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INFLUENCE OF PASTURE SPECIES ON THE FLAVOUR, ODOUR AND KEEPING QUALITY OF LAMB AND MUTTON

By F. B. SHORLAND, Z. CZOCHANSKA, M. MOY, R. A. BARTON* and A. L. RAE*

Lambs fed white clover species (*Trifolium repens* cv. Grasslands Huia or cv. Grasslands 4700) showed a stronger flavour in the fat and lean and a greater intensity of odour in the casseroled 12th rib chop than lambs fed on perennial ryegrass (*Lolium perenne*). The results for hoggets generally supported those for lambs. Differences in flavour of the lean of the lambs appeared within three weeks from the beginning of the experiment. Differences in intensity of flavour and odour between the shoulder, loin and leg of the hoggets were small.

After storage for 8 months at −15°, the thiobarbituric acid (TBA) values of the lean and fat of the 12th rib chop were highly significantly greater in lambs fed white clover than in those fed perennial ryegrass. Similar differences were found in hoggets using carcasses that had been stored for one to two months. In the hoggets, significantly higher TBA values were found in the fatty tissues of the leg than in the loin and shoulder. Differences between the TBA values of the lean were not significant.

Introduction

In a previous paper[1] it was shown that Southdown × Romney lambs grazed on white clover (*Trifolium repens*) for a period of 5 months until slaughtered when approximately 9 months old, had a significantly higher intensity of flavour and odour in the cooked 12th rib chop, compared with those grazed on perennial ryegrass (*Lolium perenne*).

The purpose of the present investigation was to determine the length of time of grazing required to produce significant differences in flavour and odour and the distribution of flavour and odour in various parts of the carcass. It was previously found that grazing white clover instead of perennial ryegrass also influenced the composition of the fat.[1] For example, the white clover-fed lambs had significantly higher iodine values in their subcutaneous fat and significantly reduced contents of lauric and myristic acids. To obtain further information about differences induced by grazing different pasture species, the keeping quality of the lean and fatty tissues was assessed by means of the thiobarbituric acid (TBA) test.

Experimental

In the first experiment, three groups, each consisting of fifteen 3½-month old Romney wether lambs, chosen at random, were grazed respectively on perennial ryegrass (*Lolium perenne*, cv. Grasslands Ruanui), white clover (*Trifolium repens*, cv. Grasslands Huia) and a new cultivar of white clover tentatively called Grasslands 4700.[2] Five animals from each group were slaughtered at the end of the third, sixth and ninth weeks respectively. The 12th rib chop was taken from the left side of each carcass and evaluated for flavour and odour.

In the second experiment, two groups of 6 Romney wether hoggets (approximately 13 months of age) were grazed respectively on perennial ryegrass and Grasslands 4700 white clover. After grazing for 10 weeks the animals were slaughtered. For evaluation of differences of flavour and odour in different parts of the carcass, samples were taken from the left side of each carcass as follows: shoulder (5th rib chop), loin (12th rib chop) and leg (mid-leg across the aitch bone). The 11th rib chop was also taken to provide a further comparison between sheep grazing perennial ryegrass and white clover. All samples from both trials were stored at −15° for 1 to 2 months prior to analysis.

The methods outlined by Cramer *et al.*[1] were used to evaluate flavour and odour of the casseroled chops. On each daily tasting session, three chops (one from each treatment in the first experiment, or one from each region of the same carcass in the second experiment) were cooked simultaneously to an internal temperature of 74°. The lean and fat were separated after cooking, cut into 6 mm cubes and presented as coded samples to members of a trained taste panel for scoring intensity of flavour. The meat residue and bones of each chop were placed in a wide-mouthed stoppered jar for odour evaluation.

The pooled scores of the five-member taste panel were used in the statistical analysis of the results. In these analyses, variation between days was eliminated by the analysis of variance and mean separation was determined by Duncan's multiple range test.

Tests were carried out on the keeping quality of the fat and lean by means of the thiobarbituric acid procedure outlined by Tarladgis *et al.*[3]

Results

Flavour and odour intensity

Effect of a particular pasture species on the cooked meat

The mean scores for intensity of flavour and odour of the casseroled chops from lambs and hoggets as affected by the pasture treatment and length of time of grazing are shown in Table I.

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After three weeks of grazing, significant differences ($P < 0{\cdot}05$) in flavour of the lean were found between the white clover varieties and perennial ryegrass. At this stage, the differences in intensity of flavour in the fat and in intensity of odour, although the means show the same trend as for flavour intensity in the lean, fell short of statistical significance (actually $P < 0{\cdot}10$ for fat and $P < 0{\cdot}20$ for odour). After 6 weeks grazing, differences between the white clover cultivars and perennial ryegrass were apparent in flavour intensity of both lean and fat but not in intensity of odour ($P < 0{\cdot}10$). After 9 weeks grazing, the differences were significant for both intensity of odour and for flavour intensity in the fat, but fell just short of significance for the lean. In the older animals (hoggets) the intensity of flavour of the lean and fat was highly significantly greater in the animals fed white clover than with those fed perennial ryegrass, but the differences in odour did not reach the 5% level of significance ($P = 0{\cdot}09$).

The results indicate that the intensity of flavour of the lean and fatty tissues as well as of the odour, is greater in animals which grazed white clover cultivars than those fed on perennial ryegrass. No significant differences were observed between lambs fed on the two cultivars of white clover.

Variations in different parts of the carcass

The average scores for the intensity of flavour and odour of the casseroled meats from the shoulder, loin and leg from the Romney wether hoggets are shown in Table II.

Differences between cuts in the intensity of flavour in the lean and in odour were small and not significant. For intensity of flavour in the fat, the mean scores of both the shoulder and the loin were significantly greater than those of the leg.

Thiobarbituric acid (TBA) values

Effect of pasture treatment on the 12th rib chop

In Table III are shown the results of the TBA values of the lean and fatty tissues of the three groups of lambs fed respectively perennial ryegrass, Grasslands Huia and Grasslands 4700 white clover and the results for two groups of hoggets fed respectively perennial ryegrass and Grasslands 4700 white clover.

Analysis of variance of the TBA values of the lean and of the fat of cooked meat of the lambs showed that the differences between the three pastures composed of perennial ryegrass, and the two white clover species respectively and between the length of time on which the lambs were grazed were highly significant. The interaction between the pasture treatments and the length of time of grazing was not significant. The TBA values for lambs fed white clover were highly significantly greater than for those fed on perennial ryegrass. Likewise the TBA values for the lean and fat of the hoggets were higher in the animals fed on white clover. Lambs fed on the Grasslands Huia cultivar yielded significantly greater TBA values than those fed on Grasslands 4700 cultivar.

TABLE I

Length of grazing period required for flavour differences to appear in cooked loin chops from lambs and hoggets fed on perennial ryegrass (PR), (Grasslands Ruanui), as compared with those fed on white clover species Grasslands 4700 (NC) and Grasslands Huia (OC)

Grazing period	Flavour intensity								Odour intensity			
	Lean				Fat							
	PR	NC	OC	Differences	PR	NC	OC	Differences	PR	NC	OC	Differences
Lambs												
3 weeks	3·8	5·4	4·8	NC, OC > PR**	3·6	4·4	5·0	***	4·7	5·8	5·1	***
6 weeks	3·1	4·5	4·5	NC, OC > PR**	3·8	5·2	6·3	NC, OC > PR**	3·5	4·7	5·3	***
9 weeks	3·2	3·9	4·2	***	3·7	5·7	4·8	NC, OC > PR**	4·1	6·1	6·3	NC, OC > PR*
Hoggets												
10 weeks	4·7	5·4	—	NC > PR*	5·3	6·5	—	NC > PR*	4·9	5·7	—	***

* $P < 0{\cdot}01$
** $P < 0{\cdot}05$
*** $P > 0{\cdot}05$

TABLE II

Intensity of flavour and odour of the casseroled chops of the shoulder, loin and leg of hoggets

	Flavour intensity				Odour intensity	
	Lean	Differences	Fat	Differences		Differences
Shoulder	4·7	***	5·5		5·2	***
Loin	5·1	***	5·9	Sh, Ln, > Lg**	5·3	***
Leg	5·0	***	4·9		4·9	***
Standard Error	0·16		0·24		0·22	

*** $P > 0{\cdot}05$; ** $P < 0{\cdot}05$
Sh = shoulder
Ln = loin
Lg = leg

TABLE III

Effect of pasture treatment and length of grazing period on the TBA values of the lean and fat of the loin chop
The lamb carcasses were stored for 8 months and the hogget carcasses 1–2 months. Abbreviations as described in Table I

Grazing period	Lean				Fat			
	PR	NC	OC	Differences	PR	NC	OC	Differences
Lambs								
3 weeks	2·22	3·73	4·23	*	3·95	4·85	5·74	*
6 weeks	3·56	4·78	5·74	*	5·13	6·70	8·36	*
9 weeks	3·19	4·00	4·69	*	4·18	7·11	6·34	*
Overall means	2·99	4·17	4·89	*(OC > NC > PR)	4·42	6·22	6·81	*(OC > NC > PR)
Hoggets								
10 weeks	2·04	2·58	—	*	0·43	0·48	—	**

The TBA values of the lean and fatty tissues of the lambs after 6 weeks grazing were higher than after three weeks grazing, but at 9 weeks the values decreased.

Effects on the cooked meat of hoggets

The shoulder, loin and leg of the hoggets were examined for the TBA values of the lean and the fat of the cooked meat. In Table IV the results for the perennial ryegrass fed and the Grasslands 4700 white clover fed groups are presented.

The TBA values of both the lean and the fat for all cuts were again highly significantly greater for the animals fed white clover. The differences between cuts in TBA values of the lean were small and not significant. However, in the groups fed perennial ryegrass and white clover, the TBA values of the fat of the leg were highly significantly greater than those of the loin and shoulder.

TABLE IV

TBA values of the lean and fat of the cuts of the cooked meats of hoggets
Abbreviations as described in Table I

		Lean	Fat
Perennial ryegrass group	Shoulder	1·42***	0·41*
	Loin	1·16***	0·33*
	Leg	1·48***	0·55*
White clover (Grasslands 4700) group	Shoulder	2·05***	0·93*
	Loin	2·08***	0·92*
	Leg	2·06***	1·25*

Discussion

The present work confirmed the findings of Cramer *et al.*[1] that the casseroled 12th rib chop from sheep fed white clover possessed a highly significantly stronger flavour and odour than that of sheep fed perennial ryegrass.

It is now shown that significant flavour differences in the lean can be produced within 3 weeks (see Table I). The flavour of the fat and odour of the cooked chops of the lambs after 3 weeks' grazing on white clover were also greater and as the experiment continued these differences became significant. The results for the hoggets, likewise, demonstrated the stronger flavour and odour of the cooked meat from the animals fed white clover, compared with those fed on perennial ryegrass.

Differences in the intensity of flavour and of odour between the hogget cuts were generally small, and indicated that regional differences in the distribution of these characteristics were of little importance.

It is difficult with flavour studies involving taste panels to detect small differences or even to distinguish between meats of different species of animals.[4] It appeared desirable therefore, to use other criteria capable of more precise measurement in order to reach firm conclusions as to the effect of pasture treatment on lambs. It was found that the TBA test which is generally regarded as useful in the evaluation of keeping quality,[5] reflected differences in pasture treatment. Lamb carcasses which had been stored for 8 months at −15° were used to ensure the development of reasonably high TBA values coinciding with the maximum period of storage consistent with an acceptable product. The highly significantly greater TBA values of the lean and fat in the lambs fed white clover after only 3 weeks grazing, indicated that the effect of pasture treatment takes place very quickly, while the maximum effect occurs perhaps within 6 weeks of grazing. The results for the hoggets also indicated that even after only a short period of storage (1–2 months at −15°), the pasture treatment effect on the TBA value is apparent.

Although the TBA values of the fat of the leg were significantly higher than in the loin and shoulder of the hoggets, the TBA values generally for the fat and lean respectively were not widely different between cuts. In the hoggets, however, the TBA values for the lean were two to three times greater than those found in the fatty tissues.

From the present results it is clear that a measure of control of the intensity of flavour and odour as well as to some extent of the composition of the meat may be obtained by varying the pasture species. It was previously shown by Rae *et al.*[6,7] that supplementation of perennial ryegrass with white clover results in increased growth of sheep and increased fatty tissue in the carcasses[8] together with a lowering of the melting point of the fat and increased iodine value.[9] The increased rate of growth of the lambs may be more important than a change in flavour characteristics and keeping quality of the meat. However, should there be a demand for lamb with mild flavour and odour with high keeping quality, the use of perennial ryegrass rather than white clover for grazing is indicated.

The results moreover in showing the rapid response of flavour and odour to pasture treatment suggest that the fast rate of liveweight growth may be achieved without detriment to flavour and odour by using pasture with a high proportion of white clover during fattening, followed by a short period of grazing on perennial ryegrass alone immediately prior to slaughter.

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References

1. Cramer, D. A., Barton, R. A., Shorland, F. B., & Czochanska, Z., *J. agric. Sci., Camb.*, 1967, **69**, 367
2. Barclay, P. C., *Proc. N.Z. Soc. Anim. Prod.*, 1967, **27**, 139
3. Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L., *J. Am. Oil Chem. Soc.*, 1960, **37**, 44
4. Wasserman, A. E., & Tolley, F., *J. Fd Sci.*, 1968, **33**, 219
5. Pearson, D., *J. Sci. Fd Agric.*, 1968, **19**, 357
6. Rae, A. L., Brougham, R. W., Glenday, A. C., & Butler, G. W., *J. agric. Sci., Camb.*, 1963, **61**, 187
7. Rae, A. L., Brougham, R. W., & Barton, R. A., *N.Z. Jl agric. Res.*, 1964, **7**, 49
8. Barton, R. A., & Ulyatt, M. J., *J. agric. Sci., Camb.*, 1963, **61**, 191
9. Shorland, F. B., Czochanska, Z., Barton, R. A., & Rae, A. L., *J. agric. Sci., Camb.*, 1967, **68**, 221

HUMAN OLFACTORY RESPONSES TO 5α-ANDROST-16-EN-3-ONE— PRINCIPAL COMPONENT OF BOAR TAINT

By NERYS M. GRIFFITHS and R. L. S. PATTERSON

Analysis of the olfactory responses of men and women to a pure sample of 5α-androst-16-en-3-one showed that 7·6% of women were unable to detect the odour compared with 44·3% of men. For subjects able to detect the odour, there was a highly significant difference between sexes in the hedonic scores, but not in the individual threshold values. Women found the odour significantly more unpleasant than did men, and the relevance of this result to the meat industry is discussed.

Introduction

The compound believed to be principally responsible for the unpleasant odour sometimes produced during cooking of pork or bacon from boars has been isolated and identified as 5α-androst-16-en-3-one.[1] The steroid occurs in the fat and its concentration depends partly on age, increasing from extremely low levels in young animals, to about 0·5 ppm at bacon weight and often to considerably more in mature boars. The compound has not been detected chemically in the fat or lean of castrates (hogs) or females.

Economic advantages can be gained from the use of boars as meat animals because they generally have leaner carcasses than castrates, and show slightly better growth rates and food conversion ratios. Furthermore, if the need for castration could be eliminated, a considerable amount of time and labour would be saved, and the risk of infection and set-back in growth which may result from the operation would be avoided.

Consumer reaction to the palatability of boar meat is now being studied but, as a preliminary experiment, the odour response of 3 groups of subjects to 5α-androst-16-en-3-one has been investigated. The purpose of the experiment was to obtain accurate information on the reaction of men and women to the odour of the compound in the absence of other cooking odours. None of the subjects was familiar with either the odour of the pure compound or, as far as was known, with the odour of heated boar fat.

Experimental

The first group of subjects containing 50 males and 50 females, some of whom were laboratory personnel, were presented with a watch glass on which 800 ng of androstenone had been applied to an area of 5 cm^2 from a solution in diethyl ether. The experiments were carried out at room temperature and care was taken in presenting the samples to minimise handling of the watch glass. The subjects were asked how pleasant they would find this smell if associated with food. The responses were marked on a nine-point hedonic scale[2] where 1 = extremely pleasant and 9 = extremely unpleasant. Those subjects unable to smell the sample were presented with a 3·2 μg sample (4-fold increase), and the question was repeated.

30 subjects (15 males and 15 females), most of whom were experienced in odour description, were selected from those who could smell the 800 ng sample and their odour thresholds for androstenone were determined by presenting them with a series of samples of increasing concentration. The first sample was a control residue from the ether solvent and the subjects were asked to say when they perceived an odour which was positively different from the control after the solvent had evaporated. The approximate threshold for each subject was first determined using 8-fold steps (i.e. 25 ng, 200 ng, 1600 ng). A more precise measure of each subject's threshold was obtained by presenting stimuli increasing in concentration by 2-fold steps; this geometric increase has

been shown to provide a reasonably good perceptual scale unit.[3] The threshold was measured on three separate occasions, slightly varying the range presented in order to eliminate possible ordering effects. Immediately after each threshold test the subject was asked to give a hedonic response to an 800 ng sample.

Two further groups of subjects were examined in the same way and were asked to give hedonic ratings for the odour in answer to the question regarding its acceptability in food. The first of these groups comprised 43 women and 54 men and the second, 52 men and 52 women, each group again including a high proportion of laboratory personnel.

Results

7·6% of women were unable to detect the odour of the androstenone sample in contrast to 44·3% of men (Table I). The mean hedonic score for women was 7·26 compared with 6·22 for men, showing that women judged the smell significantly more unpleasant. 4 of the 24 male subjects in the first group, who were unable to detect the odour at the 800 ng level, reported a very weak odour when the sample concentration was increased four-fold, but they were still unable to give a positive hedonic rating for the odour.

TABLE I

Hedonic responses of men and women to the odour of 800 ng 5α-androst-16-en-3-one

Number of subjects tested: 156 men and 145 women

Hedonic score	Description of odour	Proportion of responses, %		
		Men		Women
1	Extremely pleasant	0		0
2	Very pleasant	0·6		0
3	Moderately pleasant	1·3		0·7
4	Weakly pleasant	3·8		2·8
5	Neutral	10·9		5·5
6	Weakly unpleasant	16·0		13·1
7	Moderately unpleasant	13·5		26·9
8	Very unpleasant	5·1		27·6
9	Extremely unpleasant	4·5		15·9
	Percentage of subjects unable to detect the odour	44·3	***	7·6

Mean hedonic scores: Men 6·22 S.E. 0·16
Women 7·26 S.E. 0·11 ***

*** $P < 0·001$

The mean threshold values recorded for the 30 selected individuals extended over a 2000-fold range of concentration (0·049–100·0 ng) and most subjects were consistent in their replicate responses. It should be noted that the units in the threshold determinations are not true concentrations in air; in fact the concentrations of vapour which were perceived above the samples must have been very small because of the low vapour pressure of this relatively high molecular weight compound (mol. wt. 272).

In general, for both sexes, the more sensitive the subject, the greater was their dislike of the odour, but for subjects with the same odour threshold value, women found the odour of the 800 ng sample noticeably more unpleasant than did men (Fig. 1).

The significance of the threshold values and of the hedonic scores was examined by analysis of variance (Table II). In the case of the threshold values, the function analysed was the linear y' in $y/y_0 = 2^{y'}$ where y is the observed threshold

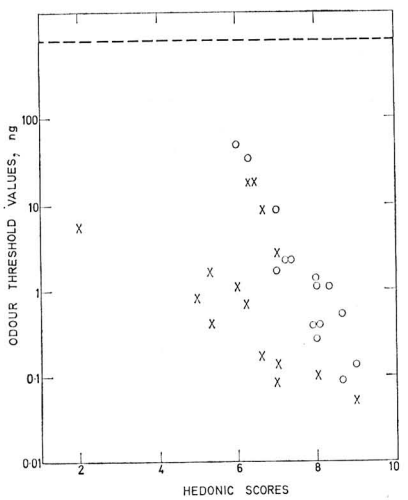

FIG. 1. *Relationship between odour threshold and hedonic rating for 5α-androst-16-en-3-one determined for 15 men and 15 women (mean of three determinations)*

× males, ○ females; — — — level for hedonic scoring (800 ng)

TABLE II

Analysis of variance of responses to the odour of 5α-androst-16-en-3-one by subjects capable of detecting the odour of 800 ng on a watch glass

Source of variation	Degrees of freedom	Transformed threshold values		Hedonic scores	
		Mean square	*F* ratio	Mean square	*F* ratio
Between sexes	1	6·40	0·29†	46·95	9·86†**
Between subjects within sex groups	28	21·89	78·80††***	4·76	32·99††***
Within subjects	60	0·28		0·14	
Total	89				

** $P < 0·01$ *** $P < 0·001$
† Between sexes/between subjects
†† Between subjects/within subjects

level and y_0 the lowest threshold level found (0·049 ng). There was a highly significant difference between sexes in the hedonic scores, but not in the threshold values (the subjects were selected from those who could smell the 800 ng sample in the first test). It is noteworthy that both threshold values and hedonic responses were consistent within subjects from one trial to another, compared with difference between subjects, shown by the very high F ratios in the second line of the Table.

Discussion

During the odour threshold study, the majority of subjects described the odour using terms such as animal, sweaty, urine or ammonia-like, but about 40% of both men and women described the odour as sweet, fruity or perfume-like at the first concentration at which the odour could be positively identified, before describing it as animal, sweaty, etc., at the next higher concentration. This difference in reaction did not appear to be correlated with either their odour threshold or their ultimate hedonic rating of the odour.

The result that women find the odour of the compound more unpleasant than men is of practical importance because generally it will be women who will decide in the kitchen during the cooking of pork or bacon whether or not it is acceptable. It is possible that boar meat, which might be discarded during cooking when the taint is most noticeable, might well be acceptable at the table to a considerable proportion of men and of less perceptive women, particularly as cooling by this time will have reduced the intensity of the odour. An adverse reaction of this nature by those most concerned with the purchase of pork could be of major importance to the meat industry, and future testing programmes on boar meat should be orientated to sample the opinion of those consumers most likely to be involved in the preparation and cooking of pork and bacon.

