2{\cdot}S$; R″ is alkyl which may be substituted by aryl (optionally carrying alkyl, halogen or NO_2), or a substituted primary or secondary amino group]. Details are given of the prep. of *Et*$_2$ 2-(*trichloroacetylthio*)*ethyl phosphothionate*, b.p. 110°/0·01 mm. F. R. BASFORD.

Fungicidal compositions. Poudreries Réunies de Belgique (B.P. 828,904, 8.3.56. Belg., 16.4.55).—Improved fungicidal compositions, which have high cryptogamic properties combined with absence of phytotoxic action on plants, are essentially dispersions of 1-chloro-2,4-dinitronaphthalene as active ingredient in an inert solid diluent or in an oil emulsion. H. L. WHITEHEAD.

Thiomethylals. Farbenfabriken Bayer A.-G. (B.P. 827,493, 19.2.58. Ger., 1.3.57).—Compounds $4,3,1\text{-}C_6H_3ClR{\cdot}S{\cdot}CH_2{\cdot}SR'$ (R is H or Cl; R′ is cycloalkyl of 5—6 C, alkyl of 5—12 C, halogenoalkyl or halogeno-substituted $PhS{\cdot}CH_2$), useful as insecticides and plant protectants (claimed) are obtained by interaction of $SR'{\cdot}CH_2Cl$ with $4,3,1\text{-}C_6H_3ClR{\cdot}SH$, or of R′SH with $4,3,1\text{-}C_6H_3ClR{\cdot}S{\cdot}CH_2Cl$. 1-*Chloro*-4-(*dodecylthiomethylthio*)*benzene*, b.p. 167°/0·01 mm., is prepared, (a) 0·1% solution of which is 100% lethal to mosquito larvae, aphids and spider mites. F. R. BASFORD.

Cyanoformamide. American Cyanamid Co. (B.P. 829,029, 10.1.57. U.S., 27.1.56).—$CN{\cdot}CONH_2$ is economically obtained in good yield by heating C_2N_2 with a mixture of water (≮1) and an aliphatic carboxylic acid, e.g., HCO_2H, AcOH, $CH_2Cl{\cdot}CO_2H$, lauric acid or succinic acid (≮1 mol.) at 50—90°, optionally in an inert polar solvent, e.g., MeCN. The product has good lethal activity to nematodes and rodents. F. R. BASFORD.

Streptomycin antibiotic copper complex. C. Pfizer & Co. Inc. (B.P. 834,325, 10.7.57. U.S., 25.7.56).—A streptomycin antibiotic is mixed with 0·5—3·0 mol. proportions of insol. Cu salt, e.g., with 1 mol. proportion of $CuCl_2$ or $CuSO_4$. The products are useful as fungicides and bactericides in agriculture. H. S. R.

Animal Husbandry

Dry matter digestion *in vitro* of forage crops. K. W. Clark and G. O. Mott (*Canad. J. Plant Sci.*, 1960, **40**, 123—129).—An artificial rumen technique was applied to medium maturing timothy grass. With one exception there was no significance in digestibility between the clones harvested in the early-leaf stage but significant differences were obtained at later stages of maturity which may be a function of the leaf–stem ratio, the amount of leaf firing and thickness of culms as well as changes in chemical composition. The technique is useful for the plant breeder provided certain precautions are taken. All lines to be screened should be included in a single trial to ensure max. control of variables. Samples should be uniformly processed, preferably freeze-dried, and digested with a single sample of rumen fluid. E. G. BRICKELL.

Digestibility of forage on burned and non-burned bluestem pasture as determined with grazing animals. E. F. Smith, V. A. Young,

K. L. Anderson, W. S. Ruliffson and S. N. Rogers (*J. Anim. Sci.*, 1960, **19**, 388—391).—Burning the pasture in spring increased the apparent digestibility of the dry matter and crude fibre of the subsequent herbage. The digestibility of the N-free extract was increased in half the trials but that of protein was unaffected by burning. A. G. POLLARD.

Effects of stage of maturity, dehydrating vs. field-curing and pelleting on lucerne hay quality as measured by lamb gains. J. H. Meyer, W. C. Weir, L. G. Jones and J. L. Hull (*J. Anim. Sci.*, 1960, **19**, 283—294).—In lucerne hay harvested at different stages of maturity the critical turning point in feeding value for sheep occurred at the 10% bloom stage. Differences between values at the bud and the bloom stages were more apparent in field-cured than in artificially-dried material. In general artificially-dried was superior to field-cured hay; pelleted hay gave better results than did chopped hay. A. G. POLLARD.

Chemistry and microbiology of forage-crop silage. A. G. Kempton and C. L. San Clemente (*Appl. Microbiol.*, 1959, **7**, 362–367).—Changes in the volatile fatty acid, amino-acid and volatile base contents of green material during ensilage are recorded and the influence of moisture, pH and types of silo on these changes are discussed. Spoiled silage [high butyric acid (**I**) content] contained the same no. of lactic acid bacteria as did well preserved silage throughout the fermentation. The production of **I** in spoiled silage is ascribed to the action of lactate-fermenting *Clostridium* spp. A. G. POLLARD.

Oestrogenic activity in dehydrated and sun-cured forages. E. M. Bickoff, A. L. Livingston, A. N. Booth, A. P. Hendrickson and G. O. Kohler (*J. Anim. Sci.*, 1960, **19**, 189—197).—The apparent oestrogen contents of various commercially dehydrated and sun-cured forages are determined (mouse uterus test). Differences in values were attributable to growth conditions, stage of growth at harvest, conditions of drying and storage and to the presence of oestrogen potentiators and inhibitors in the forage. A. G. POLLARD.

Comparison of the clipping and chromogen–chromic oxide methods for pasture evaluation using various forage mixtures. G. C. Marten, W. F. Wedin and J. D. Donker (*Agron. J.*, 1960, **52**, 542—544).—The chromogen–Cr_2O_3 method of measuring dry matter consumption by grazing dairy cows was more sensitive than the clipping (mower-strip) method. The clipping method was unreliable for measuring dry matter consumption. A. H. CORNFIELD.

Vegetation and cattle responses to different intensities of grazing on short-grass ranges on the Central Great Plains. G. E. Klipple and D. F. Costello (*U.S. Dep. Agric.*, 1960, *Tech. Bull.* 1216, 82 pp.).—A report covering the years 1940 to 1953. (39 references.) E. G. BRICKELL.

Net energy of blackstrap molasses for fattening steers as determined by a comparative slaughter technique. G. F. Lofgreen and K. K. Otagaki (*J. Anim. Sci.*, 1960, **19**, 392—403).—Molasses was added at rates of 10, 25 or 40% to fattening ration for steers. The 10% rate increased fat deposition without appreciable increase in rate of gain in live-wt. The higher proportions of molasses diminished both fat deposition and rate of gain in wt. as compared with the basal ration. With rise in amounts of molasses fed the experimentally determined total digestible nutrient and digestible energy of the ration declined somewhat (5—8%); the net energy decreased markedly (up to 50%). A. G. POLLARD.

Effect of origin, processing and storage on the unidentified growth factor activity of a variety of fish meals. T. W. Sullivan, B. D. Barnett, H. R. Bird, N. L. Karrick and L. McKee (*Poultry Sci.*, 1960, **39**, 1037—1041).—Unidentified growth factor activity was found in a wide variety of fishery by-products (menhaden meals, herring meal, shrimp meal, tuna wastes, salmon wastes, etc.). Storage in air at room temp. for 18 months did not alter the growth-promoting activity of menhaden meal samples. The normal heat treatments used in processing fish meals did not reduce the potency of the growth factor in tuna meals. A. H. CORNFIELD.

Selenium in its relation to muscular dystrophy and dietary liver necrosis. I. Muscular dystrophy in the lamb. II. Studies with laboratory animals. J. F. Proctor (*Dissert. Abstr.*, 1960, **21**, 10).—The incidence of muscular dystrophy in lambs born of ewes on a low-Se diet was reduced from 54% to 5% by adding 1 p.p.m. of Se as Na_2SeO_3 to the food of the ewes; to 10% by 100 i.u. of vitamin E/ewe/day; and to 14% by ¼ lb. of linseed oil meal (containing 1·18 p.p.m. of Se)/ewe/day. Muscular dystrophy was completely prevented by 50 i.u. of vitamin E or 0·5 mg. of Se given daily to each lamb from birth to 60 days of age. Vitamin E given to ewes or lambs increased plasma tocopherol; Se did not. Se given to ewes increased the Se content of their milk. Oral administration of Se to lambs with muscular dystrophy had a curative effect. Rabbits fed a semi-purified diet containing *Torula* yeast developed muscular dystrophy, not prevented by addition of Se and/or vitamin E to the diet. The incidence of muscular dystrophy in rats on this diet was reduced from 100 to 60%, and that of liver necrosis from 100 to 20%, by 1 p.p.m. of Se in the food; rough hair was prevented, and haemoglobin in the blood increased. M. D. ANDERSON.

Feed spoliage. Inhibition of mould growth by γ-radiation. B. D. Webb, H. D. Thiers and L. R. Richardson (*Appl. Microbiol.*, 1959, **7**, 329—333).—Mould growth in ground maize having >15·5% of moisture was prevented by irradiation in polythene bags, with a dosage of 0·25 Mrad; with moisture contents up to 22% the dosage needed was up to 1 Mrad. A. G. POLLARD.

Relative growth and appearance of young dairy calves fed two levels of milk with a simple or complex calf starter. H. N. Harrison, R. G. Warner, E. G. Sander, J. K. Loosli, S. T. Slack and K. L. Turk (*J. Dairy Sci.*, 1960, **43**, 1084—1093).—In a 7-week factorial growth experiment with 160 heifer-calves, average gains in wt. at a 250-lb. level of milk feeding were slightly lower than gains at the 350-lb. level. The simple and complex starters fed and compared at both levels, proved equally efficient. About 16% of the calves at the low milk-level would have required extra care to thrive as well as the rest. The cost of the 250-lb. milk-level was 28% lower than that of the higher level. (21 references.) P. S. ARUP.

Effects of feeding Aurofac on growth of Sindhi × Jersey male calves to six months of age. J. G. Velu and A. Reed (*J. Dairy Sci.*, 1960, **43**, 1094—1098).—Graduated supplementation with Aurofac (containing Aureomycin and vitamin B_{12}) caused significant improvements in gains in wt. (by 18%) and height at withers (by 24%) of calves reared in India. P. S. ARUP.

[A] **Effects of age and diet on secretion of pregastric esterase in calves.** J. W. Young, H. A. Ramsey and G. H. Wise. [B] **Effects of continuous nursing, length of nursing period and rate of milk consumption on secretion of pregastric esterase by calves.** H. A. Ramsey, J. W. Young and G. H. Wise (*J. Dairy Sci.*, 1960, **43**, 1068—1075, 1076—1083).—[A] The output of the enzyme (determined by sham-feeding with non-fat milk) is unaffected by the age (up to 180 days or possibly 1 year) or by the diet, whether exclusively whole milk or whole milk and hay or grain. The secretion, however, appears to be stimulated by feeding from a nipple-pail to a greater extent than by the consumption of hay or grain. No connexion is found between the total vol. of saliva secreted and the output of the enzyme. (14 references.)

[B] When calves on the above diets were sham-fed with non-fat milk (50 lb. in ten consecutive 5-lb. increments) the esterase content decreased progressively, especially after the third increment; the enzyme, therefore, probably accumulates between feeding periods. The enzyme content of single 5-lb. units was increased by ~200% when the feeding times were increased from ~1 to 11 min. The secretion is higher at morning than at evening feeding. P. S. ARUP.

Effect of chlorpromazine on performance of lactating dairy cows. L. J. Bush, M. G. Yang and G. V. Odell (*J. Dairy Sci.*, 1960, **43**, 1118—1123).—In 4-day trials, tranquillisation could be effected by single daily oral doses of 5 g., but not when the doses were divided into two equal portions. Daily dosing with 0·5 g. during 3 weeks had no effect on milk production. (16 references.) P. S. ARUP.