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References

1. Patterson, R. L. S., *J. Sci. Fd Agric.*, 1968, **19**, 31
2. Peryam, D. R., & Girardot, N. P., *Fd Engng*, 1952, **24**, 58
3. Beck, L. H., Kruger, P., & Calabresi, P., *Ann. N.Y. Acad. Sci.*, 1954, **58**, 225

NOTE ON DIFFERENTIATION OF MYOFIBRILLAR PROTEINS

By ANGELA CHAMPION, A. L. PARSONS and R. A. LAWRIE

The detection of qualitative differences between the myofibrillar proteins of muscles, which superficial examination would classify as similar on the basis of their 'redness' or 'whiteness', emphasises the need for more subtle criteria for understanding meat quality.

Introduction

Qualitative differences in both static and dynamic biochemical and physiological parameters between 'red' and 'white' muscle have been recognised for many years.[1–4] Moreover quantitative differences in such components as intramuscular fat, moisture and protein have been shown to characterise muscles which cannot readily be classified as 'red' or 'white'.[5,6] It is only relatively recently, however, that qualitative differences in the proteins of superficially similar muscles have been established. Thus, Parsons and co-workers[7] showed that the myosins of porcine *longissimus dorsi* and *psoas major* could be differentiated electrophoretically after processing although they were indistinguishable beforehand. They concluded that while the classification of muscles as 'red' and 'white' could explain some of the properties of meat, the latter also reflected more subtle biochemical differentiation. A similar view was recently expressed by Burleigh & Schimke[8] from a study of various glycolytic and oxidative enzymes from mammalian muscle. The present communication briefly reports differences in the electrophoretic patterns obtained from the myofibrillar proteins of several muscles from rabbit, ox and pig.

Experimental

Samples

Fresh samples of *longissimus dorsi* (lumbar and thoracic) and *psoas major* muscles of both ox and pig were obtained from a local slaughterhouse. Rabbits were killed in the laboratory by decapitation after injection of sufficient myanesin to cause relaxation. The *longissimus dorsi*, *psoas major* and *semitendinosus* muscles were dissected out immediately *post mortem* and stored for 4 days at 0°.

Preparation of myofibrils

Muscles were homogenised in 3 times their own weight of cold 30 mM sodium β-glycerophosphate (adjusted to pH 6·5 with 1 N-HCl)[9] in a high-speed blender for 3 min. The homogenates were centrifuged at 1000 × g for 15 min, the supernatants were discarded and the pellets were resuspended in sodium β-glycerophosphate buffer. The suspensions were then centrifuged at 600 × g for 3 min to remove coarse material.[10] These centrifugings were repeated. The myofibrils were then washed 3 times by alternatively adding buffer and centrifuging at 2000 × g for 15 min, to remove all traces of sarcoplasmic protein. All operations were carried out in a cold-room at 0°.

Preparation of samples for electrophoresis

The final suspensions of myofibrils contained 2·5–3·0 mg of protein/ml. Protein estimations were made by the micro-Kjeldahl method, a value of 16·7% of N in protein being used for converting N into protein. Aliquots (2 ml) of the suspensions were pipetted into 6 ml of 8 M urea contained in 25 ml conical flasks. The mixtures were left for 24 h at 0°, with occasional shaking, and then centrifuged at 35000 × g for ¾ h. The supernatants were decanted into small specimen tubes and retained for electrophoresis.

Starch-gel electrophoresis

A vertical apparatus[11] was used as previously described,[7] with the following non-discontinuous buffer system: inner and outer gel buffer, 0·01 M sodium barbitone–5 mM-HCl, pH 7·5 at 0°; upper and lower tray buffer, 0·1 M-sodium borate, pH 7·5 at 0°. All gels contained 16% starch (from Connaught Research Laboratories, Toronto, Ont., Canada), and 8 M urea was added to prevent polymerisation of the proteins under study.[12] Electrophoresis was carried out at 220 V, 25–30 mA, for 5 h in a cold-room maintained at 0°. Gels were then carefully sliced and stained for 2 min with a solution of 1% Naphthalene Black 10B containing 2% Nigrosine. After washing free of background stain with several changes of a solution of methanol–acetic acid–water (5 : 1 : 4 by vol.) the upper halves of the gels were photographed (Ilford HP4 film and grade 3 printing paper).

Results

Facsimiles of typical starch-gel electrophoretograms of myofibrillar proteins of the muscles studied are represented in Fig 1. It is apparent, first af all, that there are general species differences between the proteins. Thus, for example, there are two strong bands of low mobility near the origin in all three rabbit muscles, a feature not present in pig or ox samples. The patterns for the pig muscles studied are characterised by diffuse areas at the origin and five protein components of relatively similar mobility. The 3rd and 4th of these (counting from the origin) are present in relatively high concentration. The patterns for the ox muscles are characterised by possessing four major bands of which the three least mobile are present at relatively high concentration.

Within each species group, however, more subtle differences in the patterns between the muscles are found. Thus, for example, rabbit *longissimus dorsi* and *psoas major* have a fast-moving component not present in *semitendinosus*; both *psoas major* and *semitendinosus* have two components not present in *longissimus dorsi*; and the relative concentrations of the components common to all three muscles are different.

Apart from differences in the extent of the diffuse areas near the origin, the patterns for the three muscles of the pig are similar, although there are again minor differences in the relative concentrations of the components.

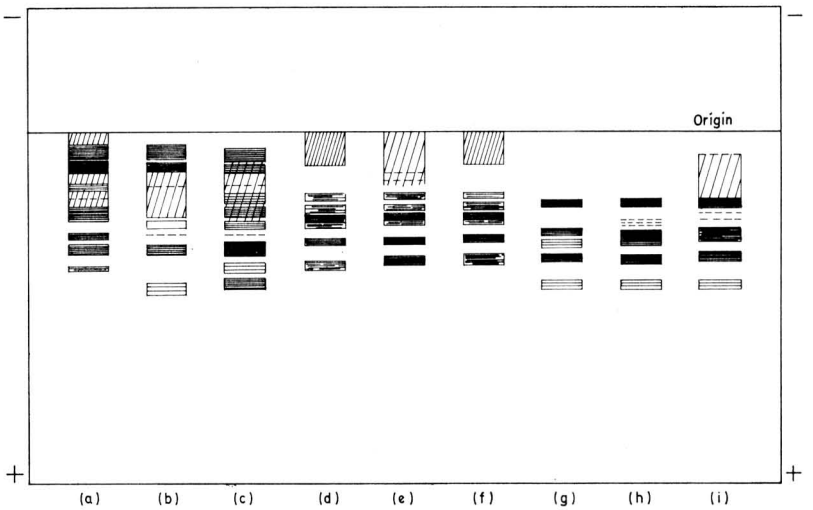

FIG. 1. *Facsimiles of starch-gel electrophoretograms in 8* M *urea of myofibrillar proteins from rabbit, pig and ox muscles*

(a) rabbit *semitendinosus;* (b) rabbit *longissimus dorsi*; (c) rabbit *psoas major*; (d) pig *longissimus dorsi* (thoracic); (e) pig *longissimus dorsi* (lumbar); (f) pig *psoas major*; (g) ox *longissimus dorsi* (thoracic); (h) ox *longissimus dorsi* (lumbar); (i) ox *psoas major*

Rather greater differences distinguish the ox muscles studied. Both *longissimus dorsi* (lumbar) and *psoas major* are distinct from *longissimus dorsi* (thoracic) in having traces of components between the two less mobile major bands; and the *psoas major* has a diffuse area near the origin not present in either of the other two ox muscles.

Discussion

The differences in the pattern of myofibrillar protein between rabbit *semitendenosus*, on the one hand, and rabibt *longissimus dorsi* and *psoas major*, on the other, were not unexpected. The former is a so-called 'red' muscle; the latter two are 'white'. That there are subtle differences between them, and between the three ox muscles studied (which are all superficially 'red') emphasises the need for closer investigation of the individual muscles of which carcasses are composed. Moreover, the apparent similarity in the patterns for the three porcine muscles (which can be grouped as superficially 'white') should not necessarily be taken to signify the identity of the proteins. Thus, even although the isolated myosins of porcine *psoas major* and *longissimus dorsi* (lumbar) yield similar starch-gel electrophoretograms when fresh, differences in their respective patterns arise when the muscles are processed.[7] In assessing the eating and keeping quality of meat, it is clearly desirable to take account of qualitative, as well as quantitative, differences in the composition of individual muscles.

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References

1. Batelli, M. F., & Stern, K., *Biochem. Z.*, 1912, **46,** 317
2. Needham, D. M., *Physiol. Rev.*, 1926, **6,** 1
3. Lawrie, R. A., *Biochem. J.*, 1953, **55,** 298, 305
4. Scopes, R. K., *Biochem. J.*, 1968, **107,** 139
5. Lawrie, R. A., *Br. J. Nutr.*, 1961, **15,** 453
6. Lawrie, R. A., Pomeroy, R. W., & Cuthbertson, A., *J. agric. Sci., Camb.*, 1963, **60,** 195
7. Parsons, A. L., Parsons, J. L., Blanshard, J. M. V., & Lawrie, R. A., *Biochem. J.*, 1969, **112,** 673
8. Burleigh, I. G., & Schimke, R. T., *Biochem. J.*, 1969, **113,** 157
9. Scopes, R. K., *Biochem. J.*, 1964, **91,** 201
10. Perry, S. V., & Grey, T. C., *Biochem. J.*, 1956, **64,** 184
11. Smithies, O., *Biochem. J.*, 1959, **71,** 585
12. Mueller, H., & Perry, S. V., *Biochem. J.*, 1952, **85,** 431

FORMULATION OF A PROTEIN-RICH VEGETABLE MIXTURE FOR PREVENTION OF PROTEIN-CALORIE MALNUTRITION

By M. M. HANAFY, Y. SEDDIK and M. K. AREF

Several mixtures of crushed decorticated groundnuts (*Arachis hypogaea*), lime-roasted chickpea (*Cicer arietinum*) and the white dregs of sesame (*Sesamum indicum*) are quantitatively and qualitatively compared with 'standard' proteins.

Using a selection of both children and adults, a blend ratio 3 : 2 : 1 (groundnut–chickpea–sesame) was selected as being fully palatable and readily acceptable.

Introduction

Protein malnutrition in Egypt has resulted from the limited sources of protein of high biological value, particularly those of animal origin, and in recent years, the high rate of population growth has aggravated the situation further.[1] The solution to this problem is urgent but it must be based on local means. Because of the difficulties and high cost of expanding animal husbandry and fisheries as well as the development of the industries, transport and storage facilities required for adequate utilisation of animal products, greater reliance has to be placed on vegetable sources of protein. These are already available and have an economic advantage over animal sources, namely that the cost of the raw material and its storage and transportation is much lower.

The devised food should not only be inexpensive and rich in protein but must also be of high biological value in order to balance the diets of those more likely to suffer from protein-calorie malnutrition, viz. the children of the lower socio-economic sectors of the community. The choice of legumes and oil-containing seeds was therefore necessary.

The groundnut (*Arachis hypogaea*) was chosen, rather than the soyabean, which is not widely cultivated in Egypt. The agricultural economy is strongly oriented towards the cotton crop, and cotton pests might be encouraged by the cultivation of the soyabean.

The press cakes resulting from extraction of the oil from the cottonseeds are not an acceptable food to Egyptians and the elaborate processing required to yield a cottonseed flour suitable for incorporation in food would add to the cost. Furthermore, the press cakes are in great demand for cattle feeding.

The groundnut is a most nutritious food. As a source of energy 100 g of shelled groundnut supplied 546–603 calories[2,3] and its protein content ranged from 25 to 30%.[4] Its production in Egypt reached 45,230 metric tons in 1963. However, the biological value of groundnut protein is only 56, and the most apparent deficiencies are in lysine, methionine and tryptophan.[5]

The addition of the chickpea (*Cicer arietinum*) which is rich in lysine can correct this deficiency in a mixture of groundnut–chickpea.[6] Chickpea is available and highly acceptable in Egypt where its production in 1963 was 8180 metric tons. It is mainly consumed (after lime-roasting) by children as a pastime food between meals. It is also used in the confection of some types of candied sweets, and in some vegetable meals. The amino acid composition of its protein (more than 23% of the dry weight)[7] though high in lysine, is low in both tryptophan and methionine.[8]

The further addition of sesame (*Sesamum indicum*) which surpasses all other seeds as a source of methionine and contains a high level of tryptophan[8] will correct to some extent the deficiencies of these two amino acids. Sesame production in Egypt was 25,550 metric tons in 1963. Caldwell reviewed[9] the importance of sesame in human nutrition and the feeding trials for the evaluation of the biological value of its protein, used alone or in various combinations with maize meal and groundnut meal. Kuppuswamy *et al.*[10] used sesame protein as a supplement for groundnut protein, Hassan recommended[11] the use of sesame for its high protein content (20–25% of the seed) and Dean thought[12] that as a condiment the contribution of sesame might improve the dietary value where the total protein from other sources was small.

Experimental

Composition of the mixture

The composition of crushed groundnut and chickpea, as given by Chatfield,[2] has been used for calculation of that of the desired mixture in which the white dreg of sesame (tehina*) was incorporated. Because of the variations in composition of tehina introduced as a result of differences in processing, preference was given to the figures of El-Dokany[13] who studied the full composition of samples of this dreg obtained from the same supplier. The present nitrogen estimations and calculated protein content for each of these foodstuffs were in agreement with the reported composition (Table I).

* Tehina, prepared from crushed decorticated sesame seeds, is a popular local dish in Egypt

TABLE I

Composition of groundnut, chickpea and sesame butter/100 g edible portoin

	Groundnut	Chickpea	Sesame dreg
Nitrogen content (detd)	4·61	3·28	5·15
Conversion factor used	5·46	6·25	5·30
Protein content (calc.)	25·1	20·5	27·2
Reference	Chatfield[2]		El-Dokany[13]
Protein, g	25·6	20·1	27·8
Fat, g	43·3	4·5	57·8
Calories	546	358	667

For the amino acid patterns of the proteins of these three foods, the Tables of Orr & Watt[8] have been used. These patterns are illustrated in Table II together with the calculated amino acid composition of mixtures of groundnut, chickpea and sesame dreg in various proportions and the F.A.O.[5] provisional amino acid pattern, for comparison.

Because of the possible application of these combinations by untrained persons, only the simplest ratios were tried. The amino acid deficiencies of the groundnut can be seen from Table II as well as the possible improvements which may be introduced by the addition of chickpea and sesame.

It may be seen that increasing the proportion of chickpea (formulae 1 and 2) to that in the 2 : 1 groundnut–chickpea mixture,[6] did not alter the content of sulphur-containing amino acids; while supplementation of groundnut with sesame alone (formulae 6 and 7) led to an aggravation of the lysine deficiency.

Mixtures of the three foodstuffs (formulae 3, 4 and 5) seemed more or less similar in amino acid pattern and similar to the F.A.O. reference protein.[5] The choice between them depended on acceptability tests.

Acceptability tests

Dishes of groundnut–chickpea–sesame (GCS) mixture were prepared containing various proportions (by wt.) of the edible portions of slightly roasted, peeled, lime-roasted chickpeas, and white dreg of sesame, sweetened with 20% sugar. The seeds were crushed well and thoroughly mixed with the sugar and sesame butter.

They were presented to 72 individuals (adults and children) who were not told of their contents. Young children were asked to point out their preferred dish and were also observed for visible signs of their preference. Older subjects were asked to arrange the dishes in descending order and state the reasons for their choice.

Results and Discussion

Most preferred GCS mixture No. 3 ($G_3C_2S_1$ = groundnut 3: chickpea 2: sesame 1). 81 g of this mixture can supply 20 g of protein and about 400 cal, i.e. an adequate supplement to a 'poor' diet, suitable for a school meal or a worker's snack. It may be prepared in different forms: as powder for enrichment of other dishes, as sweet candy, gruel or cake. The sweetened mixture may be baked into biscuits. Instead of sugar, salt may be added and GCS may then be offered as a condiment (dokka) eaten with bread.

The use of plant protein sources in general is associated with certain difficulties as the nutritive value is influenced by the presence in the seeds of factors with adverse physiological action such as trypsin inhibitors and haemaglutinins in legumes[14] and a goitrogenic factor in the red cuticles of groundnuts.[15] The latter also contain a tannin which contributes to the bitter taste of groundnut flour prepared without removal of the cuticles.[16]

In the proposed mixture, the groundnut was incorporated after peeling and this process was facilitated by mild roasting which enhanced the palatability and acceptability. It had no effect on the amino acid composition of the protein,[17] and is even reported to improve the nutritive value by inactivation of the trypsin inhibitor and increasing the availability of the limiting sulphur amino acids.[18]

Vegetable protein concentrates often provide media for the growth of moulds and other micro-organisms.[19] The growth of *Aspergillus flavus* on groundnut flour produces aflotoxin compounds extremely toxic to animals,[20,21] but hand selected peeled groundnut seeds are free from these toxins. The flour is usually obtained as a by-product after oil extraction by technical processes entailing drastic heating which, in the presence of free carbohydrate, causes some loss and reduced availability of lysine and possibly other amino acids.[14] No such effects are produced during mild controlled heating.

With chickpeas, lime-roasting not only improves their palatability but their calcium content is thereby greatly enhanced. Hanafy *et al.*[22] demonstrated by controlled clinical trials that the improvement in growth of infants on groundnut–chickpea mixture, compared with groundnut alone, was not due to the calcium, yet the contribution from the chickpea is valuable in meeting the daily requirements of this mineral where consumption of milk and dairy products is restricted.

Sesame is not known to contain any substance having adverse effects; however, the outer epidermis of the seeds has a high content (approximately 3%) of oxalate regarded by many as a suspect material in human nutrition.[9] The decortication of sesame seeds in the process of manufacture

TABLE II

Essential amino acids pattern (mg/g N) of the protein of groundnut, chickpea and sesame and various formulated mixtures compared with the F.A.O. reference protein

	Ile	Leu	Lys	Met	Total S acids	Total 'aromat'.	Thr	Try	Val
F.A.O. pattern	270	306	270	114	270	360	180	90	270
Groundnut	257	380	223	55	149	540	168	69	311
Chickpea	359	459	430	83	172	512	222	51	308
Sesame	288	480	151	175	305	677	175	90	207
1. G_2C_1*	283	400	276	62	155	533	183	64	310
2. G_1C_1	299	412	307	66	158	527	189	61	309
3. $G_3C_2S_1$	289	420	260	87	187	561	183	69	289
4. $G_2C_2S_1$	286	429	269	93	195	566	186	68	284
5. $G_1C_1S_1$	301	439	245	110	217	588	185	73	269
6. G_2S_1	268	416	197	98	205	589	171	77	274
7. G_1S_1	273	433	185	118	231	612	172	80	256
$G_3C_2S_1$ (by analysis)**	244	438	256	83	150	579	200	69	276

* G, groundnut; C, chickpea; S, sesame; 1, 2, 3, ratio by wt.
** Graciously performed by Nestlé, S.A., Vevey, Switzerland

of the white dreg (sesame butter) removed the objectionable oxalate.

The amino acid pattern aimed at in this GCS mixture was that of the reference protein adopted by the F.A.O. 1957 Committee.[5] Table III compares the amino acid composition of GCS with the F.A.O. (1957) pattern as well as with other standards such as the proteins of cow's milk, human milk and whole hen's egg.

The biological value of the GCS protein, calculated on the percentage deviation of its limiting amino acid from whole egg protein[23] was 66·5.

The protein score or percentage deviation of the limiting amino acid from the F.A.O. pattern was 69·3, on the basis of the total sulphur-containing amino acids and not the methionine alone.

In order to express by means of a single index the amount of protein in a food as well as its quality or biological value, Platt *et al.*[24] introduced the term net dietary protein calories % (NDpCal %) or the concentration of fully utilisable protein as a percentage of the energy content of the food. They calculated that when the NDpCal % of the foodstuff falls below 4·6 it can no longer meet the requirements for protein of the adult and that the minimum for the human infant is an NDpCal % of 8. GCS mixture (without added sugar) provides 24·7% protein with an energy value = 20% of the total calories and consequently has an NDpCal % of 9·3. The sweetened mixture with only 20% protein giving 16·3% of the total calories, has an NDpCal % of 8·75, which is still higher than the minimum for infants.

In 1965, the Joint F.A.O./W.H.O. Expert Group adopted,[25] in preference to the provisional F.A.O. (1957) pattern,[5] that of whole hen's egg in terms of the relationship of each essential amino acid to the total of essential amino acids or *A*/*E* ratio. Such a comparison (Table IV) shows that the limiting factor is again the sulphur-containing amino acids but they are not less than 77% of the egg protein.

The pattern of GCS is also compared with that of human milk since, as stated by the joint Expert Committee F.A.O./W.H.O. (1965),[26] the data are not sufficiently precise to provide a basis for choosing between the essential amino acid patterns of these two protein sources (hen's egg and human milk). In GCS the amino acid with the lowest *A*/*E* ratio is threonine, although it is at the satisfactory level of 82% of that in human milk or in egg protein.