Milk fever in dairy cows. VI. Effect of three prepartal dosage levels of vitamin D on milk fever incidence. J. W. Hibbs and H. R. Conrad (*J. Dairy Sci.*, 1960, **43**, 1124—1129).—In a 7-day dosing experiment involving 164 parturitions, by Jersey cows with an overall 64% expectation of milk fever, planned to end on the day after parturition, no statistical difference is found as between protection rates afforded at the three levels of dosing (15, 20 and 30 million units per day). As the average rates are ~67, 82 and 79%, respectively, a min. dose of 20×10^6 units is recommended. Similar results are recorded for three different prep. Protection increases to a high level during the first three days, remains constant up to the last day, and then decreases rapidly. P. S. ARUP.

Effects of time and level of supplementation on beef steers fed lucerne soilage or hay. G. P. Lofgreen, J. H. Meyer and N. R. Ittner (*J. Anim. Sci.*, 1960, **19**, 156—163).—For steers receiving the max. proportion of lucerne hay or soilage, supplementary concentrates of barley + sugar beet pulp were more effective when given continuously than when only in the second half of the feeding period if the criterion is the increase in energy of the wt. gained and not the actual gain in wt. A. G. POLLARD.

Nutritive values of beef cattle rations containing artificially dried maize. D. C. Clanton, M. L. Hemstrom and J. Matsushima (*J. Anim. Sci.*, 1960, **19**, 376—380).—In feeding trials with steers,

maize dried artificially at 130—190° showed no differences from that dried at ordinary temp. in digestibility or metabolic energy.
A. G. POLLARD.

Effect of arsanilic acid in counteracting selenium poisoning in beef cattle. J. A. Minyard, C. A. Dinkel and O. E. Olson (*J. Anim. Sci.*, 1960, **19**, 260—264).—Evidence is presented of increased growth of calves on seleniferous pastures or Se-bearing rations following the administration of arsanilic acid. A. G. POLLARD.

Influence of three different feeding levels during growth and gestation on reproduction, weight gains and carcass quality in swine. H. L. Self, R. H. Grummer, O. E. Hays and H. G. Spies (*J. Anim. Sci.*, 1960, **19**, 274—282).—Restricted feeding (⅓ and ⅔ normal) did not affect significantly the litter size, average birth wt. or no. of pigs weaned. Comparative effects of the three feeding levels on rates of gain in wt., feed consumption and efficiency and carcass characteristics are recorded. A. G. POLLARD.

Black-eyed peas as swine feed. H. Heitman, jun., and J. A. Howarth (*J. Anim. Sci.*, 1960, **19**, 164—166).—Addition of black-eyed cowpeas (*Vigna sinensis*) to a ration for pigs of about 80 lb. live wt. at the rate of 20 or 50% resulted in reduced feed consumption and utilisation and lowered rates of gain in wt. No toxic symptoms were apparent. A. G. POLLARD.

Chlortetracycline and oxytetracycline at high levels in a protein supplement for growing-finishing swine. J. H. Conrad and W. M. Beeson (*J. Anim. Sci.*, 1960, **19**, 363—367).—Addition of chlortetracycline to the protein supplement for pig rations (50—250 g./ton) increased the rate of gain in wt. to similar extents at all levels of administration. Oxytetracycline did not affect growth rates. Both antibiotics increased the daily feed intake with altering the feed efficiency. A. G. POLLARD.

Effect of organic and inorganic sources of unidentified growth factors on the growing pig. D. L. Jeter, J. H. Conrad, M. P. Plumlee and W. M. Beeson (*J. Anim. Sci.*, 1960, **19**, 226—237).—A semi-purified ration containing maize starch, Cerelose and soya-bean protein (Ca 0·68%, Zn 16 p.p.m.) was inadequate for the normal growth of weanling pigs and caused parakeratosis. Both effects were corrected by increasing the Zn content to 50 p.p.m. The ash of brewers' yeast gave partial protection against the disorder probably due to its Zn content. Distillers' solubles contained a potent unknown growth factor other than Zn which was extracted by methanol. A. G. POLLARD.

Comparison of different methods of iron administration on rate of gain and haemoglobin level of the baby pig. R. C. Wahlstrom and E. W. Juhl (*J. Anim. Sci.*, 1960, **19**, 183—188).—Injection of an Fe–dextran solution (Fe 100 mg.) in day-old pigs resulted in higher haemoglobin levels up to 28 days and heavier pigs at this age, than did 250 mg. of peptonised Fe injected at 1 and 21 days of age or a pill containing Fe 290 mg. with $CoSO_4$ and $CuSO_4$ given at 1, 10 and 21 days. Fe–dextran injections containing 100 or 150 mg. of Fe given at 1 or 7 or 1 and 21 days of age produced similar body wt. at 56 days. A. G. POLLARD.

Interrelationships between calcium, zinc, iron and copper in swine feeding. J. A. Hoefer, E. R. Miller, D. E. Ullrey, H. D. Ritchie and R. W. Luecke (*J. Anim. Sci.*, 1960, **19**, 249—259).—Effects of adding Zn (50 or 75 p.p.m.), Fe (100 p.p.m.) and Cu (125 p.p.m.) to rations of varying Ca content (0·55—1·31%) for weanling pigs are examined. With all levels of Ca parakeratosis developed and was corrected by Zn; Fe was ineffective with the highest levels of Ca whereas Cu had a significant effect especially at high Ca levels. Additions of the trace elements increased the haemoglobin and haemotocrit values. A. G. POLLARD.

Quality and yield of modern and old-type chickens. G. L. Gilpin, A. M. Harkin, R. A. Redstrom and E. H. Dawson (*Poultry Sci.*, 1960, **39**, 924—930).—Some quality aspects and meat yields of roasted 3- and 5-lb. male and female chickens of both a fast-growing, modern breed and a slow-growing, old-type breed reared on both a 1856-type and a 1930-type ration are reported.
A. H. CORNFIELD.

Chick diet studies. I. Discrimination between diets of differing nutritional value. F. D. Wharton, jun., J. C. Fritz and L. J. Classen (*Poultry Sci.*, 1960, **39**, 1018—1023).—Chicks were not instinctively capable of distinguishing between a complete diet and one deficient in vitamin D, thiamine or choline, especially when the deficient diet was given for some days prior to a choice of diets. There was a slight tendency, particularly towards the end of the 4-week test, for the complete diet to be preferred to the choline- or thiamine-deficient diets when birds were given the choice from one day of age.
A. H. CORNFIELD.

Dietary interrelations between methionine, glycine, choline, protein level and energy content of the chick diet. W. R. Featherston and E. L. Stephenson (*Poultry Sci.*, 1960, **39**, 1023—1029).—Addition to a maize–soya-bean oil-meal diet of 0·1% of methionine hydrochloride increased, whilst 0·3% of glycine had no effect on, wt. gains of chicks to 4 weeks of age. Methionine increased feed efficiency in only one of four trials. Choline (6·9—26·9 g. per 100 lb. of feed) increased wt. gains in only one of three trials, and had no significant effect on feed efficiency. A 21%-protein diet usually produced better wt. gains than did 18%- or 24%-protein diets. Addition of 3—16% of animal fat sometimes increased wt. gains and improved feed efficiency. A. H. CORNFIELD.

Effect of dietary energy and protein levels and energy source on White Leghorn hens in cages. F. R. Frank and P. E. Waibel (*Poultry Sci.*, 1960, **39**, 1049—1056).—Caged hens maintained adequate egg production on diets containing protein 14·9% and 782 or 1167 kcal. of productive energy per lb. The latter diet gave somewhat higher egg production. Hen wt. and serum cholesterol levels were higher with the higher energy diet but were not affected by dietary protein level (10·2—29·9% of protein). Hens receiving 10% of bleachable fancy tallow, beef tallow, hog grease, maize oil or safflower oil showed greater depot and carcass fat than did those receiving a maize–soya-bean meal diet. A. H. CORNFIELD.

Effect of energy level and laying house temperature on the performance of White Leghorn pullets. C. F. Petersen, E. A. Sauter, D. H. Conrad and C. E. Lampman (*Poultry Sci.*, 1960, **39**, 1010—1018).—Egg production of hens fed the high-energy (910 kcal./lb.) diet in mash or crumbled form and the low-energy (650 kcal./lb.) diet in crumbled form was very similar, whilst the low-energy diet in mash form resulted in lower egg production. Maintaining a reasonably uniform laying house temp. resulted in somewhat higher egg production than did a more variable temp. Egg wt. and albumin index were not affected by the treatments. Litter condition was best with the high-energy and uniform temp. treatments.
A. H. CORNFIELD.

Effect of water restriction on chicks fed different levels of molasses. E. Ross (*Poultry Sci.*, 1960, **39**, 999—1002).—Restricting the water intake of chicks to three 30-min. periods per day resulted in reduced wt. gains and feed consumption to 6 weeks of age in comparison with chicks receiving water *ad lib.* The extent of the reduction due to water restriction increased with level of molasses (15—30%) in the diet. Restricting water with the no-molasses or 15% molasses feeds had little effect on total water consumption, but increased the moisture content of the droppings; with the 30% molasses diet restriction lowered the total water consumption, but had no effect on the moisture content of the droppings. A. H. CORNFIELD.

Storage of mixed feeds containing cane final molasses. I. Effect on chick growth and feed utilisation. E. Ross (*Poultry Sci.*, 1960, **39**, 985—993).—A maize–soya-bean oil-meal diet showed little loss in nutritive value (as measured by wt. gains and feed efficiency of chicks) during storage for 9 months. When mixed with 15% of cane final molasses the nutritive value decreased slightly after 7 months' storage. When mixed with 30% of cane final molasses the nutritive value decreased considerably after 5 months' storage.
A. H. CORNFIELD.

Tolerance of growing chicks for high levels of different forms of zinc. R. H. Roberson and P. J. Schaible (*Poultry Sci.*, 1960, **39**, 893—896).—The presence of Zn (1000 p.p.m.) (O^{2-}, SO_4^{2-} or CO_3^{2-}) in the diet did not influence growth rate, feed efficiency or mortality of chicks to 4—5 weeks of age. Growth rates and feed efficiency were reduced slightly with 1500 p.p.m. of Zn as CO_3^{2-} or SO_4^{2-} and reduced considerably by Zn in all forms at 3000 p.p.m. Mortality was serious only with the CO_3^{2-} form.
A. H. CORNFIELD.

Comparison of dehydrated birdsfoot trefoil and lucerne meals as constituents of poultry mash. G. M. Wood and R. T. Smith (*Agron. J.*, 1960, **52**, 501—503).—Addition of 3·1% of dehydrated birdsfoot trefoil meal to the diet of male chicks had no effect on wt. gains to 10 weeks of age, but with 6·2% wt. gains were depressed. Dehydrated lucerne meal 3·1—6·2% in the diet depressed wt. gains. Birdsfoot trefoil meal had higher contents of carotene and most trace elements than had lucerne meal. A. H. CORNFIELD.

Nutritional value of hydrolysed poultry manure for broiler chickens. K. E. Wehunt, H. L. Fuller and H. M. Edwards, jun. (*Poultry Sci.*, 1960, **39**, 1057—1063).—Growth rate of chicks was improved when hydrolysed broiler litter or autoclaved hen and broiler manures were added to diets sub-optimal in protein or lacking other sources of unidentified growth factors. Feed efficiency was either unaffected or depressed. The crude protein of the manure was utilised less efficiently than that of soya-bean oil-meal or casein–gelatin but true protein was utilised equally in all supplements. About 50% of the crude protein in the hen manure and 33% of that in the broiler manure existed as true protein. The manures were nearly equal to

a combination of fish solubles and dried distillers' solubles as a source of unidentified growth factors. A. H. CORNFIELD.

Organic acids as potentiators of antibiotics. J. D. Yates and E. L. Stephenson (*Poultry Sci.*, 1960, **39**, 994—999).—Addition of terephthalic acid (8 g./ton) to diets containing five different antibiotics (1—100 g./ton of feed) had no effect on wt. gains or feed efficiency of chicks to 4 weeks of age. Addition of terephthalic acid, phthalic acid, isophthalic acid and *o*- and *m*-aminobenzoic acid (each at 8 g./ton) to a diet containing oxytetracycline (20 g./ton) did not affect chick performance (**I**); **I** was unaffected by *p*-aminobenzoic acid at 2 g., but depressed at 8 g., per ton of feed.
A. H. CORNFIELD.