TABLE III

Essential amino acids (mg/g N) of a mixture of 3 groundnut: 2 chickpea: 1 sesame (GCS) compared with various reference patterns

Amino acid	Cow's milk	Human milk	Whole egg	F.A.O. pattern	GCS	GCS as % of*	
						Egg	F.A.O.
Isoleucine	407	411	415	270	289	70	100
Leucine	630	572	553	306	420	76	100
Lysine	496	402	403	270	260	65	96
Total 'aromatic'	634	652	627	360	561	89	100
Phenylalanine	311	297	365	180	342	93	
Tyrosine	323	355	262	180	219		
Total S acids	211	274	346	270	187	54	69
Cystine	57	134	149	126	101		
Methionine	154	140	197	144	86	44	
Threonine	292	290	317	180	183	58	100
Tryptophan	90	106	100	90	69	69	77
Valine	440	420	454	270	289	64	100
Total essential	3200	3127	3215	2016	2258		

* Values above or equal to reference pattern are scored as 100%

TABLE IV

***A*/*E* ratio (mg amino acid/g of total essential amino acids) in groundnut-chickpea-sesame mixture (GCS) compared with whole egg and human milk**

Essential amino acid	Human milk	Whole egg	GCS	GCS as % of*	
				Egg	Human milk
Isoleucine	132	129	128	99	97
Leucine	184	172	186	100	100
Lysine	126	125	115	92	90
Total 'aromatic'	226	195	248	100	100
Phenylalanine	114	114	151	100	100
Tyrosine	112	81	97		
Total sulphur acids	87	107	83	77·5	95·5
Cystine	43	46	44		
Methionine	44	61	39	64	88·5
Threonine	99	99	81	82	82
Tryptophan	34	31	31	100	91
Valine	147	141	128	91	87

* Values above or equal to reference pattern are scored as 100%

It has been proposed by Hassan *et al.*[27] to use de-fatted flours of the oil seeds and thus reduce the cost. Their proposed mixture contained more sesame and consequently less lysine which would be diminished further by the severe processing for oil extraction.

It is known that cereals, which form more than half of the Egyptian diet,[28] are mainly poor in lysine and it has been suggested[29] that cereal flours should be supplemented with this amino acid alone. Therefore a protein supplement intended to improve the type of diet commonly consumed by the low-income groups must contain adequate lysine.

Apart from the protein, GCS is a food with high energy value (100 g of GCS without sugar may be calculated to supply 503 cal of which 295 are from a fat content of 32·8%). This, like all plant oils, is more unsaturated than animal fat in general and consequently less atherogenic.

Protein deficiency is still the most common nutritional problem of children living in the tropics.[30] It Egypt, it has been shown to result from the use, in the critical weaning age, of starchy gruels mainly from cereals with a low protein content.[31] The example of 'Incaparina'[32] is a great stimulus for a solution based on local means. The mixture proposed here can be prepared by the consumer, as a highly nutritious inexpensive food, with a familiar well-liked flavour, that can be easily incorporated into the diet of weaned babies, children and adults.

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References

1. U.A.R. Economic and Agricultural Statistics, 'Monthly Bulletin of Agricultural and Economic Statistics'. Annual book of Agricultural Economy 1963, 1965 (Cairo: Dept. of Statistics)
2. Chatfield, C., 'Food Composition Tables, Minerals and Vitamins for International Use', F.A.O. Nutritional Studies No. 11, 1954 (Rome: U.N. Food and Agriculture Organisation)
3. McCance, R. A., & Widdowson, E. M., *Spec. Rep. Ser. med. Res. Coun., No. 297*, 1960 (London: H.M.S.O.)
4. Rosen, G. D., in 'Processed Plant Protein Foodstuffs', (Ed. Altschul, A. M.) 1958, p. 419 (New York: Academic Press)
5. 'Protein Requirements', F.A.O. Nutritional Studies No. 16, 1957 (Rome: U.N. Food and Agriculture Organisation)
6. Hanafy, M. M., Ibrahim, A. H., El-Khateeb, S., & Seddik, Y., *Alex. med. J.*, 1965, **11**, 241
7. Deschamps, I., in 'Processed Plant Protein Foodstuffs', (Ed. Altschul, A. M.) 1958, p. 717 (New York: Academic Press)
8. Orr, M. L., & Watt, B. K., 'Amino acid content of foods'. Home Economics Res. Rep. No. 4, 1957 (Washington D.C.: U.S. Dept. Agric.)
9. Caldwell, R. W., in 'Processed Plant Protein Foodstuffs', (Ed. Altschul, A. M.) 1958, p. 535 (New York: Academic Press)
10. Kuppuswamy, S., Joseph, K., Narayo Rao, M., Rama Rao, G., *et al.*, *Fd Sci.*, 1957, **6**, 86
11. Hassan, A., *Gaz. Egypt. paediat. Ass.*, 1960, **8**, 343
12. Dean, R. F. A., in 'Processed Plant Protein Foodstuffs', (Ed. Altschul, A. M.) 1958, p. 219 (New York: Academic Press)
13. El-Dokany, A. M., 1965, Thesis, University of Alexandria
14. Liener, L. E., in 'Processed Plant Protein Foodstuffs', (Ed. Altschul, A. M.) 1958, p. 79 (New York: Academic Press)
15. Mougdal, N. R., Srinivasan, V., & Sarma, P. S., *J. Nutr.*, 1957, **61**, 97
16. Kuppuswamy, S., Srinivasan, M., & Subrahmanyan, V., 'Protein in Foods', 1958 (New Delhi: Indian Council of Medical Research)
17. Hirsch, J. S., Niles, A. D., & Kemmerer, A. R., *Fd Res.*, 1952, **17**, 442
18. Cama, H. R., & Morton, R. A., *Br. J. Nutr.*, 1950, **4**, 297
19. Mickelsen, O., *Nutr. Rev.*, 1968, **26**, 129
20. Davidson, C. S., *Med. Sci., Tokyo*, 1963, **14**, 32
21. Davidson, C. S., *Nutr. Rev.*, 1964, **22**, 97
22. Hanafy, M. M., El-Khateeb, S., Seddik, Y., & Zein, M. S., *Alex. med. J.*, 1967, **13**, 217
23. Mitchell, H. H., & Block, R. J., *J. biol. Chem.*, 1946, **163**, 599
24. Platt, B. S., Miller, D. S., & Payne, P. R., in 'Recent advances in human nutrition', (Ed. Brock, J. F.) 1961, p. 351 (London: J. A. Churchill)
25. F.A.O./W.H.O. Joint Expert Group *F.A.O. Nutr. Mtg Rep. Ser. No. 37*, 1965
26. F.A.O./W.H.O. Joint Expert Committee of Nutrition, *F.A.O. Nutr. Mtg Rep. Ser. No. 19*, 1958
27. Hassan, A., Morcos, S. R., & El-Zamzami, S., *Gaz. Egypt. paediat. Ass.*, 1960, **8**, 487
28. Sabry, Z. I., in 'Meeting protein needs of infants and children', Publication 843, 1961, p. 183 (Washington D.C.: Natn. Acad. Sci. Natn. Res. Coun.)
29. Rosenberg, H. R., in 'Protein and amino acid nutrition', (Ed. Albanese, A. A.) 1959, p. 381 (New York: Academic Press)
30. Jelliffe, D. B., *Monograph. Ser. W. H.O. No. 29*, 1955 (Geneva: U.N. World Health Organisation)
31. Hanafy, M. M., *J. Egypt. med. Ass.*, 1947, **30**, 440
32. Scrimshaw, N. S., Behar, M., Wilson, D., De Leon, R., & Bressani, R., in 'Meeting protein needs of infants and children', Publication 843, 1961, p. 57 (Washington, D.C.: Natn. Acad. Sci. Natn. Res. Coun.)

EVALUATION OF A PROTEIN-RICH VEGETABLE MIXTURE FOR PREVENTION OF PROTEIN-CALORIE MALNUTRITION

By M. M. HANAFY, Y. SEDDIK and M. K. AREF

Experiments showed that a crushed groundnut–chickpea–sesame (3 : 2 : 1) mixture is an adequate source of protein comparable with casein as a constituent of a 20% protein diet for promoting growth and nitrogen retention in young rats, synthesis of their serum proteins and haemoglobin, their maturation, reproduction, lactation and breeding of three generations of normal healthy animals.

Introduction

A solution to the problem of protein shortage in areas where protein-calorie malnutrition is prevalent, should be based on the increased production of local inexpensive sources of protein of high biological value, which can adequately supplement the ordinary diet of the poor and the susceptible groups in the community.[1] The mixture of groundnut–chickpea–sesame (GCS) devised by Hanafy *et al.*,[2] as an improvement on the groundnut–chickpea milk-substitute,[3] has been formulated to possess such advantages. After appraisal of its value from the study of its composition in essential amino acids[4] it was subjected to extensive feeding experiments to evaluate its nutritive value on the rat, and the results are presented here. The results of its subsequent successful application in human feeding trials have been reported.[2,5]

Experimental

Weanling Swiss albino rats (40–50 g), from the colony bred in the Shatby Paediatric Hospital, Alexandria, were kept in separate cages and fed *ad libitum* on the experimental diets prepared from a basal ration consisting of (per 100 g): 80 g rice starch, 10 g cotton seed oil, 4 g cellulose, 4 g salt mixture, and 2 g vitamins mixture, in which, either groundnut (diet G), casein (diet Cas), a 2 : 1 mixture of groundnut–chickpea (diet GC) or a 3 : 2 : 1 mixture of groundnut–chickpea–sesame (diet GCS), was incorporated in place of rice starch to bring the protein content of the diets to 10 or 20%, and the amount of oil was adjusted to make them isocaloric (Table I).

The animals were maintained on these diets, their general appearance and behaviour being noted. Their daily food intake was recorded and their growth was compared by measurement of the protein efficiency ratio (*PER*) and the efficiency of food utilisation (feed conversion efficiency = *FCE*) according to the standardised procedures described by Campbell.[6] The serum protein[7] and haemoglobin concentration at various ages were estimated on samples of blood obtained from the tail, the latter by a modification of Sahli's method in which comparison of a constant dilution of acid haematin with a fixed standard was done spectrophotometrically. Nitrogen balance determinations were performed when the animals were 8 weeks old in experiments lasting 3 days. The productive performance was also studied on animals kept on the experimental diets from weaning and mated at age 100 days, with follow up of successive generations maintained on the same diets as their parents. The protein value of the diets was also compared by the lactation efficiency method of Asenjo & Goyco.[8] The effects of the various diets were compared statistically using the Student's '*t*' test.

TABLE I

Composition of the test and control diets and percentage of calorific constituents and calories supplied

	10% protein diets				20% protein diets			
	Cas	GCS	GC	G	Cas	GCS	GC	G
Constituents (g/kg):								
Casein	105	—	—	—	210	—	—	—
Groundnut	—	203	283	390	—	405	566	780
Chickpea	—	136	142	—	—	270	284	—
Sesame dreg	—	68	—	—	—	135	—	—
Cottonseed oil	160	53	60	20	300	80	60	—
Rice starch	595	430	415	480	350	40	—	14
Cellulose (sawdust)	80	50	50	50	80	10	30	20
Salt mixture	40	40	40	40	40	40	40	40
Vitamin mixture	20	20	20	20	20	20	20	20
Calorific constituents:								
Fat, %	16	19	19	21	30	35	31	38
Carbohydrate (available), %	60	54	54	49	35	24	33	17
Calories/100 g	424	427	427	425	490	491	491	490

Results

The mean *PER* and *FCE* values obtained with the experimental diets at 10% protein level are shown in Table II.

The serum protein and haemoglobin concentration are seen in Table III to increase gradually from weanling to adult rats. Maximum values were reached at age 14 weeks on all diets except for the serum proteins with the 10% protein diet G which were still rising at 18 weeks. The 20% protein diets have also been compared with the standard Purina laboratory chow (diet Pur with 23% protein) used in the breeding of the present stock. It may be seen that on all these high-protein diets, greater values were obtained and these at an earlier age than on the 10% protein diets. Table IV compares the nitrogen retention of the various groups at the age of 8 weeks.

The reproductive performance on the 10% protein diets, showed that the number of young/litter and the mean birth weight of the newly born rats were less than in the stock animals on diet Pur, and all the litters died 3–5 days after birth. A subsequent attempt to mate the animals resulted in conception followed by death of the pregnant females. These observations were not dependent on the nature of the protein but on its level in the diet. With 20% protein diets the reproductive performance was comparable with that on Purina. After being weaned, groups of 8 rats (3 males and 5 females) were maintained on the same diets as their parents and the rest were returned to the stock. They were mated when mature, and a third generation was successfully raised also on the same experimental diets. However, the eight rats of the second generation on diet G succumbed at various intervals (possibly from respiratory infection) and none reached the age for mating. The *FCE* of the male rats in the three generations are given in Table V.

For diets at 10% protein level on five litters on each diet, GCS had a mean lactation value of 2·00 g/g protein consumed while casein had a value of 2·38 g. The difference between the two means is statistically significant at the 5% level.

TABLE II

Mean *PER* and *FCE* of 10% protein diets

	No. of rats	Cas	GCS	GC	G
Mean *PER*	6	2·61	2·34[a]	2·18[b]	1·90[c]
	3	2·53	2·32[a]	2·10[b]	1·80[c]
	8		2·36		
Mean *FCE* (7–14 weeks)	3	5·27	6·03[a]	6·28[i]	6·90[c]
Mean *FCE* (14–26 weeks)	3	10·96	10·90[i]	10·90[i]	10·60[i]

[a] Significantly different from Cas
[b] Significantly different from GCS
[c] Significantly different from GC
[i] Indistinguishable from other diets

TABLE III

Mean blood proteins concentration of 6 rats per group from weaning to adult values on various diets, compared with Purina chow (Pur) containing 23% protein

Age, weeks	10% protein diets				20% protein diets				Pur
	Cas	GCS	GC	G	Cas	GCS	GC	G	
Serum protein concentration (g/100 ml):									
3	5·0	5·1	5·0	5·0	5·2	5·2	5·0	5·1	5·2
7	6·2	5·9	5·7	5·6	6·5	6·4	6·4	6·4	6·5
10	6·6	6·5	6·7	6·2	7·2	7·1	7·2	7·2	7·2
14	7·4	7·2	7·2	6·7	7·4	7·4	7·5	7·4	7·7
18	7·5	7·3	7·3	7·1	7·6	7·5	7·5	7·5	7·7
Haemoglobin concentration (g/100 ml):									
3	9·3	9·4	9·5	9·4	9·5	9·5	9·6	9·6	9·5
7	11·5	11·0	10·8	11·2	11·6	11·8	11·9	11·8	11·8
10	13·7	13·2	13·2	13·4	14·8	14·7	14·8	14·6	14·8
14	14·8	14·9	14·8	14·9	16·2	16·0	15·9	15·8	16·3
18	14·9	14·9	15·0	14·8	15·9	16·0	16·0	16·0	16·3

TABLE IV

Mean results of nitrogen balance experiments for three days on groups of six rats aged eight weeks kept after weaning on various diets

Nitrogen retention/day	10% protein diets				20% protein diets				Pur
	Cas	GCS	GC	G	Cas	GCS	GC	G	
mg N/rat	168	143[a]	147[i]	135[c]	265	249	238	249	315
mg N/100 g body weight	113	104[a]	107[i]	104[c]	137	132	134	134	159
% of N intake	66	62[a]	60[b]	61[i]	45	42	41	41	46
% of N absorption	76	72[a]	72[i]	71[i]	56	54	53	53	57

[a] Significantly different from Cas
[b] Significantly different from GCS
[c] Significantly different from GC
[i] Indistinguishable from other diets containing groundnut

TABLE V

Mean *FCE* of various 20% protein diets on the males of three successive generations kept on the same experimental diets, compared with generations on Purina (Pur)

	Cas	GCS	GC	G	Pur
FCE between age of 7 and 14 weeks:					
First generation	2·9	3·2	3·2	3·4	4·1
Second generation	3·1	3·2	3·2	—	4·0
Third generation	3·1	3·2	3·3	—	4·0
FCE between age of 14 and 26 weeks:					
First generation	8·4	8·8	8·9	9·0	10·0
Second generation	8·2	8·7	8·9	—	10·3
Third generation	8·4	8·8	9·0	—	10·4

Discussion

Tests on the growing rat are acknowledged to possess considerable significance in human nutrition.[9]

By relating weight gain to food consumption, the *PER* and *FCE* methods were refinements of the simple growth procedure. Because varying the level of the protein in the diet gives different *PER* values,[10] it has become customary to determine *PER* at a level of 10% of dietary protein. Although it is possible to classify proteins by growth response only, the *PER* method is more critical because the proteins are fed near the minimum range (6–10% protein). Although 10% was not the level of protein usually found in a mixed diet, the differences in the quality of proteins were magnified.[10]

The *PER* of casein obtained in these experiments (2·59) was approximately the same as that quoted by Campbell[11] whose procedure had been followed. For groundnut the present results (1·87) agree with the accepted estimates by Buss & Goddard[12] and Sure.[13] The overall mean *PER* for GCS is 2·34* denoting that the protein mixture is of high biological value. On this basis addition of chickpea to groundnut is an improvement which is furthered by addition of sesame, although as also shown by the lactation value, GCS does not quite reach the level of casein.

According to the nitrogen balance technique, with 10% protein diets, the improvement of groundnut by introduction of the chickpea and sesame was not definite; casein resulted in significantly better retention. At 20% protein, the nitrogen retention was almost uniform from all the experimental diets and comparable with that from the 23% protein Purina chow.

The gradual increases in serum protein and haemoglobin concentrations with age of the rat to adult values[14] has been confirmed but almost no difference was detected between the diets tested. The reason may be that maintenance of blood proteins including haemoglobin seems to have a priority over tissue proteins.[15] However, Bressani & Behar[16] have shown that 'Incaparina' mixture when fed at 10% protein level produced in rats lower total serum protein than a milk diet with the same protein concentration, but the blood cell counts and haemoglobin were similar.

The lactation value combines effects on mother and litter and is sensitive to small qualitative differences; also the results are obtainable in only two weeks. Reproductive performance was shown to depend more on the level of protein in the diet than on the protein quality. The 100% mortality in the young parents on 10% protein diets may be attributed to failure of lactation and/or inability of the weak small-sized young rats to obtain sufficient nourishment. The critical level of casein for normal reproductive performance was found by Nelson & Evans[17] to be 5%, but the test diet was given only during the period of gestation. In the present study, the 10% diets have been administered from weaning, and the animals raised on them were thus subjected to a more prolonged deprivation more likely to exhaust their protein reserves.

The growth pattern, appearance, vigour and activity of the animals in the three successive generations on the 20% diets were normal in every respect. Such a long-term study was intended to search for any cumulative deleterious effects that could escape attention in a shorter experiment as demonstrated by Chaves[18] on the third generation of rats on *Phaseolus vulgaris*. The successful breeding of three generations of normal rats on diets GC and GCS have shown their freedom from any such effects.

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References

1. F.A.O./W.H.O. Joint Expert Committee on Nutrition, *F.A.O. Nutr. Mtg Rep. Ser. No. 19*, 1958, p. 22 (Rome: U.N. Food and Agriculture Organisation)
2. Hanafy, M. M., Aref, M. K., Seddik, Y., Zein, M. S., & El-Kashlan, K. M., *J. trop. Med. Hyg.*, 1967, **70,** 238
3. Hanafy, M. M., Ibrahim, A. H., El-Khateeb, S., & Seddik, Y., *Alex. med. J.*, 1965, **19,** 241
4. Hanafy, M. M., Aref, M. K., & Seddik, Y., *J. Sci. Fd Agric.*, 1970, **21,** 9
5. Hanafy, M. M., Seddik, Y., & Aref, M. K., *J. trop. Med. Hyg.*, 1968, **71,** 167
6. Campbell, J. A., Nutr. Document R. 10/Add 27, 1961 (New York: W.H.O./F.A.O./U.N.I.C.E.F./P.A.G.)
7. Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., *et al., J. biol. Chem.*, 1950, **183,** 305
8. Asenjo, C. F., & Goyco, J. A., *Proc. VIth Int. Congr. Nutr.*, 1964, p. 488 (Edinburgh: Livingstone)
9. Block, R. J., & Mitchell, H. H., *Nutr. Abstr. Rev.*, 1946, **19,** 249
10. Frost, D. V., in 'Protein and amino acid nutrition', (Ed. Albanese, A. A.) 1959, p. 225 (New York: Academic Press)
11. Campbell, J. A., in 'Evaluation of protein quality', Publication 1100, 1963, p. 31 (Washington, D.C.: Natn. Acad. Sci. Natn. Res. Coun.)
12. Buss, L. W., & Goddard, V. R., *Fd Res.*, 1948, **13,** 506
13. Sure, B., *J. agric. Fd Chem.*, 1955, **3,** 789
14. Albanese, A. A., Holt, L. E., jun., Kajdi, C., & Frankston, J. E., *J. biol. Chem.*, 1943, **148,** 299
15. Robscheit-Robbins, F. S., *Fedn Proc. Fedn Am. Soc. exp. Biol.*, 1942, **1,** 219
16. Bressani, R., & Behar, M., *Proc. VIth Int. Congr. Nutr.*, 1964, p. 181 (Edinburgh: Livingstone)
17. Nelson, M. M., & Evans, H. M., *J. Nutr.*, 1953, **51,** 71
18. Chaves, N., in 'Meeting protein needs of infants and children', Publication 843, 1961, p. 13 (Washington, D.C.: Natn. Acad. Sci. Natn. Res. Coun.)

AMINO ACID SUPPLEMENTATION OF A PROTEIN-RICH MIXTURE OF CRUSHED GROUNDNUT, CHICKPEA AND SESAME

By M. M. HANAFY, Y. SEDDIK and M. K. AREF

Supplementation of a 3 : 2 : 1 crushed groundnut–chickpea–sesame mixture (in a 10% protein diet) with methionine to raise the level of sulphur-containing amino acids from 187 to 228 mg/g N resulted in improved growth and lactation in the rat, but further supplementation with both methionine and tryptophan to the levels proposed by the FAO committee (1957) did not increase this effect.

Introduction

The GCS mixture (3 groundnut : 2 chickpea : 1 sesame) formulated by Hanafy *et al.*[1–3] has been devised to have a content of essential amino acids similar to that of the reference protein proposed by the F.A.O. Committee on Protein Requirements (1957).[4] However it is evident that, compared with the F.A.O. pattern, GCS contained smaller amounts of methionine and tryptophan, although it has been suggested that the level of these two amino acids in the F.A.O. pattern was too high.[5] A trial was therefore performed on rats to test the effect of supplementation of GCS with methionine and tryptophan.

Experimental

The GCS diet was prepared by mixing, to obtain 1 kg of diet the following ingredients: 203 g crushed groundnut, 136 g chickpea, 68 g sesame dreg, 53 g oil, 430 g rice starch, 50 g cellulose, 40 g salt mixture, and 20 g vitamin mixture.

The resulting mixture contains 10% protein and supplies 427 cal/100 g. Its content of essential amino acids (in mg/g N) may be compared with the F.A.O. (1957) pattern:

	Iso	Leu	Lys	Met	Total S acids	Total 'aromatic'	Thr	Try	Val
GCS	289	420	260	86	187	561	183	69	289
F.A.O.	270	306	270	144	270	360	180	90	270

This shows that GCS needs 83 mg of sulphur-containing amino acids and 21 mg of tryptophan/g of nitrogen, to equal the F.A.O. pattern.

By adding 740 mg DL-methionine to 1 kg of GCS diet, the content of sulphur acids was raised to 228 mg/g N, to form diet (+ M). The third diet (+ MT) was formulated to cover completely the difference between GCS and F.A.O. pattern in both sulphur acids and tryptophan, by adding 1500 mg DL-methionine and 375 mg DL-tryptophan to 1 kg of GCS. The amino acids added were the less expensive DL-derivatives as it has been shown that in the rat the D-form of these acids (methionine or tryptophan) can be utilised for growth.[6]

24 male weanling rats were distributed into three similar groups by allocating one animal from every three littermates to one of the three diets: GCS, diet (+ M) and diet (+ MT). The food consumption and body weights were recorded weekly. The protein efficiency ratio (*PER*) was determined in the 4 weeks following weaning, and the feed conversion efficiency (*FCE*) between 7 and 14 weeks and between 14 and 26 weeks of age.

Serum proteins[7] and haemoglobin estimations (by a modification of Sahli's method) were made at the start of the experiment (3 weeks), then at ages 7, 10, 14 and 18 weeks. After 5 weeks on the experimental diets, when the animals were two months old, N balance determinations were carried out for three days.

To determine the lactation value[8] 15 adult females were selected from the stock, aged about 14 weeks with mean body weight 216 g, and mated for the first time with adult males of about the same age. Immediately after parturition the litter size was reduced to six. Mothers and litters were weighed within 24 h after parturition, distributed into three groups placed on diets GCS, (+ M) and an isocaloric 10% casein diet. They were weighed again after 14 days i.e. before the young started to share any of the mother's ration. Student's '*t*' test was used for statistical comparisons.

Results

Throughout the whole feeding period all the animals in the first experiment were in good physical condition, had good appetite and gained weight steadily. The gain in weight was larger on diets (+ M) and (+ MT) and paralleled the food consumption. It was also evident from the growth curves (Fig. 1) that the two supplemented diets were equal.

The mean *PER* and *FCE* values are shown in Table I. The results by the two methods confirmed that diet (+ M)

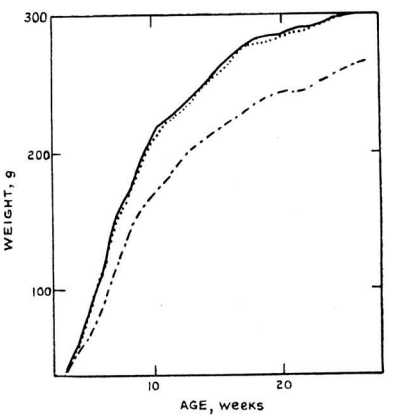

FIG. 1. *Growth of rats with 10% protein diets*
---- GCS; . . . . (+ M); ——— (+ MT)

TABLE I

Mean protein efficiency ratio (*PER*) and feed conversion efficiency (*FCE*) in three groups of eight male rats given diets GCS, (+ M) or (+ MT)

	Age, weeks	Mean ± standard deviation		
		GCS	(+ M)	(+ MT)
PER	3–7	2·36 ± 0·15	2·59[a] ± 0·14	2·61[b] ± 0·14
FCE	7–14	7·23 ± 0·51	5·00[a] ± 0·62	4·82[b] ± 0·58
FCE	14–26	11·50 ± 0·86	13·30[a] ± 1·60	13·60[b] ± 1·80

[a] Significantly different from GCS
[b] Significantly different from GCS but not from (+ M)

favoured growth more than unsupplemented GCS but that addition of more methionine plus tryptophan did not stimulate growth any further. In the period 14–26 weeks, the *FCE* of all three diets was low; GCS was better than either (+M) or (+ MT). This is probably because the rapid phase of growth had been reached earlier on the supplemented rations and after that stage the diets were used more for maintenance than for increase in weight.

The mean results of the serum proteins and haemoglobin estimations (Table II) show the expected gradual rise to adult values irrespective of the type of diet consumed, i.e. the three diets promoted to an equal degree the synthesis of blood proteins. Table III shows the mean nitrogen retention per day in each group: the differences between the retentions observed on the three diets are not statistically different at the 5% level.

For the lactation value, the mean results for five litters on each diet were found to be (per g of food consumed): 2 g on GCS, 2·2 g on diet (+ M) and 2·38 g on an isocaloric 10% casein diet. The differences between the diets are significant i.e. addition of methionine to GCS improves it though not to an equal level with casein.

TABLE II

Blood proteins of groups of 8 rats at various ages kept after weaning on diets GCS, (+ M) or (+ MT)

Diet	Age, weeks				
	3	7	10	14	18
Mean serum protein concentration (g/100 ml)					
GCS	5·1	5·5	6·8	7·1	7·1
(+ M)	5·0	5·4	6·7	7·1	7·1
(+ MT)	5·1	5·5	6·6	7·0	7·2
Mean haemoglobin concentration (g/100 ml)					
GCS	9·5	10·8	13·4	15·0	15·1
(+ M)	9·5	10·9	13·1	14·9	15·0
(+ MT)	9·8	11·1	13·6	15·0	15·0

TABLE III

Mean daily N retention after three days N balance in groups of eight rats (at age of two months) kept after weaning on diets GCS, (+ M) or (+ MT)

	mg/rat/day	mg/100 g body weight	Nitrogen retention, %	
			of N intake	of N absorption
GCS	156	104	68·7	76·9
(+ M)	175	100	70·9	80·4
(+ MT)	177	100	70·2	80·3

Discussion

In the report on protein requirements by the Joint F.A.O./W.H.O. Expert Group (1965)[9] it was stated that 60–70 mg tryptophan/g N seems preferable to the level of 90 mg given in the F.A.O. provisional pattern provided that the diet is otherwise adequate in niacin. It also added that there is enough evidence to conclude that the proportion of sulphur-containing amino acids (methionine + cystine) in the provisional pattern is also too high and that instead of 270 mg a level of methionine + cystine in the range of 190–220 mg/g N would probably be an improvement.

It has been experimentally demonstrated in the present work that raising the sulphur amino acids content in a diet from 187 to 228 mg/g N, results in better growth and higher lactation value in the rat. This has been achieved by the addition of 740 mg methionine/kg of diet (≡ 410 g of mixture of three parts groundnut, two parts chickpea and one part of sesame) or the addition of 1·8 g methionine/kg GCS mixture.

It has also been demonstrated that the addition to GCS of still larger supplements of methionine together with tryptophan (original GCS content = 69 mg/g N) to bring the level of these amino acids to that in the F.A.O. (1957) reference protein pattern,[4] fails to improve the protein quality further.

Thus, the fact that the F.A.O. pattern is overgenerous in these two amino acids has been confirmed. This point has also been raised by Bressani & Scrimshaw.[10] In 1960, Howe *et al.*[11] found that decreasing the tryptophan content in the F.A.O. pattern by 50% did not impair its nutritive value in weanling rats and Swendseid *et al.*[12] also reported that lowering the tryptophan content of the F.A.O. mixture by 20% did not decrease the efficiency of the mixture measured by nitrogen balance in young men.

It should be noted that the *PER* of the (+ M) diet reached the accepted value for casein. Moreover, while the serum proteins, haemoglobin concentrations and nitrogen retention failed to provide evidence for the improvement in protein quality, the growth methods (*PER* and *FCE*) were more useful. The most sensitive biological method seemed to be the lactation value which showed that even the improved (+ M) diet was still less efficient than casein for lactation in the rat.

However the lactating rat may require more sulphur acids than a growing rat[13] and Hartsook & Mitchell[14] noted that the rat's requirements of methionine + cystine changed with age. It should be remembered also that the requirements are proportionately larger in the rat than in humans.[15] More-

over, with wheat—the staple food in Egyptian diets—the addition of methionine has an adverse effect.[16]

It may be concluded that the content of sulphur acids in GCS is almost equal to the lower limit of the range proposed by F.A.O./W.H.O. (1965),[9] and that although the supplementation of GCS with methionine to raise the S acids in the diet from 187 to 228 mg/g N, has resulted in an improved growth and lactation in the rat, this amino acid enrichment may not be necessary for humans.

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References

1. Hanafy, M. M., Aref, M. K., Seddik, Y., Zein, M. S., & El-Kashlan, K. M., *J. trop. Med. Hyg.*, 1967, **70,** 238
2. Hanafy, M. M., Seddik, Y., & Aref, M. K., *J. trop. Med. Hyg.*, 1968, **71,** 167
3. Hanafy, M. M., Aref, M. K., & Seddik, Y., *J. Sci. Fd Agric.*, 1970, **21,** 9
4. 'Protein Requirements', F.A.O. Nutritional Studies No. 16, 1957 (Rome: U.N. Food and Agriculture Organisation)
5. 'Evaluation of protein quality'. Publication 1100, 1963, p. 17 (Washington: Natn. Acad. Sci Natn. Res. Coun.)
6. Berg, C. P., in 'Protein and amino acid nutrition', (Albanese, A. A. Ed.) 1959, p. 57 (New York: Academic Press)
7. Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., *et al.*, *J. biol. Chem.*, 1950, **183,** 305
8. Asenjo, C. F., & Goyco, J. A., *Proc. VIth Int. Congr. Nutr.*, 1964, p. 488 (Edinburgh: Livingstone)
9. F.A.O./W.H.O. Joint Expert Group Report *F.A.O. Nutr. Mtg Rep. Ser. No. 37*, 1965, p. 37
10. Bressani, R., & Scrimshaw, N. S., in 'Meeting protein needs of infants and children'. Publication 843, 1961, p. 35 (Washington: Natn. Acad. Sci. Natn. Res. Coun.)
11. Howe, E. E., Gilfillan, E. W., & Allison, J. B., *J. Nutr.*, 1960, **60,** 549
12. Swendseid, M. E., Watts, J. H., Harris, C. L., & Tuttle, S. G., *J. Nutr.*, 1961, **75,** 295
13 Nelson, M. M., & Evans, H. M., in 'Milk: the mammary gland and its secretion', 1961, Vol. II, p. 137 (Kon, S. K., & Cowie, A. T., eds) (New York: Academic Press)
14. Hartsook, E. W., & Mitchell, H. H., *J. Nutr.*, 1956, **60,** 173
15. Mitchell, H. H., in 'Protein and amino acid nutrition', 1959, p. 20 (Albanese, A. A., ed.) (New York: Academic Press)
16. Hegsted, D. M., in 'Mammalian protein metabolism', 1964, Vol. II, p. 165 (Munro, H. N., & Allison, J. B., eds) (New York: Academic Press)

EVALUATION OF WHISKY DISTILLERY BY-PRODUCTS III.*—Effect of calcium supplements on the digestibility and intake of ruminant diets containing malt distiller's grains

By T. B. MILLER, G. A. EL HAG and G. PRATT

Digestibility and intake data have been obtained from sheep fed on rations consisting of fresh and ensiled malt distiller's grains (*MDG*) fed alone and with hay and silage. The effects of calcium supplements have been measured with all rations, and calcium lactate has been compared with calcium carbonate. The results of digestibility trials with cattle have been included; in these the effect of additional calcium has been estimated with rations of barley straw + *MDG*.

When *MDG* was fed alone the intakes of fresh and ensiled forms were similar and there was a significant decrease in intake with duration of feeding. Fresh *MDG* had a significantly higher digestibility, and additional calcium increased the digestibilities of fresh and ensiled samples.

The intakes of rations containing hay + *MDG* and silage + *MDG* were positively correlated with digestibilities of the whole ration. Additional calcium resulted in higher intakes of hay and *MDG* but silage intakes were unaffected. The effect of added calcium was greater with hay + fresh *MDG* than with other combinations of constituents. Calcium carbonate and calcium lactate were equally effective in increasing intake.

The addition of NaCl during the ensilage of *MDG* reduced the retention of magnesium by sheep given grains with hay and silage.

Addition of calcium to cattle rations of barley straw and *MDG* produced an increase in the intake but digestibility of the diet was not affected. The rations with calcium provided 0·75 × maintenance requirements of the cattle for metabolisable energy and about twice the maintenance requirements for digestible crude protein.

Introduction

Malt distiller's grains (*MDG*) have been evaluated by chemical analyses and by the *in vitro* digestibility technique.[1,2] The results showed that *MDG* fresh from the distillery contains relatively high levels of structural carbohydrates, lignin, lipids and protein with correspondingly low levels of soluble carbohydrate, potassium and sodium. Before being fed to farm animals the wet material may undergo varying degrees of fermentation depending on the duration of ensilage on the farm and whether salt is added.

The relatively high lipid content is an important factor in determining the digestibility of *MDG*. Extraction of the lipids results in a marked rise in the *in vitro* organic matter digestibility. A similar increase in digestibility was obtained by increasing the level of calcium salts in the digesta. These results showed that the native lipids in *MDG* behaved in accordance with the findings of other workers[3,4] who added fat to the rations of ruminants and found a depression in digestibility which was reversed by the addition of calcium.

Regular supplies of *MDG* have been available in the north-east counties of Scotland for many years and the material is used as a major constituent in the winter rations of dairy cows. The relatively high protein content of *MDG* is important in overcoming deficiencies of protein in hay, silage, roots and high energy concentrates. The relatively low cost of *MDG* has also encouraged its use in inexpensive winter rations for beef cows and, more recently, the material has been used as a diluent in 'barley beef' rations.

The evaluation of *MDG* in such diverse feeding systems and rations is a most time-consuming task. As a preliminary step experiments have been conducted to determine the digestibility and intake of *MDG* alone and *MDG*/roughage rations in which the rations are offered *ad libitum*. In view of the results of laboratory studies, which have shown the significance of calcium/lipid interactions with digestibility, the effect of calcium supplementation of rations has received particular attention.

Earlier work (Walker, H. F., personal communication) on estimating the feeding value of *MDG* using digestibility trials with sheep indicated the difficulties in feeding the material. When the grains alone were given, intakes dropped to very low levels. Satisfactory intakes were obtained only when *MDG* was given as a supplement to a basal roughage ration and digestibilities were estimated by difference. In using the latter method it is assumed that the digestibilities of roughage and supplement are additive and interactions between the components of the ration are ignored. Such assumptions are not tenable in view of the established interaction between contents of calcium and lipid, and cellulose degradation by rumen micro-organisms.

In the present work the results of three experiments with sheep and one experiment with cattle are presented. In the sheep experiments *MDG* has been studied as the sole energy-yielding constituent of the diet and as a supplement to rations containing hay and silage. The value of *MDG* as a supplement to poor quality roughage has been estimated with cattle. The effect of adding calcium has been studied with all rations, and calcium carbonate has been compared with calcium lactate as supplements to hay/*MDG* rations with sheep.

Experimental

Evaluation of fresh, salted and unsalted *MDG* with sheep (Experiment 1)

Six Suffolk × Blackface wether lambs were used in a 3 × 3 latin square experiment to estimate the digestibility and intake of fresh, salted, and unsalted *MDG* after short-term ensilage. The fresh *MDG* was stored at −20° within 12 hours of leaving the distillery and the salted and unsalted materials were

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ensiled for 28 days prior to storage at −20° to avoid secondary fermentation before feeding. The sheep were fed and housed in crates designed by Duthie.[5] Hay was fed during the first week after the sheep were crated and *MDG* was introduced in increasing quantities over the second week when hay was removed from the ration. Potassium and sodium deficiencies in the appropriate ensiled *MDG* samples were overcome by feeding 2·5 g KCl and 9·5 g NaCl per head daily. A vitamin concentrate was fed to provide vitamin A and vitamin D slightly in excess of Agricultural Research Council (A.R.C.) requirements.[6] During each of the three experimental periods test rations were offered at 10% above voluntary intake and were fed for a preliminary sub-period of 7 days after which rations, residues, faeces and urine were weighed and sampled over the following 7 days.

The rations fed in period 3 were continued for a fourth period during which 8 g $CaCO_3$ was included in the ration but this treatment was restricted to only one period because two sheep showed deleterious effects of prolonged *MDG* feeding. One of the animals died subsequently of haemolytic jaundice due to copper poisoning.

Evaluation of fresh, salted, and unsalted *MDG* in hay and silage-based diets of sheep and the effect of added calcium (Experiment 2)

Eight Suffolk × Blackface wether lambs were used in a 3 × 2 × 2 factorial experiment to measure the intake and digestibility of *MDG* and roughage when *MDG* was fed *ad libitum* with hay and silage. The experiment was conducted over 24 weeks which was divided into 3 periods of 8 weeks. At the beginning of each period animals were allocated at random to a ration based either on hay or silage. Unsalted, salted, and fresh *MDG* were fed to all eight animals during the first, second, and third periods respectively. The salted and unsalted *MDG* had been ensiled for 6–8 months. Each period was subdivided into four sub-periods of: (a) 4 weeks acclimatisation feeding, (b) 1 week collection, (c) 2 weeks acclimatisation and (d) 1 week collection. Two animals on both hay- and silage-based rations were fed 33 g calcium lactate together with the *MDG* during sub-periods (a) and (b) only, and the other two animals on the experiment received this treatment during sub-periods (c) and (d) only. The daily rations of roughages and *MDG* were offered simultaneously at levels of about 10% above voluntary intakes. Rations, residues, faeces and urine were measured and sampled over the 7 day collection sub-periods.

Comparison of calcium lactate and calcium carbonate as additives (Experiment 3)

A 3 × 3 latin square experiment was conducted with six Suffolk × Blackface wether lambs. Each lamb was fed salted *MDG* and hay *ad libitum*. The three treatments included: (a) control – no supplementary calcium, (b) 30 g calcium lactate per day, and (c) 9·6 g $CaCO_3$; the salts were analysed and each quantity provided 4 g Ca. The experiment was conducted over 6 weeks consisting of three periods of 2 weeks.

Effect of calcium supplements on the intake and digestibility of *MDG*/barley straw ration with cattle (Experiment 4)

Eight 18-month old Ayrshire steers were used in this experiment. All animals were fed barley straw *ad libitum*. Supplies of *MDG* were delivered to the farm at 2 week intervals and half of each consignment was ensiled without additives whilst the remainder was salted (20 lb NaCl/ton wet *MDG*). Four steers were fed the salted short-term ensiled *MDG* and the other four received the unsalted material at 12 kg/day.

The experiment was conducted over a period of 6 weeks. During the first 3 weeks 2 animals on salted and 2 on unsalted *MDG* were fed 40 g $CaCO_3$/day with the *MDG* and the other 4 animals were not given calcium supplements. These treatments were reversed during the subsequent 3 weeks. Each 3 week sub-period consisted of 2 weeks preliminary feeding and 1 week collection during which the animals were harnessed and rations, residues and faeces were weighed and sampled.

Feed and excreta samples were analysed chemically according to the methods described by Miller.[1] Calorific values were determined using the Gallenkamp Autobomb. The intakes of roughage and *MDG* were measured by estimating the proportion of roughage and *MDG* in the mixed residues. The latter were estimated by interpolation of the potassium content which was low in *MDG* and relatively high in roughages. Metabolisable energy (*ME*) was calculated from the gross digestible energy (*GDE*) on the assumption that energy lost as methane and in the urine amounted to 18% of *GDE*.

Results

Experiment 1

When *MDG* was fed alone (except for the addition of Na and K supplements) the mean voluntary intakes of the sheep were relatively low. Moreover, the dry matter (*DM*) intakes decreased from 54·2 g/kg$^{0.73}$ live weight in period 1 to 36·2 g/kg$^{0.73}$ live weight in period 3 (Table I).

There was no significant difference between fresh, salted, and unsalted forms of *MDG* with respect to *DM* intake calculated *ME* intake and digestible crude protein (*DCP*) intake. However, the intakes in period 1 were significantly higher than those of periods 2 and 3 with respect to *DM* ($P < 0.01$) and calculated *ME* ($P < 0.001$).

The digestibility coefficients in Table II show significantly higher values for fresh *MDG* in all constituents except ether extract. The digestibility of ether extract was similar for each material and appreciably higher than the digestibility of other constituents.

Digestibilities of salted and unsalted *MDG* samples did not differ significantly. The added calcium showed marked increases in the digestibility of all constituents although statistical analysis of the data was not possible. The increased

TABLE I

Intakes of dry matter (*DM*), metabolisable energy (*ME*) and digestible crude protein (*DCP*) by sheep

	DM, g/kg$^{0.73}$	*ME*, kcal/kg$^{0.73}$	*DCP*, g/kg$^{0.73}$
Fresh *MDG*	41·7	95·0	6·2
Salted *MDG*	47·5	101·3	6·8
Unsalted *MDG*	39·1	85·6	5·7
Period 1	54·2**	121·0***	7·6
Period 2	38·0	81·7	6·0
Period 3	36·2	79·3	5·1
S.E. of mean	4·55	5·21	0·53

** $P < 0.01$
*** $P < 0.001$

TABLE II

Gross composition and digestibility coefficients of six constituents and of the gross energy of fresh, salted and unsalted *MFG*

	Fresh *MDG*	Salted *MDG*	Unsalted *MDG*	S.E. of mean
Ca intake (g/day):				
without supplement	0·61	0·70	0·49	
+ $CaCO_3$	4·72	5·14	4·28	
Organic matter:				
% *DM*	96·9	96·1	96·9	
Digestibility %	59·3**	57·0	55·6	0·48
Digestibility % + $CaCO_3$	65·0	60·5	59·0	
Gross energy:				
kcal/g *DM*	4·99	4·83	4·94	
Digestibility %	63·3***	58·6	59·6	0·55
Digestibility % + $CaCO_3$	66·5	65·2	62·4	
Crude protein:				
% *DM*	18·9	18·1	18·9	
Digestibility %	78·0*	75·8	75·8	0·84
Digestibility % + $CaCO_3$	83·4	79·2	81·4	
Crude fibre:				
% *DM*	21·9	22·0	22·6	
Digestibility %	45·4*	42·5	41·0	0·90
Digestibility % + $CaCO_3$	52·0	48·0	43·7	
Cellulose:				
% *DM*	24·0	23·3	23·6	
Digestibility %	51·4**	46·6	44·5	0·82
Digestibility % + $CaCO_3$	58·0	51·1	50·0	
Ether extract:				
% *DM*	8·1	8·5	8·3	
Digestibility %	88·1	88·0	88·8	0·55
Digestibility % + $CaCO_3$	93·1	91·3	90·3	
N-free extractive:				
% *DM*	48·0	47·5	47·1	
Digestibility %	54·9**	50·2	49·0	0·46
Digestibility % + $CaCO_3$	59·1	53·8	51·5	

* $P < 0·05$
** $P < 0·01$
*** $P < 0·001$

digestibility with added calcium was greater for the structural components – crude fibre and cellulose. The digestibilities of ether extract were over 90% with added calcium. The latter result could be a reflection of the level of insoluble calcium soaps in faeces which were not quantitatively extracted with ether, though the corresponding increase in the digestibility of nitrogen-free extract (*NFE*) indicated that this explanation is unlikely since the organic component of calcium soaps would elevate the faecal *NFE*. Moreover, calculation of the digestible energy content of *MDG* from the digestible nutrients yielded values which were not significantly different from the directly determined values using the bomb calorimeter.