Mycostatin in the drinking water for the treatment of crop mycosis in turkeys. S. Wind and H. Yacowitz (*Poultry Sci.*, 1960, **39**, 904—905).—Addition of Mycostatin (nystatin) (62·5—250 p.p.m.), dispersed with Na lauryl sulphate, to the drinking water for 5 days controlled crop mycosis in turkeys. Consumption of water was not reduced by the treatment. A. H. CORNFIELD.

Relative activity of the broad-spectrum antibiotics in birds as measured by clinical effects. E. H. Peterson (*Poultry Sci.*, 1960, **39**, 960—970).—When administered in a practical-type chick diet or a low-Ca diet, chlortetracycline (CTC) was twice as effective as oxytetracycline (OTC) against caecal coccidiosis on an equiv. intake basis. The clinical activity of CTC was increased 3 times and that of OTC twice with a low-Ca diet. Addition of 0·5% terephthalic acid to the low-Ca diet increased CTC activity 10-fold and OTC activity 3—5-fold. The activity of CTC was 4 times that of OTC with the practical-type diet containing terephthalic acid, but only 3 times as great with the low-Ca diet containing terephthalic acid.
A. H. CORNFIELD.

Effects of Methimazole on weight gains, carcass composition, thyroid gland weight and blood components of cockerels. J. L. Sell and S. L. Balloun (*Poultry Sci.*, 1960, **39**, 930—937).—Addition of the goitrogen Methimazole (1-methyl-2-mercaptoimidazole) (0·02 g./lb. of feed) for 4 weeks to the diet of cockerels, commencing at 6 weeks of age, decreased wt. gains and feed efficiency, increased thyroid wt. but had no effect on eviscerated carcass composition. Feeding U2911 (Carboestrol, 0·001 g.) or dienoestrol diacetate (0·032 g./lb. of feed) alone and in combination with Methimazole did not affect wt. gains but increased the fat content of cockerel carcasses. Feeding 0·075% of thiouracil or Methimazole (0·120 g./lb. of feed) to day-old cockerels for 4 weeks reduced growth rates. Methimazole did not increase thyroid wt. as much as did thiouracil.
A. H. CORNFIELD.

Effects of oral administration of diethylstilboestrol and dienoestrol diacetate on growth rate and efficiency of feed utilisation of Beltsville small white turkey broilers. C. R. Creger, R. H. Mitchell, M. L. Jones, R. L. Atkinson, J. H. Quisenberry and J. R. Couch (*Poultry Sci.*, 1960, **39**, 1041—1045).—Incorporation of diethylstilboestrol (0·02 g./lb. of feed) in the diet of turkey broilers for 4 weeks commencing at 10 weeks of age increased wt. gains slightly, but had no effect on feed efficiency. Dienoestrol diacetate (0·032 g./lb. of feed) increased both wt. gains and feed efficiency. The addition of 4·5% of animal tallow increased feed efficiency in every case, but wt. gains were improved only in the presence of the oestrogens.
A. H. CORNFIELD.

Compatibility of Nicarbazin with methyl esters and animal fats in broiler diets. R. W. Lewis, J. J. Woods and J. R. Couch (*Poultry Sci.*, 1960, **39**, 910—916).—Addition of 1·5—4·0% of prime tallow to diets containing 0·0627% of Nicarbazin retarded chick growth (floor-reared) whilst similar levels of a methyl ester product (from soya-bean and cottonseed oil) had no effect on or increased wt. gains. In battery trials reduction in wt. gains of chicks increased with level of Nicarbazin (0·0125—0·0250%). The reduction was greater in the presence of animal fat than in the presence of the methyl ester product. A. H. CORNFIELD.

Pharmacologically induced resistance to heat shock. III. Effects of rauwolfoids and chlorpromazine on heart rate. R. E. Burger and F. W. Lorenz (*Poultry Sci.*, 1960, **39**, 981—985).—Pre-treatment of adult cocks with chlorpromazine (100 p.p.m.) or reserpine-free mother liquor (250 p.p.m. in the diet) for 14—19 days delayed the onset of increasing heart rate due to exposure to acute thermal stress and also prolonged survival of the birds. A. H. CORNFIELD.

Protein requirement for egg production as influenced by management, genetic background and dietary energy level. P. A. Thornton and W. A. Whittet (*Poultry Sci.*, 1960, **39**, 916—921).—Egg production (%) and feed efficiency with respect to egg production were unaffected by varying the protein level of the hen's diet from 13% to 17% or the energy level from 700 to 900 kcal./lb. Results were similar under both floor and cage conditions. Reducing protein level to 11% decreased egg production with the high-energy and increased it with the low-energy diet. In another test using the high-energy diet fed to 4 strains of birds in cages egg production was similar with all strains and with dietary protein levels from 13% to 17%, but was lower with 11% dietary protein.
A. H. CORNFIELD.

Effect of restricted light and hormones on subsequent egg production of winter-hatched turkeys. J. A. Harper and J. E. Parker (*Poultry Sci.*, 1960, **39**, 900—903).—Turkey hens hatched in Nov.–Dec. were subjected to restricted light (9 h. daily) for 4 weeks during the following June and thereafter exposed to 17 h. of light daily. Egg production by these hens during Aug.–Sept. was higher than by comparable hens which had not been subjected to restricted light during June. Egg production by turkey hens given injections of progesterone (0·005—0·040 g.) at 25·5 weeks of age declined with dosage during the following month. Diethylstilboestrol (0·025 g.), progesterone (0·01 g.) and deoxycorticosterone acetate (0·025 g.), alone or in combination, did not overcome refractoriness in turkey hens reared during a period with increasing daily light.
A. H. CORNFIELD.

Influence of season and age of layers on egg weight, shape index, albumin quality and shell thickness. W. J. Mueller, A. J. G. Maw and E. G. Buss (*Poultry Sci.*, 1960, **39**, 854—860).—Egg wt. increased and albumin quality declined continuously from Dec. of the pullet year until April of the second year of production. The shape indices of eggs produced during the pullet year were higher than those produced during the second year of laying. Season had no effect on shape index. Shell thickness during the pullet year was lower than during the second year of laying. Correlation coeff. of the various factors between the two years were high except for albumin quality. A. H. CORNFIELD.

Effect of pre-incubation treatments on the hatchability of chicken eggs. J. D. McConachie, F. N. Jerome and W. F. Pepper (*Poultry Sci.*, 1960, **39**, 886—889).—There were no significant differences in hatchability between eggs laid before 9 a.m. and those laid between 9 a.m. and 3 p.m., between three successive 6-day periods of collection, or between three pre-incubation treatments. There was a significant difference in hatchability between White Leghorns and Columbian Rocks. A. H. CORNFIELD.

Effects of timing and method of application on efficiency of Co-Ral sprays for cattle grub control. R. O. Drummond and B. Moore (*J. econ. Ent.*, 1960, **53**,729—731).—In spraying cattle monthly from May to Sept. timing did not affect grub control. Variation in vol. concn. and pressure of spray showed only the need for complete wetting. C. M. HARDWICK.

Effectiveness of Ruelene applied as localised "pour-on" and as spray for cattle grub control. W. M. Rogoff and P. H. Kohler (*J. econ. Ent.*, 1960, **53**, 814—817).—Ruelene (4-t-butyl-2-chlorophenyl methyl methylphosphoramidate) at 4 lb./gal. applied by pouring on to backs of cattle was at least as effective as when applied by a sprayer; at least 85% control being obtained. Both methods produced slight depression of erythrocyte cholinesterase. There was no increase in wt. gains. Some skin irritation occurred in heavily treated dairy cattle but none in Herefords.
C. M. HARDWICK.

Virucidal activity of β-propiolactone vapour. I. Effect on Venezuelan equine encephalomyelitis virus. F. W. Dawson, H. J. Hearn and R. K. Hoffman (*Appl. Microbiol.*, 1959, **7**, 199—201).—The lactone rapidly inactivates the virus and might replace formaldehyde for decontamination of enclosed virus-infested areas.
A. G. POLLARD.

Present status of pullorum disease. H. V. Roekel (*Poultry Sci.*, 1960, **39**, 868—873).—A review. A. H. CORNFIELD.

Control of northern fowl mite in community wire cages with malathion in special dust-bath boxes. J. L. Rodriguez, jun., and L. A. Riehl (*J. econ. Ent.*, 1960, **53**, 701—704).—Shallow boxes containing 5 lb. of 4% malathion placed in the cages for 14 days eradicated *Ornithonyssus sylviarum*. A light infestation was present 6 weeks later. When 10% malathion was used no mites were present after 6 weeks. Costs of using this method for spot treatment were compared with those for general dusting or spraying.
C. M. HARDWICK.

Fermentative manufacture of combined agricultural vitamin B_{12}–antibiotic preparations. E. Belik, J. Doskocil and M. Herold (B.P. 828,921, 8.5.56. Czech., 26.5.55).—A vitamin B_{12}–antibiotic prep., suitable for agricultural use (feed supplement for animals), is produced by cultivating (in one fermentation batch) in aq. nutrient medium under aerobic conditions a micro-organism which produces vitamin B_{12} (e.g., *Streptomyces olivaceus* or *Nocardia*) and also one which produces a tetracycline-type antibiotic (e.g., *Streptomyces rimosus* or *S. aureofaciens*). F. R. BASFORD.

Veterinary compositions containing acid hydrazides. Imperial Chemical Industries Ltd. (Inventor: N. Greenhalgh) (B.P. 828,344, 30.1.57).—There is claimed a veterinary prep. for use in the treatment of lungworm infestations in domestic animals (cattle, sheep, pigs, goats), comprising a composition containing <0·01 wt.-% of at least one acid hydrazide, viz., A·CO·NH·NRR′ (A is residue of a carboxylic acid and may be H, OEt, $CONH_2$ or alkyl of >7 C, optionally substituted by OH, OPh, SH, CO_2Et, $CONH_2$ or CN; R and R′ are H, or R′ is CH_2SO_3H, or R and R′ together comprise :CXY; X is H or alkyl of >6 C; Y is alkyl, acyl or aryl, or X and Y together with C form a cycloalkyl radical; but when A is CH_2·CN then R and R′ are not both H). As an example of compounding, acethydrazide (1) is dissolved in water (10), and the solution is sterilised by Seitz filtration, to provide a sterile prep., suitable for parenteral administration to animals. F. R. Basford.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Losses of material during moisture determination on cereals. H. Bolling (*Getreide u. Mehl*, 1960, **10**, 102—108).—Methods of determining moisture in cereals (both sound and spoiled) are critically reviewed. Wt. losses obtained by heating at various temp. and pressures are compared with moisture determinations by the Karl Fischer method and with a direct moisture absorption method. The nature of non-aqueous substances lost in heating is investigated by paper chromatographic methods. These substances are chiefly fatty acids, e.g., oleic and linoleic acids. (17 references.) J. V. Russo.

Effect of use of fertilisers on wheat quality. H. Linser (*Getreide u. Mehl*, 1960, **10**, 97—102).—Nine years' study of N fertilisation (0—160 kg./ha. of N) of winter wheats shows that fertilisation leads to a better yield and quality of wheat, as assessed by chemical, physical and baking tests. (27 references.) J. V. Russo.

Starch-gel electrophoresis of wheat proteins. G. A. H. Elton and J. A. D. Ewart (*Nature, Lond.*, 1960, **187**, 600—601).—The work described demonstrates that starch-gel electrophoresis is a valuable method for investigating wheat proteins. Complete resolution of eight bands was obtained. C. A. Slater.

Influence of storage conditions of irradiated dry barley grain on lesions produced. M. A. Sicard and D. Schwartz (*C.R. Acad. Sci., Paris*, 1960, **251**, 897—899).—Irradiation sensitivity (evaluated from plantlet growth) of barley grains (3—10% of water) is unaffected by storage after 30-min. irradiation (1040 r./min.) provided the water content remains constant, neither is there any aggravation of lesions. Slow rehumidification of very dry grain during storage after irradiation promotes restoration. W. J. Baker.

Contamination of flour by insecticidal lacquers containing endrin and dieldrin. C. E. Dyte and P. S. Tyler (*J. Sci. Fd Agric.*, 1960, **11**, 745—750).—Flour and offal collected from a mill where such insecticidal lacquers had been applied to surfaces, were examined to determine whether contamination occurs under practical conditions. Toxic vapour is emitted from the lacquer, which passes through jute sacking and Kraft paper and is absorbed by the flour, etc. Flour which had been in contact with lacquered blocks for 2 weeks was toxic to beetles and the toxicity persisted, without noticeable decreases, during 18 months. Endrin contents of samples of flour (12) from the mill ranged from 79 to 323 p.p.m. and offal (two samples) 123—226. Acute toxicity to man is caused by bread containing 150 p.p.m. of endrin. (18 references.) E. M. J.

Analysis of endrin residues in flour by infra-red spectroscopy. A. N. Bates (*J. Sci. Fd Agric.*, 1960, **11**, 750—754).—The method described was designed specifically to measure the contamination in flour samples collected from a mill where they had been in contact with a lacquer containing endrin and is suitable for residues of 20—400 p.p.m. The concn. of endrin in the prepared solution was determined by a differential i.r. analytical technique. E. M. J.

Colour alterations by enzymes and acids. H. Huber (*Brot u. Gebäck*, 1960, **14**, 165—172).—Systematic studies into the factors affecting crumb colour of rye bread are described. The effects of acids and enzymes on various fractions of rye on the colour extracts are studied by spectrophotometric analysis. (27 references.) J. V. Russo.

Influence of different dough temperatures on bread roll manufacture and quality. H. Schauz (*Brot u. Gebäck*, 1960, **14**, 179—182).—The effects of varying dough temp. (22 and 28°), time of fermentation and time and speed of kneading on the size, taste and quality of the crumb of the resultant rolls are studied. The quality estimate of the baked goods was most favourable from dough at the cooler temp. Vol. of baked rolls was similar for dough temp. of 22 and 28°. J. V. Russo.

Sugars and confectionery

Wet disintegrator for direct analysis of [sugar] cane. R. Diecke (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 168—174).—The single-spindle disintegrator designed by the Sugar Research Institute gives complete extraction in 45 min. By lengthening the three blades from 5 in. to 6 or 7 in., the time can be reduced to 30 or 15 min., respectively. P. S. Arup.

Direct analysis of cane, using a wet disintegrator. S. R. Harris and P. A. Hanks (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 188—199).—The accuracy of the steps are examined in detail, and a standard procedure is presented. The hammer-milled cane (5 lb.) with water (15 lb.) is treated during 45 min. in a disintegrator of the type designed by the Sugar Research Institute, having three 6-in. blades revolving at 5700 r.p.m. Examples are given of calculations for the evaluation of cane based on the water or fibre content of the hammer-milled samples, and the Brix and pol. of the strained and filtered slurry from the disintegrator. The correction factor for hygroscopic water is 0·992. P. S. Arup.

Effect of starch on clarification and raw sugar filterability. R. I. Nicholson (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 213—221).—Raw juices with high starch content (e.g., >200 p.p.m.) tend (probably owing to their high phosphate content) to be more satisfactorily clarified than are juices containing less starch. Anti-clarification effects attributed to starch are not of major importance, and are caused, not by starch itself, but by its retrograded fractions; colorimetric starch determinations including the harmless sol. starch are, therefore, no reliable guide as to anti-clarification effects. P. S. Arup.

Insoluble solids in cane juice. B. Cortis-Jones (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 221—227).—The fine solids have been separated into four fractions heavier, and two lighter than juice by differential centrifugation at 2250—140,000 *g*. From the flocculation behaviour of the fractions by liming and heating in (insol.-free) juice, or by treatment with $CaCl_2$ in aq. sucrose–KCl, it is concluded that their removal (together with Ca phosphate) can be achieved in the course of ordinary processing. P. S. Arup.

Sucrose crystal growth. H. E. C. Powers (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 242—248).—A review covering the relationship of solubility to crystal size, irregularities in crystal growth and form, and methods of study. (10 references.) P. S. Arup.

Crystallisation velocity of sucrose at higher temperatures. P. V. Golovin and A. A. Gerasimenko (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 248—262).—Detailed prep. is given of crystals each weighing <0·1 g. each being subsequently suspended in sucrose solution (68·3—68·8%) for final growth (0·4—0·8 g.) to yield faceted crystals without defects. An apparatus comprising crystalliser, receiver and viscometer is described. A sucrose crystal, suspended on a quartz thread, heated in a tube (electric spiral) is transferred to the crystalliser containing supersaturated solution of known supersaturation, at the required temp. (thermostat). After the period of crystal wt. increase, deflection of the balanced quartz thread was observed by cathetometer. With this apparatus a method for direct measurement of crystal wt. increase was developed by use of elastic quartz thread. In pure sugar solutions, sugar crystallisation velocity determined at 70, 80, 90° increased with increase of supersaturation coeff.; in low raw grade (purity 79·28%) it increased with increase of supersaturation coeff. up to 80° only, at 90° it decreased. These findings are discussed. E. M. J.

Sucrose crystallisation by the dilatometric method. H. G. Walker, jun., R. Teranishi, R. E. Knowles, G. O. Kohler and R. M. McCready (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 270—277).—The advantages of the method for determining the crystallising performances of syrups are pointed out. An improved form of the Ingelman dilatometer is described; difficulties are, however, experienced in precisely following the whole course of the crystallisation, with the apparatus in its present form. Determinations of the effect of a no. of impurities on the initial supersaturations of syrups agree with results obtained by other investigators. (12 references.) P. S. Arup.

Formation of false grains in crystallisation of sugar and its effect. A. C. Raha (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 277—282).—Increases in crystal wt. (determined from samples taken during boilings) occur in *A* and *B* massecuites up to the time of the appearance of false grains. In *C* massecuites, the increases

(judged by the fall in purity of the mother liquor) cease some time before false graining, at a stage after the purity of the mother liquor has fallen below 50. The practical disadvantages caused by false graining are explained. P. S. ARUP.

Relationship between conditions of crystallisation, structure and shape of crystals. N. N. Sheftal and I. V. Gavrilova (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 282—286).—The theory is advanced that irregularities or departures from the simplest ideal crystal form are caused by alterations in the crystal lattice due to liquid (or gaseous) occlusions during growth. The argument is supported by observations on the effects of various conditions (chiefly the movement of the crystal relative to the mother liquor) on crystal form and purity. P. S. ARUP.

Calorimetric method for determination of relative rate of crystallisation of sucrose in presence of additives. D. Napper and A. E. Alexander (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 286—291).—The rate curves obtained for sugar solutions with the use of a Hutchinson and White microcalorimeter and a thermistor are similar to those observed by other investigators using refractometric and dilatometric methods. The effects of surface-active additives of various types are not significant. P. S. ARUP.

Non-sucrose constituents of Hawaiian raw cane sugar crystals. Chen-Chuan-Tu and K. Onna (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 291—304).—The following groups of substances (or substances) have been partly separated by crystallisation or pptn. procedures with the use of MeOH and other solvents, and characterised by paper-chromatographic and -electrophoretic methods: plant pigments, thermally- and alkali-degraded products of carbohydrates, melanoidins, simple sugars and oligosaccharides, amino-acids, inorg. compounds, proteins, aconitic acid (occurring naturally as the Ca–Mg salt) and acid deriv. or decomposition products of hexoses. (19 references.) P. S. ARUP.

Sugar crystallisation and boiling in presence of a minute quantity of manganous ions. K. Suzuki (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 305).—The addition of $MnSO_4$ (0·001—0·00001%) to sugar solutions causes the deposition of whiter (tending to bluish-white), clearer and purer (containing less reducing sugar) crystals than are usually obtained. Alterations in cryst. form are also observed. The presence of Mn facilitates factory operations and improves yields. P. S. ARUP.

Factors influencing the growth of sucrose crystals. N. Albon and W. J. Dunning (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 310—315).—Two sucrose crystals exposed at the same time to the same supersaturated solution at 70° may show a 2-fold difference in their rates of increase in length in the [010] direction. Growth process is probably structure sensitive depending on no., nature and distribution of screw dislocations. The effects of supersaturation, presence of dextrose or laevulose as impurity and influence of temp. on rate of growth are discussed. (10 references.) E. M. J.

Weighing technique to determine effect of inorganic and organic compounds on rate of sucrose crystallisation. T. Moritsugu (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 315—323).—Increases in wt. of individual crystals at 30·6° are promoted by the ions Ca^{2+}, Mg^{2+}, Mn^{2+} and SO_4^{2-} and retarded by Na^+, K^+ and CO_3^{2-}. Max. increases are obtained with $CaCl_2$ (~4%) and $MnSO_4$ (1%), and max. decreases with Na_2CO_3, K_2CO_3, Na_2SO_3, NaCl and KCl (4—7%). The effects of mixed salts are irregular. Glucose slightly accelerates growth, whilst Na aspartate, glutamate and aconitate (0·1%) retard growth by 6—7%. (11 references.) P. S. ARUP.

Measurements of crystallisation rates of sucrose from pure and impure solutions. B. M. Smythe (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 323—336).—An apparatus is described in which increases in wt. of a crystal, held by "tongs" in a stirred solution, can be measured by means of a torsion balance while the crystal is still immersed in the mother liquor. Rates of growth increase rapidly with increases in r.p.m. (of the two-bladed stirrer) up to 1000, and more slowly at higher rates. With rapid stirring and moderate to high supersaturations, the relationship between crystal growth and supersaturations is approx. linear; deviations from this order are observed at lower supersaturations. The operative factors in the control of crystal growth are considered. The effect of impurities is briefly discussed. (14 references.) P. S. ARUP.

Granulometric analysis of massecuites. J. Dedek (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 337—339).—A representative sample of known wt. (100—1000 g.) taken from the pan or crystalliser is well mixed with a known wt. of saturated sugar solution. A photomicrograph is taken of a drop of the homogeneous suspension. With due precautions, the size and wt. of the crystals can be determined with an accuracy of ±2%. The results are more accurate than those obtained by sieve-analysis. P. S. ARUP.

Measurement of crystallisation velocity in cane molasses. D. H. Foster (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 339—346).—The molasses are prepared for the tests by dilution, centrifugation and re-concn. under reduced pressure to the desired degree of supersaturation. The saturation temp. is determined by the Harman microscopical method. Individual sugar crystals (~0·6 mm. long) are measured by a photomicrographic technique before and after a growing-period in the massecuite sample at constant temp., the containing-tube being radially attached to a slowly revolving wheel. Observations on the effects of purity, degree of supersaturation and temp. on crystallisation are considered. (12 references.) P. S. ARUP.

Presence of phosphates in cane juices. P. Honig (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 356—361).—Typical data are given for the content of total P_2O_5 in ash, orthophosphate (colorimetrically determined), org. phosphate (by difference) and phosphatide-P_2O_5 in juices. At least 95% of the total P_2O_5 in cane is water-sol.; the amount present in bagasse is proportional to the residual sugar therein. The phosphate level in cane assessed by that in the juice is probably of more interest than the level in the dry matter of the cane. The total P_2O_5 % of the middle of the top thirds of the cane are generally ~90 and 80%, respectively, of that of the basal third; corresponding variations and also varietal variations in the org. (non-ortho) P are much smaller. Max. org. P levels occur during the stages of most vigorous growth. P. S. ARUP.

Cation exchange in plantation white sugar manufacture. Wei Chen, C. T. Yu and Y. C. Cheng (*Proc. 10th Congr. int. Soc. Sugar Cane Technol., Hawaii*, 1959, 362—373).—Operative conditions, including regeneration, are described in detail. With average influent juice-hardness values of 793 and 902 mg. of CaO per l., the % removals of CaO are 68 and 50, respectively. The chief benefits of the process are marked reduction of scale, improved evaporation, and higher Brix values and lower η in the conc. syrup. Economic aspects are considered. P. S. ARUP.

I. Quantitative relationship between colour and 5-hydroxymethyl-2-furaldehyde produced by heating acid glucose syrup. II. pH and browning of glucose syrup. Y. Yoshiro and M. Nakamura (*J. chem. Soc., Japan, industr. chem. sect.*, 1960, **63**, 157—161, 161—165).—I. Experimentation suggests that this compound is not the only factor responsible for browning. (10 references.)