The energy contents of the three forms of *MDG* are expressed in terms of starch equivalent (*SE*), total digestible nutrients (*TDN*) and calculated *ME* in Table III together with the *DCP*.

The fresh *MDG* showed values which were 5–7% higher than the ensiled material and the differences were highly significant. There were no significant differences between the salted and unsalted samples.

The additional calcium produced a mean increase of 4·5 units or 10·7% in *SE* and *TDN* values in all samples. The corresponding mean increase in calculated *ME* amounted to 293 kcal/kg or 13·3%. The *DCP* contents did not differ significantly between samples and the effect of added calcium on *DCP* was equivocal.

Experiment 2

During the course of the experiment the sheep adopted a significantly consistent pattern of selective feeding. The animals given the calcium supplement in the hay-based ration showed a distinct preference for *MDG* and any residues consisted of hay only whilst their counterparts on the unsupplemented ration left a residue of *MDG* with some hay. After changeover from supplemented to unsupplemented rations or *vice versa* the sheep conformed to this characteristic feeding pattern within 2 days. The behaviour of the sheep on the silage-based rations was similar though less pronounced.

TABLE III

Nutritive value of the dry matter in three forms of *MDG* when given to sheep with and without added calcium

	Treatment	Fresh	Salted	Unsalted	S.E. of control mean
SE, g/100 g *DM*	control	55·6**	53·2	53·0	0·31
	+ $CaCO_3$	60·8	57·7	55·5	
TDN, g/100 g *DM*	control	66·5***	63·4	63·3	0·34
	+ $CaCO_3$	73·2	68·1	66·4	
ME, kcal/100 g *DM*	control	2,276**	2,121	2,181	18·7
	+ $CaCO_3$	2,555	2,515	2,388	
DCP, g/100 g *DM*	control	14·8	14·3	14·6	0·18
	+ $CaCO_3$	15·4	14·3	15·8	

** $P < 0·01$
*** $P < 0·001$

The selective feeding is manifested in the intake data in Table IV by the increased intakes of *MDG* with added calcium, and in the results of analysis of variance according to the procedure of Cochran & Cox[7] given in Table V. The addition of calcium resulted in highly significant increases in the intake of total *DM*, *MDG*, calculated *ME* and *DCP* ($P < 0{\cdot}001$). Total intakes of *DM* and calculated *ME* were also increased to a significantly greater extent with the extra calcium in the hay-based rations than in the silage-based ration. The digestibility coefficients of organic matter (*OM*), gross energy (*GE*) and all proximate constituents, with the exception of crude protein (*CP*) were significantly increased by added calcium (Tables VI and VII).

Intake and digestibility data were also affected by type of roughage and whether the *MDG* was fed fresh or ensiled. Silage-based rations showed higher digestibility coefficients than the corresponding hay rations with respect to *OM*, *GE* and *CF* (crude fibre). The latter results are in accordance with the higher intakes of *ME* when silage-based rations were fed. The protein digestibilities, on the other hand, were greater with the hay-based rations and the significantly greater intakes of *DCP* when silage was fed can be attributed to the relatively higher *CP* content in the silage (Appendix, Table I).

Comparison of fresh and ensiled forms of *MDG* showed that the animals consumed significantly greater quantities of hay, silage, *MDG*, calculated *ME* and *DCP* when the fresh material was fed. Moreover, with fresh *MDG* the extra intake of hay was greater than that of silage. The added calcium also produced a greater increase in intakes when fresh *MDG* was included in the ration. The interaction between roughage, *MDG* and calcium with respect to *ME* intake indicates that the increase of 63·3 kcal calculated *ME*/kg$^{0{\cdot}73}$ when calcium was added to the hay-fresh *MDG*

TABLE IV

Daily intakes of calcium, total dry matter (*DM*), *MDG* dry matter, roughage dry matter, metabolisable energy (*ME*) and digestible crude protein (*DCP*) by sheep given rations of either *MDG* + hay or *MDG* + silage *ad libitum* with and without calcium lactate

	Wt. of sheep, kg	Intake					
		Ca, g/head	Total *DM*, g/kg$^{0{\cdot}73}$	*MDG* dry matter g/kg$^{0{\cdot}73}$	Roughage *DM*, g/kg$^{0{\cdot}73}$	*ME*, kcal/kg$^{0{\cdot}73}$	*DCP*, g/kg$^{0{\cdot}73}$
Hay + fresh *MDG*	49·0	3·43	64·5	32·4	32·1	143·3	8·0
Hay + fresh *MDG* + Ca lactate		9·85	87·5	52·6	34·9	206·6	11·7
Hay + salted *MDG*	50·0	1·67	57·2	42·4	14·8	95·7	6·6
Hay + salted *MDG* + Ca lactate		6·01	65·6	47·4	18·2	121·5	7·4
Hay + unsalted *MDG*	41·6	2·47	47·1	30·6	16·5	88·5	5·0
Hay + unsalted *MDG* + Ca lactate		6·18	59·4	42·2	17·2	133·6	6·5
Silage + fresh *MDG*	50·4	3·24	71·4	48·2	23·2	162·1	9·6
Silage + fresh *MDG* + Ca lactate		9·56	85·7	57·7	28·0	198·8	11·4
Silage + salted *MDG*	47·2	1·95	60·0	38·5	21·5	116·4	7·4
Silage + salted *MDG* + Ca lactate		5·77	64·2	43·7	20·5	139·5	8·4
Silage + unsalted *MDG*	41·6	2·55	47·9	29·6	18·3	108·8	5·2
Silage + unsalted *MDG* + Ca lactate		5·63	50·7	40·4	10·3	112·2	6·4
S.E. of mean			2·90	2·64	2·76	6·07	0·33

TABLE V

Results of analysis of variance of intake data

	Intake				
	Total *DM*	Roughage *DM*	*MDG* dry matter	*ME*	Digestible protein
Replicates (animals)	n.s.	n.s.	n.s.	n.s.	n.s.
Roughage	n.s.	n.s.	n.s.	*	**
MDG	***	***	***	***	***
Calcium	***	n.s.	***	***	***
Roughage × *MDG*	n.s.	*	**	n.s.	n.s.
Roughage × Ca	*	n.s.	n.s.	**	n.s.
MDG × Ca	**	*	*	**	**
Roughage × *MDG* × Ca	n.s.	n.s.	n.s.	*	n.s.

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

TABLE VI

Digestibility coefficients of five constituents and of the gross energy of rations of *MDG* and hay or silage when fed *ad libitum* to sheep with and without calcium lactate

Ration	Digestibility coefficients					
	Organic matter	Gross energy	Crude protein	Crude fibre	Ether extract	N-free extractive
Hay + fresh *MDG*	59·4	60·2	74·3	57·3	83·2	52·3
Hay + fresh *MDG* + Ca lactate	62·0	63·1	77·6	60·4	86·4	54·2
Hay + salted *MDG*	46·1	47·8	66·8	36·4	87·6	37·6
Hay + salted *MDG* + Ca lactate	51·8	51·7	67·2	45·6	88·4	43·6
Hay + unsalted *MDG*	44·2	51·5	68·6	37·0	87·6	44·9
Hay + unsalted *MDG* + Ca lactate	56·2	60·4	67·8	48·6	90·3	57·0
Silage + fresh *MDG*	58·6	59·8	69·8	56·5	85·6	50·4
Silage + fresh *MDG* + Ca lactate	60·8	61·2	69·6	61·6	86·4	52·0
Silage + salted *MDG*	52·7	54·6	67·2	45·8	85·9	47·2
Silage + salted *MDG* + Ca lactate	59·4	59·3	69·4	54·6	88·4	52·6
Silage + unsalted *MDG*	52·7	56·4	60·0	44·9	88·7	51·2
Silage + unsalted *MDG* + Ca lactate	53·4	58·6	63·5	51·5	89·4	50·2
S.E. of mean	2·42	1·84	1·67	2·80	1·15	2·06

TABLE VII

Results of analysis of variance of digestibility data

	Digestibility					
	Organic matter	Gross energy	Crude protein	Crude fibre	Ether extract	N-free extractive
Replicates (animals)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Roughage	*	*	***	**	n.s.	**
MDG	**	***	***	***	**	n.s.
Calcium	**	**	n.s.	***	**	**
Roughage × *MDG*	n.s.	**	**	n.s.	n.s.	**
Roughage × Ca	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
MDG × Ca	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Roughage × *MDG* × Ca	n.s.	n.s.	n.s.	n.s.	n.s.	**

n.s. = not significant
* $P < 0·05$
** $P < 0·01$
*** $P < 0·001$

ration is significantly greater than the effects of calcium on all other mixtures. There were no significant differences in the intake and digestibility data between the salted and unsalted forms of ensiled *MDG*.

The nutrient balance data in Table VIII showed significant effects with only nitrogen and magnesium. The nitrogen retention was higher with the hay-based ration ($P < 0·01$) and feeding fresh *MDG* and additional calcium had highly significant effects ($P < 0·001$). The negative balances of magnesium when salted *MDG* was fed were most pronounced and highly significant. The results for sodium balance indicate that the feeding of salted *MDG* also tends to produce negative balances.

Experiment 3

In this experiment the two sheep on the control ration (without added calcium) during the first period showed greater intakes and digestibility than those of the animals in the two treatment groups. This anomalous result was not repeated during the subsequent two periods when the added calcium showed the normal pattern of increased intakes and digestibility.

In the statistical analysis of the results in Table IX the spurious values for the control animals in the first period have been treated as missing values using the technique of Yates.[8] Intakes of *DM* were significantly higher with calcium lactate ($P < 0·001$) and calcium carbonate ($P < 0·01$) than with the control ration. The intakes of rations with lactate did not differ significantly from those with carbonate. The calcium supplements both produced significantly greater organic matter digestibility (*OMD*) values though the values for lactate and carbonate were very similar.

Experiment 4

The results of feeding barley straw + *MDG* to cattle are given in Table X. The intakes of both straw and *MDG* were

TABLE VIII

N, P, Ca, Mg, Na and K retention (g/24 h) of sheep fed on *MDG* and either hay or silage with and without calcium lactate
Mean values for 4 sheep

	N	P	Ca	Mg	Na	K
Hay + fresh *MDG*	6·37***	0·919	0·273	0·444	0·291	2·153
Hay + fresh *MDG* + Ca lactate	10·05***	1·484	1·822	0·434	0·391	2·552
Hay + salted *MDG*	1·50	0·196	0·744	−0·280**	0·008	2·557
Hay + salted *MDG* + Ca lactate	2·93	0·758	1·785	−0·673**	−0·518	0·963
Hay + unsalted *MDG*	2·66	0·074	0·095	0·270	0·192	−0·190
Hay + unsalted *MDG* + Ca lactate	6·14	0·993	0·943	0·213	0·358	1·471
Silage + fresh *MDG*	3·32***	1·055	−0·321	0·264	−0·009	−6·782
Silage + fresh *MDG* + Ca lactate	7·07***	0·642	0·256	0·129	0·274	−0·499
Silage + salted *MDG*	1·12	0·437	0·524	−0·102**	−0·421	1·170
Silage + salted *MDG* + Ca lactate	3·41	1·064	1·659	0·076**	−0·195	1·019
Silage + unsalted *MDG*	2·25	0·246	0·351	0·203	0·310	2·184
Silage + unsalted *MDG* + Ca lactate	3·78	0·724	0·095	0·282	0·112	2·511
S.E. of mean	1·207	0·345	0·545	0·123	0·405	2·191

** $P < 0·01$
*** $P < 0·001$

TABLE IX

Intake of calcium and dry matter by sheep and organic matter digestibility of hay/*MDG* rations with supplements of calcium lactate and calcium carbonate

	Ca intake, g/day	Dry matter intake, g/kg$^{0.73}$	Organic matter digestibility, %
Control	1·81	54·9	51·2
Calcium lactate	5·49	70·0	60·0
Calcium carbonate	5·61	64·2	60·3
S.E. of mean		1·75	3·02

similar with respect to the salted and unsalted grains. The effects of added calcium on increasing intakes were similar to the results of the sheep experiments and showed statistical significance ($P < 0·05$). The rise in digestibility of the rations, however, when extra calcium was fed was not significant.

The intakes of *ME* calculated from the digestible organic matter (*DOM*) intake were appreciably lower than the animals' maintenance requirements even with the addition of calcium. The maintenance requirements for protein were exceeded, and *DCP* intake values were almost twice the requirements with added calcium.

Discussion

In evaluation of a foodstuff which is generally fed as a constituent of a mixed ration it is necessary to have a measure of the nutritive value of the material when fed alone. Whilst some estimate of associative effects between ration constituents is possible through studies of different rations containing the test material it is necessary to obtain some preliminary data when the material is fed as the sole constituent of the diet.

The feeding of distiller's grains alone is only possible by compensating for the loss of certain essential nutrients such as sodium and potassium which are almost quantitatively removed during the extraction process in the distillery. Sheep are reluctant to eat *MDG* without the addition of sodium and potassium salts. In experiment 1 reasonable intakes were achieved during the first period though a highly significant decrease in intake was obtained during subsequent periods. This decrease in consumption with duration of feeding of *MDG* was also experienced in more recent trials (El Hag, S. A., & Miller, T. B., unpublished results) in which sheep were fed dried *MDG* and it was found that the level of calcium in the ration was too low to sustain satisfactory feed intake levels. The calcium intakes recorded without supplementary calcium in experiment 1 were appreciably lower than the A.R.C. requirements. It is likely that steady intakes could have been achieved in experiment 1 by adding calcium though the effects of added calcium *per se* could not have been measured satisfactorily if calcium had been included with the sodium and potassium supplements throughout the whole experiment.

The death of one sheep by copper poisoning indicates a possible hazard in the feeding of high levels of *MDG* in sheep rations. The absence of calcium supplementation could have been an important factor in causing copper toxicity in view of the findings of Kirschgessner[9] who showed that the copper uptake in the alimentary tract is reduced when the calcium content of the ration is increased.

The higher digestibility of fresh *MDG* relative to the salted and unsalted ensiled products illustrates the significance of the fermentative changes during the ensilage process. Although the *MDG* used in experiment 1 had been ensiled for only 4 weeks the degradation of soluble carbohydrate and loss of products of fermentation[1] were clearly manifested in the reduction in nutritive value when fed to the animal. The results also indicate that the practice of adding NaCl during ensilage does not have any beneficial effect on the quality of the product when fed to the animal.

TABLE X

Daily intakes of total dry matter, straw dry matter and *MDG* dry matter by cattle and organic matter digestibilities of rations of *MDG* and barley straw given with and without calcium carbonate

	Salted *MDG* + Straw		S.E. of Mean	Unsalted *MDG* + Straw		S.E. of Mean
	$-CaCO_3$	$+CaCO_3$		$-CaCO_3$	$+CaCO_3$	
Total dry matter intake, g/kg$^{0.73}$	50·0	59·4	0·91	54·0	63·9	2·68
Straw dry matter intake, g/kg$^{0.73}$	22·3	26·1	1·07	26·8	29·8	2·64
MDG dry matter intake, g/kg$^{0.73}$	27·7	33·3	1·12	27·2	34·1	2·20
Organic matter digestibility, %	51·8	53·2	2·64	48·0	48·2	
Digestible organic matter intake, kg/day	2·21	2·71		2·16	2·55	
Digestible crude protein intake, g/day	407	548		429	540	
Feeding level of energy*	0·64	0·78		0·62	0·74	
Feeding level of protein*	1·46	1·97		1·54	1·94	

* As multiple of maintenance requirement

The added calcium produced an increase in intake together with an increase in digestibility in experiment 2 when *MDG* was fed with hay and silage. This result agrees with the findings of Blaxter *et al.*[10] who showed that the appetite of sheep for long fodders is governed by the digestibility of the material. The same workers suggested that 'sheep eat to a constant distension of their digestive tracts measured by the "fill" which is in turn determined by the rate of passage of food and its digestibility'. Their results showed that an increase in percentage digestibility of energy from 40 to 60 units is accompanied by increased *DM* intake of 38 and 76 g/kg$^{0.73}$/day. A comparison of these values with the data in experiment 2 shows that numerically a similar relationship exists between *DM* intake and *GE* digestibility of the ration consumed. The relationship between digestibility and intake which has been established for long fodders may be regarded as applicable with these mixed rations of hay + *MDG* and silage + *MDG*.

The calcium intakes in experiment 2, although lower than the A.R.C. requirements, were appreciably higher than those recorded when *MDG* was fed alone in experiment 1 and the effect of calcium in increasing the digestibility of *MDG* when fed with roughages may be attributed largely to the chemical interaction of calcium and fatty acids. The fatty acids are produced by the hydrolysis of triglycerides contained in the *MDG*, and, in unpublished work, the present authors have shown that the unsaturated acids oleic, linoleic and linolenic account for about 70% of the fatty acids present. These unsaturated fatty acids have a characteristic bacteriostatic action on cellulolytic bacteria *in vitro* and this effect can be reversed by increasing the concentration of calcium salts in the digesta. The concordance of results obtained *in vivo* with those of *in vitro* experiments when the digestibility is increased by adding calcium suggests that the elimination of the bacteriostatic action of the unsaturated fatty acids is the controlling factor. The resultant increase in digestibility is accompanied by a greater rate of passage of food through the digestive tract, thus increasing the animal's appetite.

In the mixed rations fed in experiment 2 the added calcium raised the digestibility of the whole ration. The intakes of *MDG* were markedly increased with calcium in all rations and, though hay showed a slight but significant increase the consumption of silage was unaffected on the whole by calcium. Both hay and silage intakes were higher, however, when the more digestible fresh *MDG* was fed regardless of calcium intake. These results in general support the contention of Blaxter *et al.*[10] that variations in intake are associated with digestibility rather than palatability though the positive correlation between digestibility and intake is not confined to long roughage feed and can apply to ground constituents in rations containing roughage.

Calcium lactate was used as the supplement in the mixed ration fed in experiment 3 because of the high solubility of the salt and the presence of lactate in silage and ensiled *MDG*. The results with calcium carbonate in experiment 1 and the comparative study of lactate and carbonate in experiment 3 indicate, however, that the form of calcium is not important. It is also interesting to note that other workers[11] found that the addition of lactic acid to hay depressed intake whilst the addition of calcium lactate had no effect which accords with the present results that the beneficial effects in hay/*MDG* rations can be attributed mainly to the influence of calcium on the digestibility of *MDG*.

Whilst the nutrient balance data obtained from experiment 3 should be treated with some reservation in view of the limitations of the technique reviewed by Duncan,[12] they may be of some significance because of the variations in calcium and sodium levels inherent in the treatments. The highly significant increase in nitrogen retention with rations containing fresh *MDG* is partly due to the greater *DCP* intakes and increased *ME* in the fresh draff which would enhance the ammonia fixation in the rumen. The significant decrease in magnesium retention with the feeding of salted *MDG* is a particularly important finding with serious practical implications. Faecal and urinary losses of magnesium did not show any consistent pattern which would suggest a greater loss in either the faeces or urine alone. The values for sodium did not show any statistically significant effects although there is an indication that depletion of sodium is more likely with the salted material. This result may be attributed to the relatively massive urinary excretion of sodium on the high sodium ration and the very low urinary sodium when fresh and unsalted *MDG* were fed. These results do not support the common farm practice of salting the grains during ensilage which could be of a contributory factor in the incidence of hypomagnesaemia though further work is necessary to establish this result.

Experiment 4 was conducted to determine the response of added calcium in rations of cattle containing poor quality roughages and *MDG*. This mixture is typical of the inexpensive winter rations which are fed to cattle during unproductive periods. The additional calcium has not elicited

the highly significant effects which were obtained with the hay- and silage-based rations fed to sheep. This result may be attributed to the high animal variance which is characteristic of digestibility data obtained from animals on poor quality roughages. It is to be noted, however, that the extra calcium produced a significant increase in intake though the increase in digestibility was not significant which indicates that intake can be increased without a concomitant increase in digestibility. The relatively high protein and low energy content of the ration is reflected in the respective feeding levels; the calculated *ME* intake falls short of maintenance requirements, even with additional calcium whilst there is a surfeit of *DCP*. Although this feeding regime may be justified on economic grounds, it is wasteful with respect to protein and undesirable on a nutritional basis.

The calcium level of a mixed ration containing *MDG* is clearly a significant factor in determining the digestibility and intake of the whole ration. The results indicate that calcium supplementation is essential in such rations and the inclusion of high energy concentrates such as cereals which are deficient in calcium would increase the need for supplementary calcium. Moreover, the findings suggest that such rations would increase the animal's requirements of dietary calcium to a level which is greater than the A.R.C. requirements though further work on rations of varying calcium content is required to establish this conclusion.