II. The pH range was 2—7. Glucose syrup (**I**) studied in absence of 5-hydroxymethyl-2-furaldehyde (**II**) showed that the pH of **I** had a strong influence on colour formation, the max. occurring at pH 3·5; **II** did not affect the formation of colour. (16 references.) (From English summaries.) C. V.

Fermentation and Alcoholic Beverages

Maple syrup. XII. Effect of zinc on growth of micro-organisms in maple sap. H. A. Frank, J. Naghski, L. L. Reed and C. O. Willits (*Appl. Microbiol.*, 1959, **7**, 152—155).—Fermentation of the sap was inhibited to varying extents when glass containers were replaced by new or acid-cleaned galvanised vessels. Inhibition was related to the amount of Zn dissolved from the containers. A. G. POLLARD.

Cycloheximide (actidione) and its non-agricultural uses. J. H. Ford and W. Klomparens (*Antibiotics & Chemotherapy*, 1960, **10**, 682—687).—This compound has been used as a substitute for H_2SO_3 in the preservation of sweet wines. *Saccharomyces ellipsoideus* is mainly responsible for undesirable secondary fermentation and is very sensitive to this antibiotic. Odour and taste were not affected at concn. of 1—30 μg./ml. and when added (0·1—1·0 μg./ml.) at the outset of fermentation, refermentation was prevented. (39 references.) C. V.

Refrigeration and distillery operations. A. Smiley (*Air Cond. Htg Vent.*, 1960, **57**, No. 11, 86).—The rapid cooling of the mash and control of fermentation temp. is described. The production of heat, B.Th.U./h./100,000 lb. dry grain, at 4, 8, 12 . . . 60 h. after inoculation is given. C. V.

Instability in potable spirits. I. Scotch whisky. L. A. Warwicker (*J. Sci. Fd Agric.*, 1960, **11**, 709—716).—A typical instability is the cloudiness ("greying") produced by low temp. If no deposition has occurred, the haze is dispersed on raising the temp. Instability is caused also by contamination during manufacture or developed during maturation. Extraction of Mg salts from filter pads or contamination with Zn or Al salts contribute to a deposit, depending on pH and temp. A critical pH value for a particular blending of Scotch whiskies is 4·5, above which deposition occurs in a few days. Tannin extracted from wood during maturation is subsequently

deposited when certain metals are present and the pH is above the critical value. H^+ exchange removes a factor causing instability. (17 references.) E. M. J.

Organic acid metabolism in cider and perry fermentations. III. Keto-acids in cider-apple juices and ciders. G. C. Whiting and R. A. Coggins (*J. Sci. Fd Agric.*, 1960, **11**, 705—709).—Of four keto-acids detected in cider-apple juices three were identified as pyruvic, α-ketoglutaric and oxaloacetic acids; conversion to their 2,4-diphenylhydrazones and subsequent hydrogenation to the corresponding amino-acids confirmed the identifications. Quant. changes in pyruvic and α-ketoglutaric acids occurring during fermentation are described and various series of metabolic reactions are discussed. (15 references.) E. M. J.

Biochemical investigation of germination and its inhibitors. C. F. van Sumere (*Brass. et Malt., Belg.*, 1960, **10**, 20—26).—A review covering the occurrence, detection and functions of inhibitors in seeds (especially barley), the processes of cytolysis and the classification of barleys according to their content of hemicellulose and their cellulase and hemicellulase activity, and enzymic processes occurring in the embryo during germination. (12 references.) P. S. ARUP.

Application of gibberellic acid in small-scale malting and in practice. W. Kleber and M. Lindemann (*Brauwelt*, 1960, **100B**, 542—547).—The addition of 0·15 mg. of gibberellic acid (**I**)/kg. of barley given with the last steeping water or 0·06 mg. of **I**/kg. of barley sprayed on the steeping grain before the beginning of the germinative process, was optimal. The quality of the malt was unharmed and the malting process shortened by 48 h. in small-scale tests and practice. (11 references.) E. M. J.

Detection of proteolytic stabilising enzymes in beer. W. Kleber and G. Franke (*Brauwelt*, 1960, **100B**, 374—375).—A method was developed based on the work of B. S. Miller, De Ceuster and Schultze-Berndt. Haemoglobin (Merck) was taken as the standard prep. Two flasks were prepared containing the same quantities of haemoglobin, acetate buffer and beer sample and warmed to 40°. Trichloroacetic acid (**I**) was added to one, the other remained at 40° for 3 days and **I** was then added. N was determined (Kjeldahl) in each. The difference calculated on 100 ml. of beer gave the measure of proteolytic activity of the beer sample under test; e.g., 100 ml. of beer effected a protein degradation corresponding to 12·3 mg. of N. E. M. J.

Beer stabilising methods. B. D. Hartong (*Brauwelt*, 1960, **100B**, 389—394).—Methods of stabilising finished beer to preserve clarity, foam stability, colour and taste are reviewed. Natural methods depend on the prevention of cold haze formation and absorption during filtration. Other undesirable changes are covered, e.g., pptn. of protein, by fish mucilage or gelatin, enzyme treatment (pepsin or papain), or prepared enzyme mixtures, e.g., Collupulin, Maltolysin, etc. and suitable means of filtration with, e.g., filtermasse, kieselguhr, bentonite, montmorillonite, nylon, etc. The properties of foam and the effect of oxidation potential on colour and taste are discussed. E. M. J.

Beer clarification and oxidation influence. Physico-chemical activity on the beer by massefilter, kieselguhr filter, layer filter and centrifuge. J. Mühlbauer and V. v. Meduna (*Brauwelt*, 1960, **100B**, 133—137).—The above-mentioned methods, most used in beer clarification, are reviewed in relation to decrease in colour and bitter substances, loss of reductones and depreciation of foam stability. By the newer filtermasse (**I**) (with less separation into fibres) the loss of colour and reductones is smaller. By centrifugation (**IV**) colour loss is least and least increase in oxidation occurs, with second place being given to kieselguhr filtration (**II**); by layer filtration (**III**) only a small quantity of colour is lost and a smaller quantity of reductones. No loss of bitter substances occurred by **I** but 10—11 mg./l. were lost by **II** and **III**. Foam stability was less affected by **IV** and **III** than by **II** and **I**. Methods **IV** and **II** were sensitive to cold haze formation; beers filtered by **I** were most stable in this respect. (18 references.) E. M. J.

Atmospheric oxidation [of beer] and its control by ITT methods. K. Zeidler (*Brauwelt*, 1960, **100B**, 1—3).—The control of oxidation in various stages of the brewing process (e.g., storing and bottling) is described. In bottling beer, the frothing-over method gave the best results. A series of experimental tests is presented showing the value of the ITT method. In general, to maintain the requisite keeping quality and taste of the beer, the O_2 content should be $<0{\cdot}5$ mg./l. E. M. J.

Drying, storage and use of hops in brewing. M. H. de Backer (*Brass. et Malt., Belg.*, 1960, **10**, 7—12).—Losses of humulone and lupulone during drying are minimised by commencing the drying with air at 30°, slowly rising to 40°; no damage occurs at 60°, provided that the moisture has been reduced to ~10%. Re-drying of material that has absorbed moisture can be accomplished at >40°, only if the moisture % is ⩾18—20°. The most satisfactory storage temp. for fresh hops are −20 to −30°; moulds may develop at −5° on moist hops. Extraction and isomerisation of the bitters increase with increasing pH (5·1—5·8) of the wort used for hop-boiling; there is, however, a concomitant increase in the extraction of substances giving a harsh flavour in the beer; similar effects are observed on using defoliated hops, especially when the defoliated material has been ground. P. S. ARUP.

Determination of carbon dioxide content in the atmosphere of fermentation and storage cellars. W. Bretzke (*Brauwelt*, 1960, **100B**, 1037—1040).—A gas testing apparatus is described comprising a bellows-pump and a measuring tube graduated to determine 0·1—1·0 vol.-% or 1—20 vol.-%. The air sample is drawn through at controlled speed. The presence of CO_2 is shown by a colour reaction with a hydrazine compound. Data are given on concn. of CO_2 occurring in fermentation cellars: e.g., over the floor 0·7 vol.-%; 10 min. after cutting off ventilation 0·2—0·4 vol.-%; in an unventilated storage cellar 0·4 vol.-%; 0·5 vol.-% of CO_2 being the highest limit permitted for working conditions. The physiological effect of CO_2 on human beings is discussed. E. M. J.

Hop extracts and hopped beverages. Pabst Brewing Co. (B.P. 827,334, 17.7.57. U.S., 17.7.56).—Whole, undried, vine-fresh hop cones are extracted with MeOH at ⩾65°, and the extract is concentrated to give non-toxic product (green, viscous paste) immiscible with water at 20°, dispersible in water at 50°, and suitable for use in the brewing of beer. F. R. BASFORD.

Carbonation of beverages. K. A. Bownass (B.P. 827,946, 4.2.58). —Apparatus is figured. F. R. BASFORD.

Fruits, Vegetables, etc.

Statistical study of parathion and DDT residues on apples. R. J. Daum (*Dissert. Abstr.*, 1960, **20**, 4471).—Determinations were made of the effects of ratio of solvent to surface area, and of duration of tumbling, on the recovery of parathion and DDT residues from apple fruits and leaves. The total variances on fruits and leaves were due respectively 75 and 90% to variance within trees in the case of parathion, whereas with DDT variances within and between trees were about equal. The presence of extracts of apple fruits decreased the recovery of parathion, but not of DDT. The Schechter–Haller method for determining DDT residues was nearly twice as precise as the Averell–Norris method for parathion. About 14% of the total variance of parathion field samples was due to analytical determinations, as compared with 4% for DDT. The sizes of sample necessary to detect given differences of concentration were ascertained. M. D. ANDERSON.

Viscometric behaviour in relation to evaporation of fruit purees. J. C. Harper (*Food Technol.*, 1960, **14**, 557—561).—The performance of a wiped film evaporator in concentrating apricot, peach and pear purees was studied and the η characteristics of the products established. The η behaviour was expressed by $r = K\gamma^n$, where r = shear stress, dyne/sq. cm., K, n = parameters of η... for pseudoplastic fluid, γ = shear rate, du/dx, sec.$^{-1}$. Overall heat transfer coeff. correlated well with values for K. E. M. J.

Factors influencing the colour of potato chips. L. R. Townsend and G. W. Hope (*Canad. J. Plant Sci.*, 1960, **40**, 58—64).—Fundamental browning is due to reaction between amino-acids and reducing sugars but it can occur in the presence of amino-acids and sucrose provided conditions are favourable for the hydrolysis of the latter. Inorg. constituents, *per se*, have little colour-enhancing effect but may contribute toward sucrose hydrolysis. E. G. BRICKELL.

Evaluation of instruments to measure firmness of tomatoes. A. W. Garrett, N. W. Desrosier, G. D. Kuhn and M. L. Fields (*Food Technol.*, 1960, **14**, 562—564).—A highly significant correlation was found between firmness values obtained from two measurements on the same fruit; whether determined by the Asco Firmness meter (**I**) or the Firmometer. Reproducibility by the McCollum Firmness meter (**II**) could not be determined, since the tomatoes were altered in the first measurement. **I** could be used in the breeding programme as well as in quality control for fresh tomatoes, **II** is useful as a tool in selection of firm fleshed tomato varieties. (10 references.) E. M. J.

Consistency and serum separation of catsup. B. A. Twigg (*Dissert. Abstr.*, 1960, **21**, 167).—The term lyophoresis is used to describe the separation of liquid and solid phases of such suspensions as tomato catsup. About half the variations found in the consistency (evaluated organoleptically) and lyophoresis of 77 samples of tomato

catsup could be explained by differences in pH, and in contents of structural solids, non-structural solids and total pectic substances. Content of pectic substances was the factor exerting the greatest influence on consistency, and structural solids on lyophoresis. The thickening effect of decreasing pH in catsup is attributed to the accompanying increase in η of low-methoxyl pectic substances. Consistency was thickened and/or lyophoresis lessened by increase of hot-break temp., and increase in extractor screen size.
M. D. ANDERSON.

Utilisation of honey in fruit products. B. S. Bhatia, G. S. Siddappa and Girdhari Lal (*Food Sci., Mysore*, 1960, **9**, 163—169).—Sugar was partly or wholly replaced with wild honey (Malnad) or pot-honey produced under controlled conditions, in 25 samples of fruit squashes, crushes, syrups, jams, jellies, chutneys, preserves and guava cheese. After storage at 24 to 30° for 5—8 weeks the samples were examined organoleptically. A good quality product was obtained by replacing sugar solids with honey up to ~25%. Reasonably acceptable products were obtained with a replacement at the 50% level. In tomato ketchup all sugar may be replaced with honey without deteriorating effect. All samples kept well at room temp. for 1 year.
I. DICKINSON.

Alginate jellies and the like. Alginate Industries Ltd. (Inventors: R. R. Merton and R. H. McDowell) (B.P. 828,350, 14.2.57).—A water-sol. alginate (e.g., Na alginate of $>0{\cdot}2\%$ of water) is compounded with alkali metal carbonate, solid, edible acidic substance (e.g., citric acid), dispersing agent (sugar), and at least one salt, the cations of which with alginic acid give a water-insol. salt (of solubility product $>10^{-4}$ at 25°), e.g., a Ca salt (carbonate, tartrate, citrate, sulphate, H phosphate or phosphate). F. R. BASFORD.

Non-alcoholic beverages

Technological aspects of manufacture of mandarin orange concentrate. J. S. Pruthi, N. S. S. Rao and Girdhari Lal (*Food Sci., Mysore*, 1960, **9**, 169—174).—The optimum time and temp. of the inactivation of pectin methylesterase in Coorg mandarin orange juice prior to its vac. concn. was determined. A 100% destruction was obtained by heating the juice for 8 sec. at 96°, 70 sec. at 90°, 100 sec. at 85° and 110 sec. at 80°. For commercial production an optimum time of 8—10 sec. at 95—96° is suggested and was employed for the investigation of the coeff. of heat-transfer during vac. concn. This coeff. ranged between 200 and 300 B.Th.U./h. sq. ft. °F, depending upon the initial pulp content and η of the juice. The losses in carotene and ascorbic acid during concn. were 4·6 and 9·3% respectively. The juice retained most of its nutrients and had a good appearance but lacked flavour. The normal flavour can be restored by addition of 0·02% of freshly extracted cold pressed orange peel oil to the reconstituted juice or 0·08% to the concentrate. (14 references.)
I. DICKINSON.

Viability of a yeast in high density orange concentrates stored at various temperatures. R. L. Kitchel and M. W. Miller (*Food Technol.*, 1960, **14**, 547—549).—An osmophilic yeast (*Torulopsis magnoliae*) isolated from 65° Brix concentrated orange juice, fermented all concentrates tested when stored at 60 or 80°F for 30 days. If orange juice concentrate becomes inoculated with this yeast, lower temp. are necessary to protect the product. (15 references.)
E. M. J.

Equilibrium relative humidity (ERH) relationships of fruit juice and custard powders. G. S. Siddappa and A. M. Nanjundaswamy (*Food Technol.*, 1960, **14**, 533—537).—Mango and guava powders have almost similar moisture equilibrium curves, while orange juice and mango custard powders have curves that differ significantly from these and from each other. The graphical interpolation method is quicker than the wt. equilibrium method but the latter gives more precise determination of ERH and information regarding storage behaviour in relation to danger and critical points and safe margin. Mango and guava powders and mango custard powder have a wider safety margin (4, 10 and 14% R.H.) than orange juice powder (1% R.H.); all have tendency to develop mould when R.H. of storage atm. exceeds ~80%. (11 references.) E. M. J.

Consumer opinion of sweeteners in frozen concentrated lemonade and orange juice drink. R. M. Pangborn, G. L. Marsh, W. R. Channell and H. Campbell (*Food Technol.*, 1960, **14**, 515—520).—Three consumer surveys were made. An optimum sweetness level was established for lemonade of 11° Brix at a Brix–acid ratio of 16·0 and for orangeade 13° Brix and a Brix–acid ratio of 18·5. The optimum sweetness in sucrose-sweetened concentrates was determined; the effect on acceptance of dextrose replacements of 10, 20 and 30% of the sucrose; and of maize syrup replacements of 10, 20 and 30% of the sucrose. All orangeade containing dextrose was acceptable, but samples darkened on storage for 6 months at 0°F. The lemonade sample containing 30% dextrose was rejected; maize syrup in lemonade was objectionable. In all-sucrose sweetened lemonade 9·8° Brix was equiv. in sweetness to 10·8° Brix in samples with sucrose replacements of 20% dextrose or 20% maize syrup. Sucrose had the greatest and maize syrup the least sweetening capacity. (14 references.)
E. M. J.

Tea, coffee, cocoa

Cocoa-butter substitutes. Unilever Ltd. (Inventors: R. L. Best, A. Crossley, S. Paul, H. Pardun and C. J. Soeters) (B.P. 827,172, 19.2. and 7.9.55).—In the production of confectionery goods in which cocoa-butter is a normal ingredient, e.g., in the manufacture of milk chocolate, part or all of the cocoa-butter can be economically replaced by a palm oil fraction of I val. >45, softening point 30—45°, and dilatation of <1000 at 20°. The palm oil fraction may be used in conjunction with a Borneo tallow type fat. F. R. BASFORD.

Milk, Dairy Products, Eggs

The industrial way [with milk]. A. H. Johnson (*Food Technol.*, 1960, **14**, 541—546).—An address covering: the problems related to the perishable quality of milk, improving the keeping quality, ultrasonic treatment, Hofius process, irradiation, freezing, use of additives, etc., dried whole milk and the complexity of the problems of imparting keeping quality to dairy products. E. M. J.

Effect of various dietaries on milk composition and efficiency of production of dairy cows. D. K. Hotchkiss (*Dissert. Abstr.*, 1960, **20**, 4476—4477).—Cows fed diets containing concentrates with 75, 55, 35 or 15% of hay, at high, medium and low nutritional levels, gave less milk, fat-corrected milk and milk solids, at the low nutritional level. The protein content of the milk was higher with high concentrates, but both protein and lactose were affected more by nutritional level than by hay/concentrate ratio. Neither factor affected the fat content of the milk. Production efficiencies calculated from energy in the milk per unit of feed energy, less maintenance allowance, were 0·946, 1·050 and 1·154 for high, medium and low nutritional levels respectively. Similar calculations based on digestible energy gave 0·412, 0·457 and 0·508 respectively. Values for increasing proportions of concentrates were 0·403, 0·481 and 0·522.
M. D. ANDERSON.

Simplified method for determination of iodine-131 in milk. G. K. Murthy and J. E. Campbell (*J. Dairy Sci.*, 1960, **43**, 1042—1049).—After the treatment of milk with formaldehyde (1 ml. of 40% CH_2O per l.) during 2 h. at room temp., 98% $\pm$ 2% of the I is precipitated in combination with proteins by trichloroacetic acid (**I**). The ppt. is washed free from other radioactive elements by dil. **I**, after which its content of ^{131}I is determined by gross γ-counting. (19 references.)
P. S. ARUP.

Removal of iodine-131 from milk. B. J. Demott and D. G. Easterly (*J. Dairy Sci.*, 1960, **43**, 1148—1150).—In laboratory experiments, ~90% of the ^{131}I secreted in the milk (representing ~5—16% of the dose) can be removed by treatment with Dowex I-X8 (in the chloride form) at room temp. during 10 min. The amount of Cl gained by the milk (max. 71 mg./l.) is inversely proportional to the original Cl^- content of the milk, and likewise the amount of P lost (max. 16 mg./l.) to the amount of Cl^- gained. The concn. of ^{131}I in the milk is not lowered under reduced pressure at 70°. (16 references.)
P. S. ARUP.

^{90}Sr in dairy milk produced in central Europe during 1957—1960. J. Nosek and W. Santholzer (*J. Hyg.*, 1960, **58**, 261—262).—The moderate increase in the autumn had disappeared in the first half of 1958 but a marked increase then followed and continued to mid-1959 when a significant fall was noted. The reduction in radioactive fall-out preceded this fall. The areas examined related to Novy Bydzov and Hradec Králové.
C. V.

Potentiometric method for determination of chloride in milk. B. L. Herrington and D. H. Kleyn (*J. Dairy Sci.*, 1960, **43**, 1050—1057).—Results more accurate than those obtained by titration with standard $AgNO_3$ in presence of K_2CrO_4 are obtained by potentiometric titration with the use of an Ag electrode connected with a pH-meter in the presence of dil. HNO_3 (to give pH 2) or with the addition to the milk (10 ml.) of aq. 10% K-alum (20 ml.). The end-point is sharp, and interference by the proteins is practically eliminated.
P. S. ARUP.

Rapid determination of protein in fresh milk. F. Kiermeier and E. Renner (*Z. LebensmittUntersuch.*, 1960, **113**, 1—13).—The literature of the subject is reviewed. Results (from 51 samples) obtained by the formol titration (according to Schulz) and the Kofranyi methods, and by two colorimetric methods (according to Steinsholt and to Schober and Hetzel) all agree satisfactorily with

the corresponding Kjeldahl results. The formol titration method is preferred on account of its comparative rapidity; minor modifications are proposed in order to increase the accuracy and convenience of the Schulz procedure. Calibration graphs are given for the two colorimetric methods. The increase in formol titration and Kofranyi results frequently observed during the souring of milk, is due, not to the action of lactic acid bacteria, but to the proteolytic activity of *Bacillus macerans* which thrives in an acid medium. (73 references.) P. S. ARUP.

Rennet and its action on the casein of milk. XIV. Amino-acid composition of the glyco-macropeptide liberated from κ-casein by rennet. H. Nitschmann and R. Beeby (*Chimia*, 1960, **14**, 318—319).—The glyco-macropeptide (GMP) liberated by rennet from the κ-casein sub-fraction isolated from milk has a quant. amino-acid composition substantially identical with those found for the GMP from total casein and from α-casein. This supports the assumption that the splitting off of this GMP from the κ-casein protecting the Ca-caseinate micelles is the decisive reaction in the rennet-clotting of milk. (13 references.) C. L. HINTON.

Properties of rennin. L. Friedman (*Dissert. Abstr.*, 1960, **20**, 4510).—A study of the hydrolysis of cereal proteins by crude rennin showed it to contain pepsin. Rennin was inhibited by 25% acetone, but pepsin was not. Highly active prep. of purified rennin were obtained, which were homogeneous during electrophoresis, ultracentrifugation and diffusion. Column chromatography and continuous paper electrophoresis removed impurities that could not be removed chemically, but often denatured the rennin to some extent. Physical and chemical constants were determined for the purified rennin; the sedimentation constant was the only one in which rennin differed from pepsin. The rates of hydrolysis of egg albumin and haemoglobin by rennin and pepsin were different. Rennin hydrolysed a max. of 4% of the peptide bonds of bovine serum albumin, at an optimum ionic strength of 0·35—0·5. Several reagents that inhibit trypsin, chymotrypsin and papain, did not inhibit rennin. Iodination inhibited rennin; partial acetylation did not; 4M urea reduced its activity by 50%; RNA inhibited it; some phospholipid prep. activated it. M. D. ANDERSON.

Mechanism of the clotting of casein by the action of rennin. C. A. Broomfield (*Dissert. Abstr.*, 1960, **21**, 34—35).—Citrate-buffered Na caseinate is clotted by cryst. rennin at pH 5·2, in the absence of Ca, and gives a clot similar to that of Ca paracaseinate. Increase in clotting rate with ageing is apparently due to uncatalysed hydrolysis of rennin-susceptible bonds. Heating the casein solution to 90° for 10 min. did not affect clotting rate, but possibly changed its dependence on temp., and decreased the rate of uncatalysed liberation of non-protein N. Plots of rate of clotting against inverse of abs. temp. indicated the existence of two separate reactions in the clotting process, one with a very high activation energy; this stage is probably intermediate between the primary reaction and clot formation. Relation of enzyme concn. to clotting rate at different temp. suggested that there are thermal and enzymic mechanisms for destabilisation of rennin-altered casein, and that the thermal mechanism may be reversible. Turbidity and η changes during rennin action in the presence and absence of urea suggested that clotting may be a polymerisation, involving H-bond cross-linking. Na caseinate solutions in malate, acetate and phthalate buffers below pH 5·4 behaved like those in citrate buffer. M. D. ANDERSON.