Attempts to translate the findings to commercial practice are still somewhat speculative and require caution. High calcium intakes can impair the intestinal absorption of certain trace elements, notably zinc[13] and indiscriminate use of calcium supplements could cause serious zinc deficiency. Whilst the calculated *ME* content of the ration and calculated *ME* intake are increased with calcium it would be desirable to obtain a measure of the effect on true *ME* by determination of the losses of energy in urine and methane. The work of Czerkawski *et al.*[14] showed that, although the digestibility of energy is reduced by the presence of unsaturated fatty acids in the rumen, there is a concomitant decrease in energy lost as methane which these authors claim can compensate for the reduction in digestibility and thus result in a net increase in the *ME* of the food which is available to the animal. It seems likely that methane may be increased with the addition of calcium, thus reducing the true *ME* but it is necessary to await further research on the interaction of fatty acids and calcium on rumen metabolism before reliable values for the true *ME* of *MDG* can be stated.

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References

1. Miller, T. B., *J. Sci. Fd Agric.*, 1969, **20,** 477
2. El Hag, G. A., & Miller, T. B., *J. Sci. Fd Agric.*, 1969, **20,** 481
3. Brooks, C. C., Garner, G. B., Gehrke, C. W., Muhrer, M. E., & Pfander, W. H. J., *J. Anim. Sci.*, 1954, **13,** 758
4. Brethour, J. R., Sirny, R. J., & Tillman, A. D., *J. Anim. Sci.*, 1961, **20,** 319
5. Duthie, I. F., *Lab. Pract.*, 1959, **8,** 408
6. Agricultural Research Council, 'The nutrient requirements of farm livestock. No. 2. Ruminants', 1965 (London: H.M.S.O.)
7. Cochran, W. G., & Cox, G. M., 'Experimental designs', (2nd edn), 1957, (New York: Wiley)
8. Yates, F., *Emp. J. exp. Agric.*, 1933, **1,** 129
9. Kirschgessner, M., *Proc. Nutr. Soc.*, 1965, **24,** 89
10. Blaxter, K. L., Wainman, F. W., & Wilson, R. S., *Anim. Prod.*, 1961, **3,** 51
11. Montgomery, M. J., Schultz, L. H., & Baumgardt, B. R., *J. Dairy Sci.*, 1963, **46,** 1380
12. Duncan, D. L., *Nutr. Abstr. Rev.*, 1958, **28,** 695
13. Underwood, E. J., 'The mineral nutrition of livestock', 1966 (Farnham Royal, Bucks: Commonwealth Agricultural Bureau)
14. Czerkawski, J. W., Blaxter, K. L., & Wainman, F. W., *Br. J. Nutr.*, 1966, **20,** 485

APPENDIX

TABLE I

Proximate composition and major mineral constituents in the materials fed, % of dry matter

	Experiment 1			Experiment 2					Experiment 3		Experiment 4		
	Fresh *MDG*	Salted *MDG*[a]	Unsalted *MDG*[a]	Hay	Silage	Fresh *MDG*	Salted *MDG*[b]	Unsalted *MDG*[b]	Hay	Salted *MDG*[b]	Barley Straw	Salted *MDG*[a]	Unsalted *MDG*[a]
DM, %	26·3	26·0	26·1	85·3	21·3	26·8	31·2	27·6	85·5	27·7	77·7	27·7	26·6
OM	96·9	96·1	96·9	93·9	91·2	96·0	94·2	97·0	93·2	95·4	95·4	95·7	97·0
CP	18·9	18·1	18·9	8·2	14·1	21·7	20·2	21·2	11·3	18·6	3·7	20·0	22·0
CF	21·9	22·0	22·6	35·4	33·2	21·4	19·6	20·0	32·8	21·8	40·5	19·2	19·1
EE	8·1	8·5	8·3	1·3	4·3	7·2	9·4	9·0	1·9	9·1	1·8	7·2	7·8
Ash	3·1	3·9	3·1	6·1	8·8	4·0	5·8	3·0	6·8	4·6	4·6	4·3	3·0
NFE	48·0	47·5	47·1	49·0	39·6	45·7	45·0	46·8	47·2	45·9	49·4	49·3	48·1
GE, kcal/g *DM*	4·99	4·83	4·94	4·25	4·46	5·02	4·96	5·20	4·36	4·94	4·44	4·79	4·92
Na	0·06	0·38	0·06	0·26	0·20	0·02	0·98	0·06	0·68	0·73	0·04	0·71	0·01
K	0·02	0·02	0·02	1·83	2·64	0·03	0·08	0·04	1·45	0·03	0·84	0·02	0·03
Ca	0·10	0·10	0·09	0·43	0·47	0·13	0·09	0·21	0·40	0·11	0·22	0·10	0·10
Mg	0·10	0·10	0·10	0·11	0·14	0·18	0·09	0·11	0·16	0·10	0·06	0·09	0·10
P	0 29	0·28	0·29	0·26	0·34	0·50	0·30	0·34	0·28	0·30	0·07	0·32	0·31

[a] Short-term ensilage [b] Long-term ensilage

INACTIVATION OF α-AMYLASE IN WHEAT AND FLOUR WITH ACID

By P. FULLER, J. B. HUTCHINSON, E. E. McDERMOTT and B. A. STEWART

The inactivation of α-amylase in flour and wheat by hydrochloric acid treatment, followed by neutralisation with ammonia gas, has been investigated. A considerable degree of irreversible inactivation could be achieved by the hydrochloric acid gas treatment of 'dry' flour, and the aqueous hydrochloric acid conditioning of wheat. Careful control of the experimental conditions was necessary to minimise deleterious effects on the other flour constituents. The practical possibilities of such processes are considered.

Introduction

The use of home-grown wheat for bread flour is often limited by its frequently excessive α-amylase activity. This adversely affects the quality of the bread obtained, and it also causes difficulties at the bakery at the slicing stage. The wet harvest conditions of 1968 produced very high levels of α-amylase activity in many samples of home-grown wheat, and as the same trouble frequently occurs in other seasons through the same cause, there is a recurring but unresolved need to greatly reduce the α-amylase of these wheats or flours, without otherwise damaging their baking quality—a condition that excludes the severe heat treatment used in the production, for example, of low-amylase soup flours.[1] Inactivation by acid treatment of the wheat or flour has accordingly been studied at these laboratories as one possibility.

The unfavourable effects of low pH values upon the stability and activity of α-amylase from many sources including wheat and barley are well known.[2] Dadswell & Gardner[3] treated a suspension of flour with dilute hydrochloric acid to reduce the amylase activity, and Meredith[4] described a wet process for the inactivation of α-amylase in soup flours. As purification, in the case of α-amylase, reduces its stability and greatly alters the nature of the environment in which it causes trouble, the present work is concerned with a preliminary study of the conditions for the inactivation by acid of an unpurified active extract of flour from wheat sprouted in the laboratory, followed by tests of the effects of acid upon wheat and flour in their normal states.

Experimental

Flour and wheat

These were from home-grown samples of high α-amylase activity from the 1968 harvest; the wheats were milled on a Bühler mill to provide flours of about 70% extraction. Wheats chosen for the study of the effect of pH on flour-water slurries in the amylograph are described below. The baking tests were confined to flour from Maris Widgeon, one of the better breadmaking varieties of home-grown wheat.

α-Amylase extract

Bühler-milled flour, from Manitoba wheat sprouted in the laboratory, was extracted with three times its weight of 0·01 M calcium acetate solution at room temperature, with gentle stirring for 2 h. The extract was separated by centrifugation, heated at 70° for 20 min to inactivate the β-amylase, and centrifuged again to remove precipitated protein. The solution thus obtained was stored at 3° with little loss of α-amylase activity over periods up to 3 months.

Measurement of activity of α-amylase

The α-amylase activity of the extracts was determined by measuring their diastatic power towards a substrate of soluble starch, using the dinitrosalicylic acid method of Bendelow[5] to measure the degree of hydrolysis in terms of maltose equivalent. The activity of the flour and wheat was determined by measuring the dextrinising power of a saline extract using the method and units (JU) of Jongh.[6]

Bendelow's method was modified slightly in that the incubation temperature used was 25°; for the pH studies the soluble starch substrate was made up in water.

Amylograph

Viscometric measurements were made using a Brabender amylograph.

In the studies on the effect of pH on the peak viscosities of flour–water slurries, flour (60 g) was stirred mechanically for 1 h at room temperature in dilute acid prepared by diluting to 300 ml a known volume of standardised hydrochloric acid. After pH measurement, the flour–acid slurry was neutralised by the addition of the equivalent volume of standard caustic soda solution and the necessary water was added to give the usual weight of 510 g as for 60 g of flour + 450 ml of water. This (neutralised) slurry was then transferred to the amylograph to determine its peak viscosity in the usual manner at or before 95°.

In other studies, peak values were obtained for a mixture of flour (80 g) or heat-inactivated flour (70 g) with water (450 ml), using in all measurements, the standard 700 cm-g torsion head and a final temperature of 95°.

Hagberg falling number test

The apparatus and method were those described by Hagberg,[7] using flour (5 g) or wholemeal (7 g) in water (25 ml), but each result is reported as falling time, i.e. falling number less its constant component of 60 sec for the initial cooking period.

Extensometer

Rheological properties of the doughs were measured, where needed, on a Simon Research Extensometer; shortage of material limited this test to one piece of dough (75 g) per sample of flour.

Baking test

Doughs were prepared using flour (280 g), yeast (4 g), salt (3·5 g), the appropriate amount of water as determined by the Simon Research Extrusion Meter, together with an oxidising agent, potassium bromate (15 ppm of the flour).

The ingredients were mixed for 2 min in a peg mixer, fermented for 3 h at 80°F, moulded in a Mono moulder, tinned, proved for a further 60 min at 90°F and baked at 425°F for 32 min, in a Simon Rotary Test Baking oven. The bread was examined 16 h after baking.

Treatment of flour with gaseous HCl and its subsequent neutralisation

A strong-walled flask containing the flour was evacuated and connected to a sintered glass bubbler containing concentrated (sp. gr. 1·18) hydrochloric acid. Air saturated with the acid was drawn into the flask which was shaken by hand. The adsorption of acid was monitored by measuring the pH of a mixture of flour (5 g) with water (25 ml). After the required time of interaction, the acidified flour was restored to its previous pH by treatment with gaseous ammonia (aspirated from 0·880 solution) in the same apparatus.

Conditioning of wheat with aqueous hydrochloric acid

Wheat was dried in a current of air at 45° to about 9–10% moisture in a Mitchell drier and cooled. Sufficient aqueous hydrochloric acid was added later to the (well shaken) wheat to bring it to a suitable pH and to a moisture content of 15%. It was then conditioned for 16 h at room temperature to facilitate penetration of the acid into the grain. For the small samples of Table IV the wheat was ground on a Christy–Norris mill and the flour separated from one-half the sample by sifting it through a 5 xx silk sieve. The flours and wholemeals from the acidified wheats were all neutralised with ammonia, as previously described, before the examination of their α-amylase activity. Some information on the diffusion of acid into the grain was obtained by titrating 5 g of each milling fraction before neutralisation, in 25 ml water, to the pH value of the corresponding fraction from the control wheat.

Results and Discussion

Activity of α-amylase in extracts and flour-slurries

Activity measurements were carried out with the α-amylase extract at different hydrogen ion concentrations ranging from pH 3 to 8 using soluble starch as the substrate at 25° and the method described earlier. 1 ml of α-amylase extract was diluted to 50 ml with a solution of 0·02 M sodium glycerophosphate–0·005 M calcium acetate and adjusted to the required pH with glacial acetic acid. The enzyme was inactive at pH 3·5, relatively stable between pH 4 and 6, and gradually became less active as the pH was raised to 8·0. The results are shown in Table I.

Although hydrogen ion concentration is not a very important factor in determining the structure of dough or bread within the pH range 7·80–4·20, some slight progressive deterioration does take place in the direction of either increased acidity or alkalinity,[8] together with a deterioration in crumb colour and the development of 'off-tastes'. Consequently after treatment with acid, the pH of the wheat or flour should be restored, after which the inactivation of α-amylase achieved by the initial acidification should persist, otherwise the treatment will have been a failure. In order to test the permanence of inactivation, α-amylase extracts were adjusted to pH values ranging from 5·5 to 3·0, left for 1 h at room temperature and re-adjusted to pH 5·5 before measuring their residual activities; Table II includes the results obtained. In a further series the procedure was modified so that slurries of the active flour in 0·01 M calcium acetate solution (1 g flour/3 ml)—as distinct from the previous clarified extracts—were adjusted to pH values of 5·5–2·0, re-adjusted after 1 h to pH 5·5 prior to the inactivation of β-amylase and clarification by heating and centrifugation as previously described for the preparation of α-amylase extracts. The residual α-amylase activity in solution, when referred to the original activity similarly determined before acidification of the slurry, provided a measure of the inactivation of α-amylase at low pH values in the presence of the other flour constituents. The results for both series of experiments are shown in Table II.

The results show that at pH 3 over 90% of the enzyme was permanently inactivated in the extract and in the flour slurry, and that none could be detected in the flour slurry held at pH 2. After treatment at pH values of 3·5 and 4, the slurries appeared to retain more of their original activity than did the extract.

Use of the amylograph to examine the residual activity of an acid-treated flour

The approximate activity of residual α-amylase in solution can be assessed from its effect on the amylograph maximum of a starch or a heat-inactivated flour, if the response of the maximum to graded amounts of the original solution or other source of activity is known. In general the higher the activity of the extractable α-amylase the lower the amylograph

TABLE I

Activity of α-amylase (in a malted wheat flour extract) as a function of pH at 25°C

pH of incubation	Activity*	% of original activity (pH 5·5)
3·0	0	0
3·5	0	0
4·0	1·08	89
4·5	1·28	105
5·0	1·46	120
5·5	1·22	—
6·0	1·04	85
6·5	0·78	64
7·0	0·54	44
7·5	0·26	21
8·0	0·06	5

*mg maltose/5 min/25°c/2·0 ml diluted extract

TABLE II

Reversibility of acid inactivation of α-amylase in an extract from malted wheat flour, and in malted wheat flour slurries

pH before re-adjustment to pH 5·5	Extract		Flour slurry	
	Activity*	% of original activity	Activity*	% of original activity
5·5	1·28	—	1·86	—
5·0	1·30	102	1·84	99
4·5	1·26	98	—	—
4·0	0·96	75	1·52	82
3·5	0·40	31	0·94	51
3·0	0·08	6	0·10	5
2·0	—	—	0	0

* mg maltose/5 min/25°c/2·0 ml diluted extract

maxima.[9] Fig. 1 shows the response curve of a heat-inactivated flour to the addition of graded amounts of α-amylase extract. Its increasing departure from linearity as the concentration of the α-amylase increases, reflects a marked loss of sensitivity of the instrument to the enzyme. Thus the instrument is highly sensitive at low concentrations of α-amylase but almost useless at higher concentrations.

Comparison of acidification and subsequent neutralisation of three different flour–water slurries

For this experiment, three wheats were chosen as being of different types likely to indicate whether the pH values at which the α-amylase of flour slurries is inactivated vary widely between different flours. Excessive variations between flours would militate against successful commercial practice. One flour (A-1·7 JU) was milled from an English wheat and the second (B-0·11 JU) from a soft American wheat of much lower activity. The third flour (C-5·3 JU) was unusual in that the wheat, grown in Ireland, was of good appearance, and completely free from visible sprouting despite its high α-amylase activity.

Their flour slurries were acidified and neutralised, and their residual activities assessed in the amylograph as described earlier. The results are shown in Fig. 2 as relationships between the amount of acid added, and its effects upon the pH of the slurry and upon the peak amylograph viscosity after neutralisation. All three flours gave similar pH curves above and at pH 2·2. Further additions of acid had no effect upon the peak viscosity of flour C, but flours A and B showed a further small positive response. The overall similarity between the results indicated a common basic relationship between pH and α-amylase inactivation for all three flours. The initial similarity and apparent lack of response of flours A and C to small additions of acid, compared to that of the much less active flour B, reflects the lack of sensitivity of the instrument at these higher amylase activities.

Hydrochloric acid gas treatment of flour

The flours for acid treatment were milled from mixtures of some of the more active and therefore poorer English wheats harvested in 1968. They had high α-amylase activities as measured by the Jongh method, and amylograph maxima below 100. Thus considerable reductions in Jongh values were accompanied by comparatively small increases in paste viscosities as measured on the amylograph because of the insensitivity of this instrument at these levels of activity.

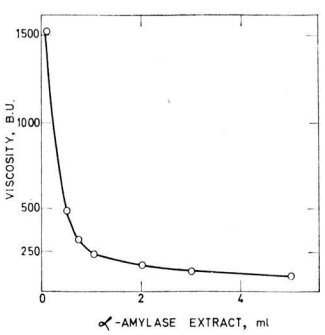

FIG. 1. *Effect of additions of α-amylase extract to the amylograph peak viscosity of a heat-inactivated flour*

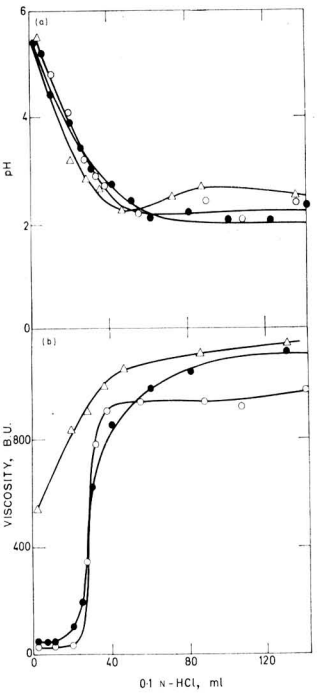

FIG. 2. *Relationship between amount of hydrochloric acid added and* (a) *pH of flour–water slurry, and* (b) *the amylograph peak viscosities of flour–water slurries after neutralisation*

● Flour A; △ flour B; ○ flour C

(More recent work here suggests that excessive activity in flour is best examined after an appropriate addition of the flour to an excess of starch.) Flour was treated with HCl gas to pH values of 2·9, 2·7, 2·6, 2·4, 2·2, 2·0 and 1·8. The acidified flour was left for measured intervals of time before re-adjustment with ammonia gas to its original pH value. The treated flours were examined with the amylograph, the Hagberg and the extensometer and their residual α-amylase was determined by Jongh's method. The results in Table III are for flours treated to pH values below 2·4. At higher pH values there was little irreversible inactivation of α-amylase (16 h at pH 2·4 inactivated a maximum of 30%). Thus the dry flour needs a lower pH value than the flour slurries of Table II to effect the same degree of inactivation.

Results for unyeasted doughs on the extensometer indicated that in most cases the acid treatment caused little gluten damage, although some loss of extensibility was noted in the dough made from flour treated to pH 1·8 for 16 h.

Although the α-amylase as measured by the Jongh method was appreciably inactivated at these low pH values, this loss in activity was not always reflected in the results obtained in the Hagberg or on the amylograph, where the peak viscosity was lower for several treated flours than would be expected from their Jongh values. The failure of the Jongh procedure[9,10] to completely extract all the α-amylase could account for this anomaly if the 'bound' enzyme is more resistant to inactivation by acid, but a likelier possibility, suggested by the double peak in three of the amylograph

TABLE III

Inactivation of α-amylase in flour treated with hydrochloric acid gas to pH 2·2–1·8, followed by neutralisation with ammonia gas

Time before ammonia treatment:	Control	10 min			30 min			7 h			16 h		
pH after HCl treatment:		2·2	2·0	1·8	2·2	2·0	1·8	2·2	2·0	1·8	2·2	2·0	1·8
Hagberg, sec	9	23	73	51	46	36	97	110	98	46	31	22	6
Peak height, B.U.	80	83	150	105	130	100	170	180	145	165*	125	185*	160*
α-Amylase (Jongh)	3·6	2·6	1·2	1·3	1·9	1·7	0·96	1·1	0·96	0·70	1·7	0·57	0·33

* Double peak on amylograph (not encountered in ordinary flours)

TABLE IV

Acid adsorption and residual α-amylase of wheat conditioned with aqueous hydrochloric acid

Conditioning HCl conc.	Wholemeal				Flour				'Tails'	
	pH	Titre*, ml	Hag., sec	Jongh	pH	Titre*, ml	Hag., sec	Jongh	pH	Titre*, ml
Control	6·40	—	79	4·5	6·25	—	10	3·7	6·5	—
0·5 N	5·10	1·20	92	4·7	5·15	1·0	13	3·3	5·15	1·20
1·0 N	4·35	2·50	82	2·8	4·35	2·35	10	3·0	4·75	2·70
1·5 N	3·75	3·90	73	3·5	3·60	3·65	9	2·6	4·35	4·50
2·0 N	3·45	5·20	82	1·8	2·75	5·20	9	1·7	3·90	5·40

* Titre in ml 0·1 N-NaOH

curves, is that the starch has been modified by the acid treatment. That the drop in Hagberg falling times for flours treated at pH 2·0 and 1·8 for 16 h is associated with a loss of starch-pasting capacity, and not with a failure to inactivate α-amylase, was confirmed by carrying out the Hagberg test in the presence of the α-amylase inhibitors, silver nitrate and iodine (work in progress).

Baking tests

As considerable inactivation of α-amylase had been achieved without apparent serious gluten damage, though with some starch modification in flours treated at low pH values for several hours, larger quantities of flour from two samples of the same variety of wheat (Maris Widgeon) of higher activities (6 and 8 Jongh units respectively) were acid treated to pH 1·8 for 16 h for baking tests. After neutralisation with ammonia the activity of the first flour had fallen to 2·7—a rather higher value than had been expected— whereas the activity of the second flour had fallen to 0·8.

The crumb of the loaves baked from the untreated flours showed the usual signs of high diastatic activity, i.e. a weak, damp, streaky crumb, whereas both the treated flours produced loaves much improved in all these respects, although they had dark crusts owing, partly at least, to their content of residual ammonium chloride.