Lactoperoxidase. II. Inactivation by heat. F. Kiermeier and C. Kayser (*Z. LebensmittUntersuch.*, 1960, **113**, 22—33).—Relationships between time and temp. of heating with respect to the rate of destruction of the enzyme are determined with the use of a laboratory pasteuriser. In the range 72—85°, the inactivation proceeds as a monomol. reaction ($Q_{10} = 38$). A linear inverse relationship is found between the temp. and the log of the time of heating for 50% or 100% inactivation. Positive tests for milk heated at 85° are probably largely due to the reduction of the heating time in modern pasteurisers. In view of the possibility of detecting 1% of the original activity in milk heated during 8 sec. at 85°, it is advisable to rely more on the recording thermometer than on the peroxidase test. For the destruction of pathogenic organisms, the (German) official regulations prescribe a temp. of 85° without reference to the time of heating. (25 references.) P. S. ARUP.

Effects of direct steam heating and vacuum treatments on the chemical composition of milk with especial reference to substances involved in oxidised flavour development. D. H. Kleyn (*Dissert. Abstr.*, 1960, **21**, 39—40).—Milk was heated to 190°F by steam infusion, or to 200—240°F by steam injection, and was then submitted to vac. treatment. The results were: decreased O content of the milk; increase in retention of ascorbic acid; increase in activation of SH, slowly disappearing over 2 weeks; increase in activation of SH by homogenisation; and delay in oxidation of ascorbic acid and development of oxidised flavour in presence of Cu. There was a lack of correlation between cooked flavour and the results of the nitroprusside test. Denaturation of whey proteins by steam heating was about the same as by usual heat treatments. M. D. ANDERSON.

Forced convection heat transfer characteristics of fluid milk products. M. L. Peeples (*Dissert. Abstr.*, 1960, **21**, 139—140).—Forced convection heat transfer characteristics of four non-fat milk products containing 8·8—20% solids and of six milk products containing 3·8—100% solids at 43—93° and at Reynolds no. range of 5000—250,000 were determined and a single equation was derived mathematically to describe all these under the conditions studied. O. M. WHITTON.

Effect of certain processing variables on spray-dried milk products. C. H. Amundson (*Dissert. Abstr.*, 1960, **21**, 166—167).—Cooked flavour in spray-dried whole milk was due to the pre-heating stage in processing, rather than to spray-drying. The flavour was attributed to S compounds produced by denaturation of protein, and protein associated with milk fat was more heat-sensitive than skim-milk protein. Cooked flavour was lessened by separation of the milk at low temp., and application of the major pre-heating treatment to the cream only. The keeping quality of the dried product was not affected. Other methods of lessening cooked flavour were only successful at the expense of storage life. Reconstitution of dried skim milk was made easier by increasing particle size, either by lowering spraying pressure, or by arranging multiple nozzles to allow a spray of relatively dry particles to descend through a mist of relatively moist particles. The aggregates thus formed were very heat-sensitive, and were produced most successfully when final removal of moisture was accomplished outside the spray chamber. M. D. ANDERSON.

Determination of carbohydrates in ice cream by paper chromatography. T. C. Chou and J. Tobias (*J. Dairy Sci.*, 1960, **43**, 1031—1041).—A procedure is described by which maize-syrup sugars can be determined with the use of n-BuOH–EtOH–H_2O (2 : 1 : 1 by vol.) as descending solvent on parallel paper strips. The spots are located by spraying the (two) locator strips with aniline phthalate and heating. The eluted sugars are determined spectrophotometrically by means of the anthrone reagent; graphs are given for μg. of lactose, fructose, glucose and maltose, and sucrose against % transmissions at 625 mμ; recoveries are 93—104%, averaging approx. 100%. Small quantities of (slowly moving) higher sugars, from isomaltose to maltoheptaose, have also been determined. (13 references.) P. S. ARUP.

Detection of nitrates and nitrites in cheese. H. Dubrow and W. Kabisch (*Milchwissenschaft*, 1960, **15**, 543—549).—NO_2^- is determined with *p*-nitraniline and NO_3^- with *m*-xylenol. Of the 338 samples tested for NO_3^-, 60% were negative while the remainder, with one exception, showed <30 mg./kg. The addition of saltpetre to cheese-milk and the breakdown processes are described; it is shown that with small additions, NO_3^- will disappear entirely. C. V.

Determination of the nitrate content of cheese. H. Kay and G. Mrowetz (*Milchwissenschaft*, 1960, **15**, 550—556).—Hänni's method using 2,4-xylenol was modified so that the NO_3^- content at concn. between 0 and 20 mg./kg. was determined with an accuracy ±1 mg./kg. The reaction with diphenylamine (**I**) was also examined. **I** also reacts with NO_2^- but since this is not normally present in ripened cheese a separate NO_2^- determination is unnecessary. (19 references.) C. V.

Behaviour of nitrate in cheese. M. E. Schulz, H. Kay and G. Mrowetz (*Milchwissenschaft*, 1960, **15**, 556—559).—Addition of 10 g. KNO_3 (**I**) to 100 kg. cheese milk was made; **I** disappeared completely in Tilsit (**II**) and Dutch (**III**) cheeses when ripened at 15°. When **II** was ripened at 7° ~20 mg. NO_3^-/kg. remained after six weeks. With 20 g. of **I**/100 kg. addition to the milk there was a complete destruction in **III** but 20 mg. of NO_3^-/kg. was found in **II** at 6 weeks. NO_2^- was colorimetrically determined both by the diphenylamine and xylenol methods. (12 references.) C. V.

Ripening of Dariworld and Cheddar cheese with special emphasis on proteolysis. D. G. Vakaleris, N. F. Olson, W. V. Price and S. G. Knight (*J. Dairy Sci.*, 1960, **43**, 1058—1067).—No difference between the rates of proteolysis during the ripening of the two cheeses is found to account for the superior texture of Dariworld cheese. The comparatively rapid development of the smooth consistency of Dariworld is probably due to physico-chemical processes occurring during the making, induced by the prolonged exposure of the curd to brine, and the final heating in aq. 5% NaCl. The development of normal acidity is necessary for the production of a good texture in both cheeses. (24 references.) P. S. ARUP.

Edible Oils and Fats

Nutritional and chemical changes occurring in heated fats: a review. E. G. Perkins (*Food Technol.*, 1960, **14**, 508—514).—The following are discussed: autoxidation, thermal polymerisation and thermal oxidation; implications of the research on heated fats. Three main questions arise: (*a*) are polymers formed in unsaturated oils during deodorisation, processing and use? (*b*) are polymeric materials absorbed on food products? and (*c*) what are the physiological and nutritional effects of these materials? (111 references.) E. M. J.

Ultraviolet spectrophotometric research on olive oils. V. Morani and C. M. Colloca (*Ann. Staz. Chim.-agr., Roma*, 1959, iii, 149, 15 pp.).—Spectrophotometric readings for solutions of oil (0·8 g.) in ethanol (100 ml.) at 262, 268 and 274 mμ are reported for a large no. of oil samples of known origin; edible pressure oils, acid and rancid oils, A and B rectified oils, and oils treated with H_2O_2, superheated steam, decolorisers and neutralisers. Values of $\Delta K = [E_{268\ m\mu} - (E_{262\ m\mu} + E_{274\ m\mu})/2] \times 1000$, range from +8 to −6 for virgin pressure oils, from +18 to +57 for clear oils (acid and rancid), from +46 to +101 for A rectified oils and from +100 to +240 for B rectified oils, but values of ΔK are significantly altered by treatment and by the development of rancidity. The utility of the method in the examination of olive oils is discussed. (13 references.) E. C. Apling.

Isolation and identification of odorous constituents of maize. J. S. Noland (*Dissert. Abstr.*, 1960, **20**, 4527).—From the product obtained by blowing high pressure steam through maize oil previously treated with alkali, odorous constituents were isolated by steam distillation and CH_2Cl_2 extraction. A complex mixture of unsaturated hydrocarbons was obtained. Oxidation by the periodate–$KMnO_4$ technique yielded *n*-butyric, *n*-valeric, isovaleric and *n*-caproic acids which were identified by gas chromatography of their methyl esters. O. M. Whitton.

Meat and Poultry

Use of antipyrine, *N*-acetyl-4-aminoantipyrine and tritium, for the estimation of body water and gut water in living ruminants. B. A. Panaretto (*Dissert. Abstr.*, 1960, **21**, 9—10).—Body water, estimated by antipyrine, decreased in goats deprived of food and water for 48 h., but not in close relation to decrease of body wt. Blood antipyrine content increased during the 48 h. Estimations of body water by antipyrine in goats deprived of food and water for 48 h. and then restored to pre-fasting wt. by administration of water by stomach tube were not compatible with state of hydration; estimates by *N*-acetyl-4-aminoantipyrine were less than simultaneous antipyrine estimates by 2—3 l. After augmentation of body water by stomach-tube administration of rumen fluid from another goat, antipyrine estimates of body water showed an increase, and *N*-acetyl-4-aminoantipyrine estimates a much smaller increase, largely because of relative absence of this compound from rumen water. Estimates of body water by antipyrine and tritium gave comparable results. Differences between antipyrine and *N*-acetyl-4-aminoantipyrine estimates were compared with vol. of water in the gut as measured post mortem. M. D. Anderson.

Treatment of meats with ionising radiations. V. Radiation pasteurisation of beef for chilled storage. C. H. Lea, J. J. Macfarlane and L. J. Parr (*J. Sci. Fd Agric.*, 1960, **11**, 690—694; cf. J.S.F.A. Abstr., 1961, i, 99).—Small-scale tests on the storage of cuts of beef and of beef fatty tissue at chilling temp. showed that microbial spoilage is retarded by doses of ionising radiations between 25,000 and 100,000 rads; development of "tainted" odour and flavour, appearance of visible bacterial growth and rate of increase in the free fatty acid content of the fat are retarded. During storage a tallowy odour and flavour developed in the raw or lightly cooked fat; the yellow carotenoid pigment bleached, and peroxide accumulated more rapidly in the irradiated than in the control fat. The margin between desirable and undesirable effects is so small as to make the application of irradiation of doubtful benefit for overseas transport of chilled beef. E. M. J.

Bacterial metabolism of nitrate and nitrite in maturing bacon. B. P. Eddy, D. P. Gatherum and A. G. Kitchell (*J. Sci. Fd Agric.*, 1960, **11**, 727—735).—During maturation processes, large losses of nitrate may occur without accumulations of nitrite; such losses are attributed to bacterial activity and take place after the cured sides have left the factory. These decreases did not occur when bacterial growth was reduced by swabbing the surface with formaldehyde solution. (11 references.) E. M. J.

Occurrence and flavour significance of acetoin in aqueous extracts of chicken. E. L. Pippen, E. J. Eyring and M. Nonaka (*Poultry Sci.*, 1960, **39**, 922—924).—When cut-up chicken was boiled with water for 3 h. much greater amounts of acetoin than of diacetyl were found in the extract, particularly in the aq. phase. Addition of diacetyl 20 and acetoin 300 μg. per 100 ml. of broth did not improve its flavour, although 100 μg. of added diacetyl per 100 ml. could be detected. A. H. Cornfield.

Fish

An accelerated oxidation method for estimation of storage life of frozen seafoods. R. E. Palmateer, T. C. Yu and R. O. Sinnhuber (*Food Technol.*, 1960, **14**, 528—532).—The method depends on the use of purified diatomaceous silica (Celite) as a support for the material, e.g., fish or fat, to be tested. The increase in surface area permits autoxidation to proceed at an accelerated rate even at subfreezing temp. The effect of temp. and their fluctuations during storage, action of antioxidants and the relation of temp. to oxidation rate in frozen seafoods are described. The 2-thiobarbituric acid test is applied to the intact fish–Celite sample without extraction. This procedure is proposed as a method for the accelerated autoxidation of fats and oils. (22 references.) E. M. J.

Chemical studies of the herring (*Clupea harengus*). IV. Creatine in herring flesh and its behaviour during heat processing. R. B. Hughes (*J. Sci. Fd Agric.*, 1960, **11**, 700—705).—Herring caught off the coast of Britain contained 350—420 mg. of creatine and 5—10 mg. of creatinine/100 g. of flesh, corresponding values for creatine in the American Atlantic coast herring being 577 mg./100 g. and in the Pacific herring 740 mg./100 g. of wet flesh. In cooking, creatine is converted into creatinine but a general overall loss (25—30% of the original creatine) occurs in the first 2 h. Herring proteins are rapidly coagulated during cooking; occlusion or absorption of the creatine on to the proteins may be occurring, thereby rendering the creatine unavailable for extraction. Flavour is not affected. (13 references.) E. M. J.

Assessment of the progressive spoilage of ice-stored shrimp. J. R. Iyengar, K. Visweswariah, M. N. Moorjani and D. S. Bhatia (*J. Fish. Res. Bd Can.*, 1960, **17**, 475—485).—The estimation of triethylamine and total volatile N was unreliable in assessing quality of shrimp caught off the Indian coast. Total bacteria plate count and pH measurement give useful information on the progress of spoilage. Rapid tests based on the colour change in Phenol Red papers, when in contact with shrimp muscle, show promise for use in the field. Other rapid tests are based on colour change in turmeric papers and catechol–$FeCl_3$ solution. (12 references.) S. G. Ayerst.

Spices, Flavours, etc.

Preservatives

Experimental preservation of poultry by chlortetracycline and radiation. A. W. Phillips, H. R. Newcomb and F. C. Bach (*Food Technol.*, 1960, **14**, 521—522).—Chlortetracycline (CTC) (10 p.p.m.) and 100,000 rads of ionising radiation were used for retarding microbial spoilage in prepackaged fresh chicken meat. Treated meat was stored at 41 °F for 7 weeks without microbial spoilage and was similar to fresh chicken in odour, colour and texture. By contrast irradiated meat and that treated only with CTC possessed pronounced yeast odour and some slime. E. M. J.

Coating of articles of food. Dow Chemical Co. (B.P. 828,129, 30.4.58. U.S., 17.6.57).—A food product, especially meat product, is protected and preserved by coating with a thin (0·01—0·1 in.), tough, transparent film comprising a vinyl chloride polymer (or copolymer thereof with ⩾15 wt.-% of vinyl acetate) (12—25), an epoxy type plasticiser (5—15) and a non-toxic plasticiser, e.g., acetyl tributyl citrate (60—75 wt.-%). The film is deposited from the molten state, at 130—165°, on to the food product kept at low temp., e.g., in frozen state. F. R. Basford.

Food Processing, Refrigeration

Low temperature handling of sterilised foods. V. Biochemical changes in storage. A. L. Brody, K. Bedrosian and C. O. Ball (*Food Technol.*, 1960, **14**, 552—556; cf. J.S.F.A. Abstr., 1961, i, 49).—Biochemical changes were studied in products processed by high temp.-short time (HTST) and conventional canning sterilisation methods and stored at 35, 50 and 80 °F. β-Carotene content in oyster stew was not affected but was decreased in sweet maize with time and increased temp. of storage. Effects on thiamine, riboflavin, ascorbic acid, in various products, colour of tomato juice, green beans, etc., were observed. HTST processing methods give

a product equivalent to or superior in quality to conventionally canned product, and storage at temp. <50°F gives better results. E. M. J.

Layer drying of grains in storage. A. P. Deshmukh (*Dissert. Abstr.*, 1960, **21**, 138).—The velocity, depth and relationship of the drying layer to the moisture content ratio, airflow (2, 6, 10 and 14 cu. ft./min. per bu.), and temp. of drying air (58—60, 72—74 and 86—88°F) for shelled maize and pea beans in a deep bin were studied. A formula is given for calculating the time required to dry a given depth of shelled maize. O. M. WHITTON.

Drying characteristics of rewetted soya-bean. Chul Choo Lee (*Dissert. Abstr.*, 1960, **21**, 139).—Moisture moves within the soya-bean by diffusion. The value of the diffusion coeff. depends on temp. and initial moisture content but is not affected by size of the grain kernel. An equation is given for the rate of drying of soya-beans for any temp., R.H., initial moisture content and size of grain kernel. O. M. WHITTON.

Curing of ham: sodium chloride accumulation. II. Combined effects of time, solution concentration and solution volume. H. E. Wistreich, R. E. Morse and L. J. Kenyon (*Food Technol.*, 1960, **14**, 549—551; cf. J.S.F.A. Abstr., 1960, i, 207).—In pork muscle accumulation of NaCl varied linearly with solution concn. and vol. The accumulation value–time relationship was logarithmic. A composite equation is derived: $\log A = 0{\cdot}4 \log t + \log C + \log (2V + 40) - 2{\cdot}55$, where A = accumulation in mg./sq. cm., t = time in h., C = solution concn. in g./l., V = vol. of solution in ml./sq. cm. of contact area. Precision is within ±10% for contact times <24 h. E. M. J.

Time–temperature tolerance of frozen foods. XXIII. Quality changes in frozen spinach. W. C. Dietrich, M. M. Boggs, M.-D. Nutting and N. E. Weinstein (*Food Technol.*, 1960, **14**, 522—527).—Time–temp. conditions ranging from 1 day at 40°F to 2 years at −20°F were studied with regard to changes in chlorophyll, ascorbic acid, carotene, reflectance colour, visual colour and flavour. Significant deterioration occurred (except for carotene) above 0°F within periods of handling as in industry practice. Rate (%) of change of chlorophyll to pheophytin in spinach compared with that in peas and green beans was 1—2 times and ½—¼, respectively, e.g., at 10° and 20°F. Some time–temp. effects caused enough deterioration for panels to rate samples as "sufficiently poor to cause consumer complaint." Variation occurred in 10-oz. packages necessitating adequate sampling for quality control. (15 references.) E. M. J.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Substances which reduce the heat resistance of bacterial spores. H. D. Michener, P. A. Thompson and J. C. Lewis (*Appl. Microbiol.*, 1959, **7**, 166—173).—Among 650 substances examined a marked ability to lower the heat resistance of *Clostridium* spp. was frequent among those known to possess mutagenic properties for higher organisms and also among org. S compounds. Antimicrobial substances and antibiotics generally were not often active in this respect. Subtilin and its Me esters, nisin, ethylene oxide, diepoxybutane, H_2O_2, formaldehyde, dodecylguanidinehydrobromide, cetyltrimethylammonium bromide and vitamin K_4 were among the most active materials tested. A. G. POLLARD.

3.—SANITATION

Insect control by γ-irradiation: an appraisal of the potentialities and problems involved. P. B. Cornwell and J. O. Bull (*J. Sci. Fd Agric.*, 1960, **11**, 754—768).—Two potential methods of controlling insect pests by irradiation, viz., indirect control by release of sterilised adults and direct treatment (**I**) of infested products are discussed. **I** is of especial interest for disinfestation of stored materials; of those handled in bulk at particular centres. Radiation treatment of grain is examined in detail and results are compared with those obtained by fumigation. Among factors which may influence success of radiation disinfestation, problems associated with bulk handling and incorporation of treatment into established handling procedures must receive primary consideration. These could be solved most easily in the treatment of packaged products where production and irradiation may be carefully integrated. (23 references.) E. M. J.

Toxic action of a mixture of Sevin and *pp'*-DDT to the house fly. S. Nagasawa (*J. econ. Ent.*, 1960, **53**, 709—711).—Topical application of the mixture gave compound response curves indicating different types of toxic action. The response changed when the concn. of Sevin were increased after the effect of *pp'*-DDT had approached max. C. M. HARDWICK.

Toxicity of insecticides to diazinon-resistant *Musca domestica*. A. J. Forgash and E. J. Hansens (*J. econ. Ent.*, 1960, **53**, 741—745).—A strain of flies in which a resistance to diazinon had developed showed markedly increased tolerance (up to 800-fold) to numerous other insecticides. (11 references.) C. M. HARDWICK.

Feed additives for control of house-fly larvae in livestock faeces. T. L. Harvey and J. R. Brethour (*J. econ. Ent.*, 1960, **53**, 774—776).—Addition of Polybor 3 (1 gm./kg.) to steer manure controlled house flies but when fed to steers at 100 g./day it did not affect fly breeding. *Bacillus thuringiensis* spores mixed with cattle or poultry manure at 100 mg./kg. reduced fly breeding. Admixture of the spores to hen rations (7·8 mg./kg.) almost eliminated fly breeding in droppings. With 20 mg./kg. additions to cattle feed, flies did not breed in manure at all. (12 references.) C. M. HARDWICK.

Tests with compounds affecting house-fly metabolism. G. C. LaBrecque, P. H. Adcock and C. N. Smith (*J. econ. Ent.*, 1960, **53**, 802—805).—The effects on development of the addition of over 200 compounds to larval and adult food were evaluated and those of ~50 are tabulated. Of these 79 affected larval development but only 10 affected adults. Amethopterin reduced the fecundity of treated females but not that of males. (11 references.) C. M. HARDWICK.

Resistance to malathion in the mosquito, *Culex tarsalis*. D. I. Darrow and F. W. Plapp (*J. econ. Ent.*, 1960, **53**, 777—781).—Bioassay of 20 compounds with malathion-resistant and -susceptible strains showed almost no resistance to other org. P compounds or chlorinated hydrocarbons. Compounds with two carbethoxy ester groups had up to seven-fold resistance. Detoxification of Acethion [*OO*-diethyl *S*-(ethoxycarbonyl)ethyl phosphorodithioate] was rapid with both strains and the variation in hydrolysis with malathion was insufficient to explain the resistance mechanism. The pH partitioning method showed that the formation of carboxylic acid derivatives is not significantly different in the two strains. This was substantiated with mice. (18 references.) C. M. HARDWICK.

Toxicity of hexavalent chromium to bluegills. F. B. Trama and R. J. Benoit (*J. Wat. Pollut. Control Fed.*, 1960, **32**, 868—877).—Literature on toxicity of Cr^{VI} to fish and micro-organisms is reviewed. The 96 h. TL_m for bluegills in soft water is ~110 mg./l. Cr where Cr^{VI} enters the system as $Cr_2O_7^{2-}$ and the 96 h. TL_m where Cr^{VI} enters soft water as CrO_4^{2-} is ~170 mg./l. Cr. Difference is explained. The 24 h. TL_m in soft water is 175—225 mg./l. Cr. Toxicity is significantly reduced by alkalinity and hardness. (28 references.) O. M. WHITTON.

Adaptation of Walter's chromatographic equations for use with effluent concentrations. G. W. Thomas (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 422).—The equations are presented. A. H. CORNFIELD.

Nitrogen and phosphorus removal from secondary sewage plant effluents by algae. V. M. Witt (*Dissert. Abstr.*, 1960, **20**, 4618).—The removal of N and P from secondary sewage plant effluents by algae at light intensity from 100 to 1100 ft.-candles, at 20—30° and at concn. of *Scenedesmus* cells 600—20,000 per cu. mm. was studied. The algae absorbed NH_3 in preference to other inorg. and org. forms of N and P as ortho-phosphate and org. P. Under optimum conditions, 20 h. of processing normally removed these substances completely. O. M. WHITTON.

4.—APPARATUS AND UNCLASSIFIED

Breakage of glass bottles pressurised with steam. H. Burton (*Dairy Ind.*, 1960, **25**, 825—827).—The occurrence and prevention is discussed. C. V.

Organic [A, C] copper and [B] cobalt compounds. Director of Technical Services, Dept. of Agriculture, Union of S. Africa (Inventor: I. S. Perold) (B.P. 827,521—3, 27.3.57).—[A] A water-sol. chelate Cu citrate compound, $C_{12}H_{10}O_{14}CuR_4$, where R is the cation of the alkali employed, for combating Cu-deficiency diseases in animals, plants or human beings, is prepared by de-ionising or de-activating $CuSO_4$ or other Cu salt, by causing it to react with citric acid in presence of water and NaOH or other alkali hydroxide or aq. NH_3. [B] The corresponding Co compound, $C_{18}H_{15}O_{21}CoR_6$, is prepared in a similar manner by substituting, e.g., $CoCl_2$ for the $CuSO_4$. [C] The corresponding chelate Cu tartrate compound, $C_8H_8O_{12}CuR_4$, is prepared as under [A] by substituting tartaric acid for citric acid. J. M. JACOBS.

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