That from the first treated flour showed no evidence of gluten denaturation, nor of starch modification, but the loaf produced from the second treated flour showed signs of gluten damage resulting from the acid treatment.

Conditioning of wheat with hydrochloric acid

In practice, flour treated as just described would need to be dried before sale because of its pick-up of water during treatment. Furthermore, as the treatment of flour from sound imported wheats of low α-amylase activity would be undesirable, the process would be even less attractive in normal circumstances, where the separate milling of imported and native wheats is uneconomic and usually impracticable. However as damp wheat is more easily dried than is flour, and whilst poor wheat could be separately treated in the mill before mixing it with the other wheats of the grist, the effects of hydrochloric acid on wheat when added to the water used to condition the grain have been examined also. Table IV gives the results for fractions milled from wheat conditioned with various concentrations of hydrochloric acid for 16 h.

These results indicate that this method is restricted by the high concentration of acid needed to bring the endosperm pH value down below 3·0 and by the loss of acid in the 'tails'. The sieving of the wholemeal gave on the average a 60% yield of flour, but the residual 'tail' fraction still contained about 50% of the acid applied to the wheat. The use of a higher ratio of more dilute acid is restricted by the economics of drying wheat to low moisture contents and by the necessity to mill the wheat at a moisture content compatible with good milling behaviour. Furthermore the milling of the acidified wheat would promote corrosion troubles.

Conclusions

The foregoing experiments indicate that the acid treatment of flour and wheat can be used to effect a considerable irreversible inactivation of α-amylase. In practical terms, a strict control of factors such as pH and length of treatment would have to be exercised in order to prevent excessive and deleterious changes in the gluten and starch properties.

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References

1. Hutchinson, J. B., *Chemy Ind.*, 1963, p. 1084
2. Greenwood, C. T., & MacGregor, A. W., *J. Inst. Brew.*, 1965, **71**, 405
3. Dadswell, I. W., & Gardner, J. F., *Cereal Chem.*, 1947, **24**, 79
4. Meredith, P., *N.Z. Jl Sci.*, 1968, **11**, 720
5. Bendelow, V. M., *J. Inst. Brew.*, 1963, **69**, 467
6. Jongh, G., *Chem. Weekbl.*, 1957, **53**, 597
7. Hagberg, S., *Cereal Chem.*, 1961, **38**, 202
8. Fisher, E. A., & Halton, P., *Cereal Chem.*, 1929, **6**, 97
9. Hutchinson, J. B., *J. Sci. Fd Agric.*, 1966, **17**, 198
10. Dodds, N. J. H., & Knight, R. A., *J. Sci. Fd Agric.*, 1967, **18**, 258

FREE SUGARS OF WHEAT ALEURONE CELLS

By D. J. STEVENS

The sugars extracted from de-fatted contents of the aleurone cells of wheat, accounted for 10% of the dry weight of the material. After separation by gel-filtration and paper chromatography, the components were identified as 42% sucrose, 31% raffinose, 20% neo-kestose, and 6% fructosyl raffinose, with traces of two other oligosaccharides, one of which was probably stachyose. Monosaccharides, maltose, and higher oligosaccharides which occur in other parts of the grain, were absent.

Introduction

Earlier investigations of the sugars present in the wheat grain have been confined almost exclusively to commercial milling products. Wholemeal flour, white flour, bran, and germ contain approximately 3%, 1%, 5%, and 17% of total sugars respectively, but identification of individual sugars can give only a rough indication of their distribution in the anatomical structural parts of the grain, since commercial fractions inevitably consist of mixtures of these. Hand-dissection may be used to obtain uncontaminated individual parts, and Dubois *et al.*[1] examined the germ in this way, and found only sucrose and raffinose in both the embryo and scutellum portions.

Although the aleurone layer is botanically associated with the endosperm, most of it appears in the bran fraction rather than in the flour, after milling, accounting for about half of its weight. The rest of the bran is made up of the outer pericarp and seed coat layers, together with variable amounts of germ and endosperm. Sugars which have been reported to occur in bran include xylose, arabinose, fructose, glucose, galactose, sucrose, melibiose, neo-kestose, raffinose, fructosyl raffinose, and stachyose.[2,3] However, Kretovitch[4] was unable to extract any sugars from material said to consist only of the bran coat and aleurone layer, and he therefore concluded that there could be none in the aleurone layer.

In a previous paper,[5] a method was described for isolating the contents of aleurone cells, and this material has now been examined for the presence of sugars.

Experimental

Materials

Reagents

Analar grade reagents were used where available. Yeast invertase solution was prepared by dialysis of rehydrated Difco 'Invertase Analytical'.

Aleurone cell contents

De-fatted material was isolated from Manitoba wheat flour by differential centrifugation in benzene–carbon tetrachloride mixtures as described earlier,[5] or, in 10-fold greater yield, from the corresponding bran by a similar method. In the latter case, bran was first ground in a hammer mill fitted with a 0·4 mm screen, then made into a slurry (20% wt./vol.) with a mixture of benzene and carbon tetrachloride of density 1·51 g/ml, and treated in a tissue disintegrator (Waring Blendor). The product was centrifuged, the upper layer was re-treated, and the bulked sediments were dispersed in carbon tetrachloride and re-centrifuged twice. The air-dried sediment was similar in every respect to that prepared from flour.

Methods

Extraction of sugars

The following procedure was adopted to avoid the possibility of enzyme- or proton-catalysed hydrolysis, which could produce sugars not originally present. Aleurone cell contents (1 g) were dispersed in hot 96% ethanol (15 ml) by means of a vortex mixer. The suspension was brought to the boil, refluxed for 1 h, and then evaporated just to dryness. Extraction was carried out with 3 successive batches of glass-distilled water (15 ml, pH 7·0) on a boiling water bath under reflux, for 1½ h, 30 min, and 15 min. After each extraction, the suspension was centrifuged, the sediment was re-extracted, and the superantants were bulked and finally made up to 50 ml. An aliquot (1 ml) was taken for quantitative analysis of total sugars, and the remainder was evaporated to ~ 5 ml under reduced pressure (Büchi Rotavapor-SB, bath temp. < 50°). Absolute ethanol was then added to precipitate protein, which was removed by centrifugation. The supernatant was applied to a column (70 × 2 cm) of cation-exchange resin (Amberlite IR-120, Na^+ form), and eluted with glass-distilled water (pH 7·0). Fractions containing sugars were bulked, made up to 100 ml, and checked for negative ninhydrin reaction, and an aliquot (1 ml) was taken for assay. The remainder was evaporated under reduced pressure to a syrup, and stored at −20°

Gel-filtration

This was carried out on Sephadex G-15, which has a nominal fractionation range up to a mol. wt. of 1,500. About 0·1 ml of the extract was diluted to 1–2 ml so as to

reduce the viscosity before applying it to the column (Sephadex K25/100). For calibration purposes, 1 ml of a solution containing 1% each of glucose, maltose, raffinose and stachyose was used. Elution was carried out with glass-distilled water (pH 7·0) at a flow-rate of ~ 15 ml/h, and 2 ml fractions were collected.

Paper chromatography

Whatman No. 1 chromatography paper was used, and samples were applied as spots or streaks, and developed by the descending solvent technique. The most useful solvent systems and volume ratios were found to be: (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) 1-butanol–ethanol–water (4 : 1 : 5); (C) 1-propanol–ethyl acetate–water (6 : 1 : 3); and (D) 1-propanol–ethyl acetate–water (7 : 1 : 2). Reagents used for the detection of sugars were *p*-anisidine hydrochloride,[6] 3,5-dinitrosalicylic acid–caustic soda (specific for reducing sugars),[7] and silver nitrate–caustic soda.[8] The last is extremely sensitive towards reducing sugars, and was used for detecting hydrolysis products.

The 5-diazouracil test of Raybin[9] for sucrose derivatives in which the fructose moiety is unsubstituted, was applied on paper as described by Bacon.[10]

Hydrolysis of sugars

Complete acid hydrolysis was carried out in 0·25 N-H_2SO_4 at 100° under reflux for 18 h. These conditions were necessary in order to sever the glucose–galactose linkage, but resulted in fructose being partly destroyed. The hydrolysate was neutralised with barium carbonate, centrifuged, and the superantant was evaported under reduced pressure. 0·5 N acetic acid was used for partial acid hydrolysis, refluxing being limited to 1 h, and the hydrolysate was evaporated without prior neutralisation. For invertase hydrolysis, 5–10 ml eluates were mixed with 0·2 ml yeast invertase solution, and left at room temperature for 5 min before evaporation. Invertase hydrolysates were used for quantitative work, since there was no destruction of fructose by this method.

Transfructosylation

Since authentic samples of fructosyl sucroses (kestoses[11]) and fructosyl raffinoses were not available, (with the single exception of 6-kestose, kindly supplied by Dr. D. Gross), they were identified and characterised chromatographically from the products of partial hydrolysis of sucrose and raffinose by yeast invertase. Invertases have been shown to transfer β-D-fructofuranosyl radicals to primary alcoholic groups.[12] Thus, under suitable conditions of partial hydrolysis, fructosyl radicals released from sucrose may be transferred to the $-CH_2OH$ groups of intact sucrose molecules to give trisaccharides. Transfer to the glucose moiety gives 6^G-fructosyl sucrose (neo-kestose), and to the fructose moiety gives 6^F-fructosyl sucrose (6-kestose) and 1^F-fructosyl sucrose (1-kestose). Yeast invertase produces mainly the 6^G- and 6^F-compounds, the 1^F-compound being produced chiefly by mould invertases. Reaction with yeast invertase was carried out as described by Gross.[13] A reducing disaccharide, 6-fructosyl glucose, also appears among the partial hydrolysis products, by attachment of a fructosyl radical to the $-CH_2OH$ group of glucose, leaving its reducing group exposed.[11]

In the case of raffinose, yeast invertase may be expected to produce tetrasaccharides mainly by transfructosylation to $-CH_2OH$ groups at carbon 6 of the galactose moiety to give 6^G-fructosyl raffinose, and at carbon 6 of the fructose moiety to give 6^F-fructosyl raffinose, by analogy with its reaction with sucrose.

Analysis of sugars

The phenol–sulphuric acid colorimetric method[14] was used, with the usual precautions to exclude cellulosic lint and paper fibres. Absorptions were measured by an EEL (Evans Electroselenium Ltd.) photo-electric colorimeter using a blue OB 10 filter. Standard curves were established for fructose, glucose, sucrose, melibiose, and raffinose. Results for mixed sugars were expressed in terms of the glucose curve. 1 ml aliquots were invariably taken, and excessively high colorimeter readings were avoided when necessary by suitable dilution of the original sample, not by dilution of the coloured reaction product since this had a non-linear effect. Spots were located on chromatograms by spraying an adjacent marker strip, and each was cut out together with a blank of the same dimensions from the same region of the paper. Each excised region was cut into small pieces which were placed in a sintered funnel (3X3) and agitated with a known amount (3–5 ml) of water. After 5 min, the eluate was filtered under vacuum into a test tube, from which 1 ml aliquots were withdrawn. (When sintered funnels were washed with acetone and dried before re-use, it was found to be essential to ensure that all traces of acetone were removed, otherwise a characteristic spurious pink coloration appeared in the eluate on addition of the phenol and sulphuric acid reagents.)

Results

The total sugar content of the initial crude extract was almost 15%, expressed as glucose, on the basis of dry weight of aleurone cell contents. This figure includes sugar derivatives as well as free sugars, since the phenol–sulphuric acid method measures virtually all classes of sugar compounds. Chromatography showed the presence of a range of ninhydrin-positive spots, but these were not investigated further. After treatment with alcohol and ion-exchange resin until no reaction was given with ninhydrin, the total content of free sugars was 10% of the aleurone cell contents.

Paper chromatography of the free sugar extract revealed four components (I–IV, in order of decreasing mobility). The relative proportions by weight of these, expressed in terms of the glucose standard curve, are given in Table I, together with their $R_{glucose}$ values and those of a range of reference sugars, in solvent systems A, B, and C. $R_{sucrose}$ values are given in solvent system D, together with those for the reference compounds prepared by transfructosylation.

It will be seen that no monosaccharides, maltose, or high mol. wt. oligosaccharides of the levosine type are present. The major components I and III have R_g values the same as those of sucrose and raffinose respectively.

All four components gave a negative reaction with the 2,4-dinitrosalicylic acid spray reagent, and were therefore non-reducing. The silver nitrate–caustic soda treatment gave a faint response, in common with other non-reducing sugars, but in all cases this became a strong positive reaction if the chromatogram were first sprayed with yeast invertase solution and dried in a warm air current. This indicated that all four components contained a terminal fructosyl group, released by the action of yeast invertase, which is a β-fructofuranosidase.

TABLE I

Relative proportions of aleurone sugar components, and chromatographic mobilities compared with standards

	Proportion by wt.,* %	Solvent system			
		A $R_{glucose}$	B $R_{glucose}$	C $R_{glucose}$	D $R_{sucrose}$
Component I	44	0·78	0·74	0·90	1·00
Component II	20	0·50	0·52	0·75	0·57
Component III	30	0·33	0·33	0·59	0·34
Component IV	6	0·20	0·22	0·45	0·20
Fructose		1·15	1·15		
Glucose		1·00	1·00	1·00	
Galactose		0·88	0·91		
Sucrose		0·78	0·74	0·90	1·00
6-Fructosyl glucose					0·80
Maltose		0·66	0·55		
neo-Kestose					0·57
6-Kestose					0·46
Melibiose		0·42	0·42		
Raffinose		0·33	0·33	0·59	0·34
6^G-Fructosyl raffinose†					0·26
6^F-Fructosyl raffinose†					0·20
Stachyose		0·13			0·12

* From standard curve of glucose
† Identification tentative, see text

Separation of the components by gel-filtration was carried out after first determining the elution volumes corresponding to mono-, di-, tri- and tetra-saccharides, using a mixture of authentic glucose, maltose, raffinose, and stachyose. Elution curves are shown in Fig. 1. Equal amounts of the reference sugars were used, and the areas of the corresponding peaks in Fig. 1 (a) were also equal. The trisaccharide peak from the aleurone extract in Fig. 1 (b) was larger than that of the disaccharide peak. From consideration of the relative amounts of each component, as given in Table I, this implied that component II was a trisaccharide, although its R_g value on paper chromatograms lay between those of the disaccharides maltose and melibiose. Samples of the eluates from each peak were examined by chromatography, and this confirmed that the compounds were distributed as indicated in Fig. 1 (b).

Identification of individual components was completed as indicated below.

Component I

This was a non-reducing disaccharide, which had the same R_g value as sucrose in all four solvent systems used. Hydrolysis by acid or invertase yielded fructose and glucose, in a 1 : 1 molar ratio. A positive reaction was given with 5-diazouracil. Component I was therefore identified as sucrose, and its proportion of the total sugars, using the sucrose standard curve, was 42%.

Component II

This was a non-reducing trisaccharide, which was hydrolysed by acid or invertase to fructose and glucose, in a 2 : 1 molar ratio, and was therefore one of the kestoses. The R_f value in solvent system D was the same as that of neo-kestose, and the 5-diazouracil test was positive. The products of partial hydrolysis by dilute acetic acid included a reducing disaccharide with the same R_f as that of 6-fructosyl glucose, which could only be produced from neo-kestose. Component II was therefore identified as neo-kestose, and

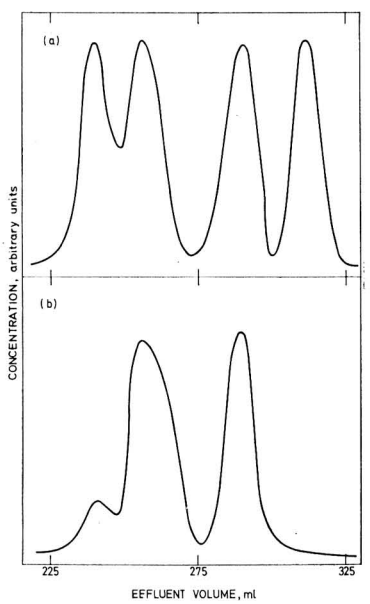

FIG. 1. *Gel-filtration elution curves of sugar mixtures on Sephadex G-15*

Peaks from right to left: (a) authentic mono-(glucose), di-(maltose), tri-(raffinose), and tetra-(stachyose) saccharides; (b) sugars extracted from aleurone cell contents; peaks from right to left: component I, components II and III, component IV

in the absence of an authentic sample, its proportion of the total sugars was expressed in terms of the glucose standard curve as 20%.

Component III

This was a non-reducing trisaccharide, which had the same R_f value as raffinose in all four solvent systems used. Acid hydrolysis gave fructose, glucose and galactose, and invertase yielded fructose and melibiose in a 1 : 1 molar ratio. A positive reaction was given with 5-diazouracil. Component III was therefore identified as raffinose, and its proportion of the total sugars, using the appropriate standard curve, was 31%.

Component IV

This was a non-reducing tetrasaccharide which was hydrolysed by acid to fructose, glucose, and galactose, and by invertase to fructose and melibiose in a 2 : 1 molar ratio, showing it to be a fructosyl raffinose. The R_f value in solvent system D was the same as that of the compound tentatively identified as 6^F-fructosyl raffinose. The 5-diazouracil test was indefinite. The proportion of the total sugars was 6%.

When the tetrasaccharide peak from gel-filtration was concentrated and chromatographed, very slight traces of two components other than component IV were revealed by using the invertase–silver nitrate–caustic soda technique. One had an R_f value between those of components III and IV, and the other was similar to stachyose in this respect.

Discussion

The four sugars found in the aleurone cell contents have all been reported to occur in flour also, assuming that the 'glucodifructose' reported in flour[15] is in fact neo-kestose, and that the fructosyl raffinose[16] is the same in each case. The aleurone cell contents differ from flour in having no monosaccharides, maltose, or 'levosine', and from germ in containing oligosaccharides other than sucrose and raffinose, which are the most widely distributed sugars in the plant kingdom.

No explanation is offered for the finding by Kretovitch that no sugars could be extracted from material consisting only of aleurone layer and outer bran layers. Although the aleurone cell walls are very thick, it is known that most of the protein in the cell contents can be extracted through the walls,[17] and an extract from a preparation of intact aleurone layer was found to give a very strong positive reaction with the phenol–sulphuric acid reagents. The sucrose, raffinose, neo-kestose and fructosyl raffinose occurring in the sugars extracted from bran by Saunders & Walker[3] may therefore be assumed to be derived mainly from the aleurone layer, and this is supported by the relative amounts of each, which are similar to those reported above. Other sugars found in bran by these workers presumably originated from other parts of the grain, but stachyose, which had not been reported previously in any wheat products, is only tentatively identified, as a trace component in the present investigation of the aleurone cell contents. Its presence in significant amounts in a bran extract may imply that there are considerable varietal differences in the amount of stachyose present in the aleurone layer, or that it is present in other parts of the grain, but has not yet been identified as such.

The important rôle of the aleurone layer in germination, has been suggested by MacLeod & Millar[18] to involve an explosive release of hydrolytic enzymes, as a result of the action of gibberellin from the embryo on the respiring aleurone layer. Since oligosaccharides containing a gluco-pyranose–fructofuranose linkage have been shown to be intimately connected with respiration,[19] it is noteworthy that all of the sugars now found to be present in the aleurone cell contents are of this type.

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References

1. Dubois, M., Geddes, W. F., & Smith, F., *Cereal Chem.*, 1960, **37,** 557
2. Ramachandra, B. S., Krishnamurthy, R. G., & Raghunatha Ras, Y. K., *Fd Sci.*, 1959, **8,** 83
3. Saunders, R. M., & Walker, H. G., *Cereal Chem.*, 1969, **47,** 85
4. Kretovitch, W., *Biochem. J.*, 1933, **27,** 1687
5. Stevens, D. J., McDermott, E. E., & Pace, J., *J. Sci. Fd Agric.*, 1963, **14,** 284
6. Hough, L., Jones, J. K. N., & Wadman, W. H., *J. chem. Soc.*, 1950, p. 1702
7. Jeaves, A., Wise, C. S., & Dimler, R. J., *Analyt. Chem.*, 1951, **23,** 415
8. Trevelyan, W. E., Proctor, D. P., & Harrison, J. S., *Nature, Lond.*, 1950, **166,** 444
9. Raybin, H. W., *J. Am. chem. Soc.*, 1937, **59,** 1402
10. Bacon, J. S. D., *Biochem. J.*, 1959, **73,** 507
11. Gross, D., Blanchard, P. A., & Bell, D. J., *J. chem. Soc.*, 1954, p. 1727
12. Bacon, J. S. D., *Biochem. J.*, 1954, **57,** 320
13. Gross, D., in 'Methods in Carbohydrate Chemistry', 1962, Vol. I, (Eds Whistler, R. L., & Wolfrom, M. L.) p. 360 (New York and London: Academic Press)
14. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F., *Analyt. Chem.*, 1956, **28,** 350
15. MacKenzie, R. M., *S. C. I. Monogr. No. 3*, 1958, p. 127 (London: Society of Chemical Industry)
16. White, L. M., & Secor, G. E., *Archs Biochem. Biophys.*, 1953, **44,** 244
17. Stevens, D. J., in preparation
18. MacLeod, A. M., & Millar, A. S., *J. Inst. Brew.*, 1962, **68,** 322
19. MacLeod, A. M., Travis, D. C., & Wreay, D. G., *J. Inst. Brew.*, 1953, **59,** 154

CAROTENE-BLEACHING ACTIVITY IN PLANT TISSUE EXTRACTS

By J. A. BLAIN

The effect of antioxidants on carotene-bleaching by extracts of various vegetable tissues other than seeds suggests that this activity in general could be more readily attributed to intrinsic haematins than lipoxidases.

Introduction

It was shown recently[1] that aqueous extracts of tomato contained heat-labile and non-dialysable factors which bleached β-carotene in the presence of oxidised linoleic acid, although in the fresh fruit juice this activity was likely to be inhibited by the natural antioxidants present. The carotene-bleaching characteristics were similar to those displayed by haematins such as peroxidase and cytochrome *c* and were unlike those of soya lipoxidase.

Carotene-bleaching or fat-oxidising activities have been noted in a large number of vegetable tissues other than seeds, and some workers have associated such activities with lipoxidases[2,3] although the possibility that activity could have resulted from the haematins normally present was not disproved.

In this study, extracts from seeds, solutions of pure haematins and extracts from a wide variety of plant tissues have been compared for carotene-bleaching activity in the presence and absence of antioxidants in an attempt to establish a criterion for true lipoxidase action.

Experimental

Materials

Reagents used in the preparation of carotene–linoleate agar plates, cytochrome *c*, peroxidase and quercetin were obtained and used as described previously.[1] Butylated hydroxyanisole (BHA) of commercial grade was prepared as an ethanolic solution at a concentration of $1{\cdot}5 \times 10^{-3}$M. The plant materials used are shown in Table I. Soya, millet, lentil, pea, broad bean, cucumber and lupin seeds and wheat germ were obtained in the dry state from commercial sources. Fruits were purchased locally in summer, and other vegetable materials were grown locally and collected and used fresh in June and July. Melon seeds were extracted from the fruit and allowed to dry before being ground. Fresh lupin seeds were extracted from the green pods.

Methods

Seeds were ground, de-fatted with ether, dried and ground to pass a 60 mesh sieve and 1 g of the meal was extracted with 20 ml of water. Wet vegetable material was ground with twice its volume of water and an equal weight of sand. All extracts were centrifuged at $5000 \times g$ and the supernatants were used, freshly prepared, in the agar plate cups.

The agar gels containing linoleate, β-carotene and antioxidant (when used) were prepared as described previously,[1] being incubated for periods of 20 h and 44 h after addition of the extracts to the cups. Bleached zones were then measured. Concentrations of antioxidants in the gels were 4×10^{-3}M for ascorbic acid and 3×10^{-5}M for BHA. Peroxidase solution contained 0·15 mg/ml (wt./vol.)

The agar gel plates for peroxidase were identical in preparation and use to those described above, except that they contained 0·02% guaiacol only, in addition to agar and buffer. After the period of incubation the plates were flooded with 3% hydrogen peroxide which produced a red–brown zone coincident with the area of peroxidase activity.

Results

Comparison of extracts of three types of seeds, in which lipoxidase activity has been studied previously, with those of other plant tissues is made in Fig. 1. It can be seen that the antioxidants ascorbic acid, BHA and quercetin did not suppress the bleached zones caused by the seed extracts, but did suppress those caused by cytochrome *c*, peroxidase and by the other plant tissues with the exception of lupin leaves extract. Subsequent experiments showed no activity for an extract from lupin leaves but confirmed the other results, and it was assumed that the apparent lupin leaf activity was due to contamination from another source.

In the case of apple skin and cress where faint zones appeared only after 44 h, it cannot be assumed that an active catalyst is lacking, since prevalence of natural water-soluble antioxidants in the extracts may produce a similar effect.[1] This is illustrated in plate F where it can be seen that if peroxidase, at the normal concentration used in the carotene plate, is present in the cup containing the apple skin extract, then only at the lower concentration of that extract is bleaching visible. When soya extract was present instead of peroxidase no such inhibition was manifest.

The effects of intrinsic antioxidants are shown more clearly in Fig. 2 for extracts of the skins of turnip, tomato and orange, where at the highest concentration of extract, no bleaching is visible, but bleaching does become obvious at lower concentrations. The inner dark zone, shown most clearly for tomato, appears to be caused by inhibition of carotene-bleaching. Fig. 2 also shows an indication of peroxidase activity obtained concurrently on the same extracts. It would seem possible, bearing in mind that sensitivy of zone detection was not identical for the two systems, that carotene bleaching for the extracts other than soya could be caused by peroxidases. It will be noted that extracts of potato skin showed an unusual double zone of bleaching. It was found that when these extracts were incubated in the gel systems containing antioxidants, the outer zone was not seen but the inner was unaffected.

To isolate the carotene-protecting effects of natural intrinsic antioxidants from the pro-oxidant effects of enzymic factors in the same materials, the water-soluble antioxidants were extracted under conditions which would inactivate enzymes. Broad beans, melon seeds, millet seeds, peas, lentil and soyabean were ground in a mill but not de-fatted.

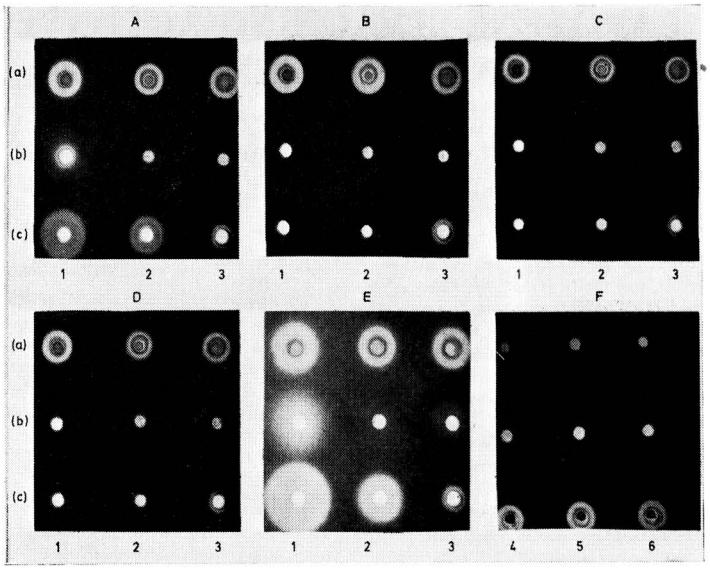

FIG. 1. *Influence of antioxidants on carotene-bleaching factors from different sources*

Plates: A, control; B, ascorbic acid; C, BHA; D, quercetin; E, control at 44 h
Cups: 1 (a) soya; 2 (a) lentil; 3 (a) pea; 1 (b) cytochrome *c*; 2 (b) apple skin; 3 (b) cress; 1 (c) peroxidase; 2 (c) cauliflower florets; 3 (c) lupin leaves
Plate F: Cups in row 4 have undiluted apple skin extract, in row 5 have 1/6 dilution and in row 6 have 1/18 dilution. In addition, cups in row (b) contain peroxidase and those in row (c) contain soya extract

Grass, orange peel, tomato skin, apple skin and lupin leaf were freeze-dried and powdered in a mortar.

Extracts of all these materials were made by adding 10 g to 100 ml of boiling water for 10 min. The centrifuged supernatant liquids were each mixed with an equal volume of standard peroxidase solution, as were also their 1 : 10 dilutions, before incubation in the carotene–linoleate agar gel cups for 20 h.

Comparison of the inhibitory effects on carotene bleaching by peroxidase is shown in Fig. 3. It can be seen that even at lower concentrations the extracts of grass, apple skin, and lupin leaf inhibited this bleaching almost entirely, while lentil, soya and broad beans were inhibitory at the higher concentrations.

Table I shows a comparison of the effects of ascorbic acid and BHA on carotene-bleaching by extracts of seeds which are recognised as lipoxidase sources—soya, lentil, pea, bean and wheat germ—with other seeds and different plant tissues. Although it has been convenient to express results in zone diameters the comparison is essentially qualitative. For any one molecular species of enzyme in the diffusion assays, $\log [E]$ has been found to be proportional to zone diameter,[4,5] but since enzymes from different sources will diffuse at different rates it was not possible to make comparisons analogous to those based on activity measurements.

Discussion

Tappel suggested[6] that despite the wide variety of plants which had been reported to contain lipoxidase those sufficiently characterised as true lipoxidase sources might be limited to soyabean, urd bean (*Phaseolus mungo*), pea, wheat, barley, groundnut and lucerne. There appears to be little published since to suggest any wide occurrence except in the seeds of legumes and some cereal grains.

While there are major differences in the fat oxidase activities of lipoxidase and haematins in that haematins can sometimes be inhibited by cyanide,[7] and at low substrate concentrations[8] (or with rigorously peroxide-free linoleate)[9] will show induction periods, the lack of simple and general criteria has tended to lead to the assumption that unsaturated fat oxidase activity in vegetable tissues is due to lipoxidases rather than haematins.

It was shown recently[1] that linoleate-coupled oxidation of carotene by soluble constituents of tomato could more readily be attributed to intrinsic haematin than lipoxidase activity. While this was based on kinetic studies as well as the cup-plate diffusion method used here, the major criterion was that lipoxidase will cause carotene-bleaching in the presence of linoleate which is unoxidised, while haematins will not.[10] It seems likely that the presence of antioxidants in the cup-plates will prevent autoxidation of linoleate during the incubation period since the areas outside the zone retain more carotene colour than in controls.

The results shown in Table I, and Figs 1 and 2 are consistent with this criterion and with the supposition that true lipoxidase activity is rare outside the leguminous and cereal seeds. Broad bean, lentil, lupin, pea, soya and wheat germ all showed carotene-bleaching activity which was not suppressed by the antioxidants although it was sometimes partly inhibited. The haematin activity, on the other hand, was suppressed along with that of extracts of all other plant tissues except lupin seed pod and potato skin. Since fresh lupin seeds appeared to be very active as a lipoxidase source it was not

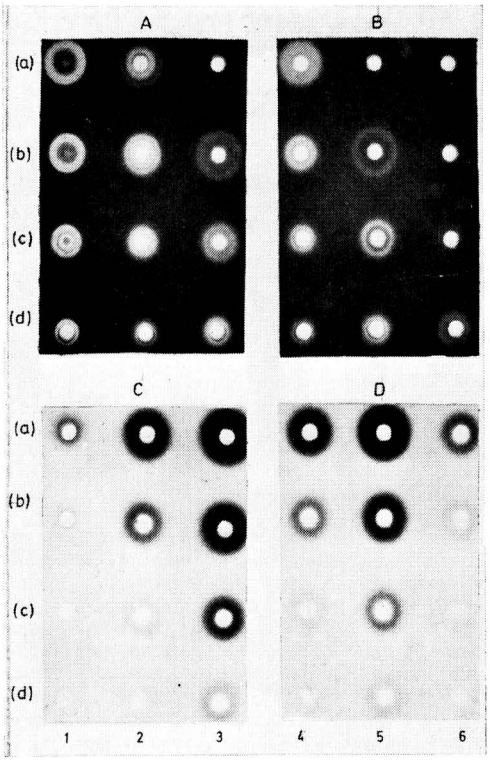

FIG. 2. *Comparison of carotene-bleaching and peroxidase activities of extracts of vegetable tissues over a range of dilutions*

Plates A and B show carotene-bleaching, C and D peroxidase activity.
Dilutions: (a) full strength; (b) $\frac{1}{4}$; (c) $\frac{1}{16}$; (d) $\frac{1}{64}$. 1, Soya; 2, potato skin; 3, turnip skin; 4, peroxidase; 5, tomato skin; 6, orange skin

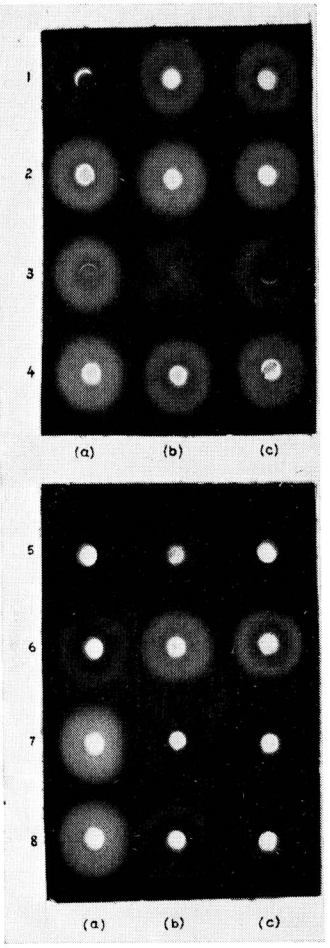

FIG. 3. *Influence of boiling water extracts of vegetable tissues on carotene-bleaching by peroxidase*

Odd numbers indicate full strength extracts, even ones the corresponding $\frac{1}{10}$ dilution
1 (a), 2 (a), broad bean; 1 (b), 2 (b), melon seed; 1 (c), 2 (c), millet seed; 3 (a), 4 (a), pea; 3 (b), 4 (b), lentil; 3 (c), 4 (c), soya; 5 (a), 6 (a) grass; 5 (b), 6 (b), orange peel; 5 (c), 6 (c), tomato skin; 7 (a), 8 (a), duplicate contro, peroxidase only; 7 (b), 8 (b), apple skin; 7 (c), 8 (c), lupin leaf

surprising that the closely associated pod showed activity also.

The case of potato skin extracts was anomalous. As can be seen from Fig. 2 these produced a double zone, the inner being more intensely bleached. With antioxidant in the gels (Table I) the outer zone disappeared. Lipoxidase activity has been previously reported in potato although the methods used would not necessarily have distinguished this from haematin activity. It seems not unlikely that a true lipoxidase is present.

Studies on unsaturated fat oxidases in plant may be complicated by the presence of natural antioxidants as was pointed out by Rhee & Watts.[2]

In the studies on tomato it was shown previously[1] that such antioxidants may be associated with inhibition of bleached zone on carotene plates, or with the appearance of unbleached inner zones. The effects of such natural antioxidants on peroxidase-catalysed bleaching and inferentially on that by other haematins, as shown in Fig. 3, indicates that where plant extracts show peroxidase activity and not carotene-bleaching activity (Table I) then the carotene-bleaching which would be anticipated from peroxidase is inhibited by the antioxidant.

This is shown in Fig. 1, plate F, where apple extract at the highest concentration suppresses bleaching by peroxidase, but permits it to a slight extent at the lowest concentration. It does not however, inhibit the activity of soya extracts and thus, although natural antioxidants are present in seeds containing true lipoxidase (Fig. 3), they will not be manifest except in extracts which are boiled to destroy the enzyme.

While observation of no definite zone for peroxidase does not indicate complete absence of the enzyme—the cup-plate method for peroxidase is not very sensitive—the results in Table I suggest that the most potent lipoxidase sources may be relatively deficient in peroxidase.

Despite the limited nature of the survey made here it is clear that lipoxidase activity should not readily be attributed

TABLE I

Effect of ascorbic acid and BHA on carotene-bleaching by extracts of vegetable tissues
Peroxidase activities are noted

Source	Zone diameters relative to soya control (100)				
	Carotene-bleaching activity				Peroxidase
	Control	Control at 44 h	Ascorbic acid	BHA	
Cytochrome *c*	61	161	0	0	—
Haemoglobin	112	—	0	0	—
Peroxidase	130	200	0	0	130
Seeds:					
Broad bean (*Vicia faba* L.)	81	92	81	69	0
Cucumber (*Cucumis sativus* L.)	70	90	0	0	0
Lentil (*Lens esculenta* Moench)	77	100	92	73	—
Lupin (dry) (*Lupinus* sp.)	79	90	57	57	0
Lupin (fresh) (*Lupinus* sp.)	114	150	82	82	108
Melon (*Citrullus* sp.)	54	62	0	0	0
Millet (*Setaria italica* L. Beauv.)	54	77	0	0	0
Pea (*Pisum arvense* L.)	80	104	85	69	—
Soya (*Glycine soja* Sieb. et Zucc.)	100	138	100	92	0
Wheat germ (*Triticum vulgare* Vill.)	125	—	81	70	—
Other tissues:					
Apple skin (*Pyrus malus* L.)	0	0	0	0	—
Bean leaves (*Vicia faba* L.)	0	0	0	0	96
Broom leaves (*Sarothamnus scoparius* L.)	0	0	0	0	97
Cabbage leaves (*Brassica oleracea* L. var. *acephala* DC.)	0	0	0	0	105
Cucumber pulp (*Cucumis sativus* L.)	58	77	0	0	0
Cucumber skin (*Cucumis sativus* L.)	115	162	0	0	96
Grass leaves (*Hierochloe odorata* L. Beauv.)	0	0	0	0	94
Laburnum leaves (*Laburnum anagyroides* Medic.)	69	77	0	0	109
Lettuce leaves (*Lactuca sativa* L.)	0	0	0	0	0
Lupin leaves (*Lupinus* sp.)	0	0	0	0	122
Lupin seed pod (*Lupinus* sp.)	121	157	50	50	79
Melon skin (*Citrullus* sp.)	0	177	0	0	109
Melon pulp (*Citrullus* sp.)	0	0	0	0	0
Pea leaves (*Pisum arvense* L.)	61	73	0	0	90
Potato skin (*Solanum tuberosum* L.)	+82(121)	—	82	71	91
Potato leaves (*Solanum tuberosum* L.)	0	0	0	0	105
Tomato skin (*Lycopersicum esculentum* Mill.)	125	—	0	0	120
Turnip skin (*Brassica rapa* L.)	122	—	0	0	109

0 = No definite zone
\+ = Inner and outer zones

to any vegetable tissue unless steps are taken to show that oxidation of unsaturated fats or coupled oxidation of carotenoids or other substances cannot be attributed to the ubiquitous haematins. This should be emphasised even more in instances where homogenates of tissue rather than, as here, water-soluble extracts are studied. Very few studies have been made on washed insoluble fractions of homogenates and present concepts of lipoxidase would have to change radically before the name could be applied to an insoluble enzyme.

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References

1. Blain, J. A., Patterson, J. E. D., & Pearce, M., *J. Sci. Fd Agric.*, 1968, **19**, 713
2. Rhee, K. A., & Watts, B. M., *J. Fd Sci.*, 1966, **31**, 664
3. Wooltorton, L. S. L., Jones, J. D., & Hulme, A. C., *Nature, Lond.*, 1965, **207**, 999
4. Dingle, J., Reid, W. W., & Solomons, G. L., *J. Sci. Fd Agric.*, 1953, **4**, 144
5. Blain, J. A., & Todd, J. P., *J. Sci. Fd Agric.*, 1958, **9**, 235
6. Tappel, A. L., 'Autoxidation and Antioxidants', (Lundberg, W. O., Ed.) 1961, Vol. I, p. 328 (New York: Interscience)
7. Boyd, D. H. J., & Adams, G. A., *Can. J. Biochem. Physiol.*, 1955, **33**, 191
8. Maier, V. P., & Tappel, A. L., *J. Am. Oil Chem. Soc.*, 1959, **36**, 8
9. Banks, A., *J. Soc. chem. Ind., Lond.*, 1944, **63**, 8
10. Blain, J. A., & Styles, E. C. C., *S.C.I. Monogr. No. 11*, 1961, p. 160 (London: Society of Chemical Industry)

ANALYSIS OF MINOR VOLATILE CONSTITUENTS OF WINE

By P. J. HARDY and E. H. RAMSHAW

The analysis of minor volatile constituents in alcoholic beverages presents difficulties because of the relatively high concentrations of a few higher alcohols. Volatile components were extracted from a freshly prepared Riesling white table wine using the solvent trichlorofluoromethane, and analysed by combined gas chromatography–mass spectrometry. The higher alcohols were then removed from the trichlorofluoromethane extracts by treatment with propylene glycol, and the remaining components, mainly esters, were concentrated further. The final concentrate, whose volume represented about 0·01% of that of the wine, was analysed again, and in all, over 40 components were identified. Some of the esters observed, including the mixed ester, 3-methylbutyl ethyl succinate, have not been reported previously.

Introduction

A large number of volatile acids, alcohols, and esters have been reported in wines since the advent of gas chromatography.[1,2] More than 20 organic acids, and a similar number of alcohols may be present in any wine, and the possible number of esters is therefore considerable. In all, some 50–60 esters have already been reported.[1] There is evidence that esters contribute positively to wine quality whereas higher alcohols may be detrimental.[3] The threshold levels for the detection of some esters are very low[4] and any meaningful analysis of volatile aroma in alcoholic beverages must therefore take into account the large number of esters present in low concentration.

The analysis of minor volatile constituents in wines is complicated by the presence of relatively large amounts of a few compounds such as water, ethyl-, propyl-, isobutyl-, 3-methylbutyl-, 2-methylbutyl-, hexyl- and 2-phenethyl-alcohols. The usual method of extracting volatiles from alcoholic beverages is by means of an organic solvent. To be of any use the solvent must not extract water or ethyl alcohol but must extract efficiently the compounds under investigation. The choice of solvent is thus of major importance. The efficiencies of extraction of alcohols, carbonyls and esters up to C_{12} from 10% aqueous ethyl alcohol using trichlorofluoromethane have been measured.[5] This solvent does not extract water or ethyl alcohol, but extracts alcohols, carbonyls and esters of C_5 and above with recoveries of between 70 and 100% in 17 h. The bulk of the content of higher alcohols can then be removed from the extract by treatment with propylene glycol,[5,6] allowing the remaining volatile components to be concentrated further.

Many of the esters which have been reported in alcoholic beverages have been identified on the basis of retention data only. Esters are a difficult class of compound to analyse by such methods since the retention times of esters of the same molecular weight but composed of different acid and alcohol moieties are often very similar. Most of these esters, can, however, be readily distinguished by mass spectrometric methods. This paper describes the application of trichlorofluoromethane to the extraction and concentration of volatile esters from a Riesling white table wine, and the subsequent analysis by combined gas chromatography–mass spectrometry.

Experimental

Preparation of wine

Grapes (*Vitis vinifera* L. cv. Rhine Riesling, syn White Riesling) were harvested from a vineyard at Springton, South Australia on 12 March, 1968. The grapes were crushed, the stems were removed, and the juice was separated from the skins using a Willmes pneumatic grape press. The soluble solids content of the juice was 16% (by refractometer), the titratable acidity 90 mequiv./l and the pH 3·2. The juice was clarified by means of pectic enzymes (0·05 ml 'Pectinol 59L'/l), and SO_2 was added to 100 ppm. The juice was filtered and inoculated with yeast (*Saccharomyces cerevisiae* No. 729 Epernay strain) a yeast used widely in Australian wineries,[7] and fermented to dryness at 15° for 13 days. After filtration and addition of 80 ppm SO_2, 1150 ml of the wine was extracted with trichlorofluoromethane in continuous downwards displacement extractors for 17 h, and the extract of volatile components was concentrated as described previously.[5] The final stages of the concentration were carried out in a small pointed test tube with an air condenser containing a nichrome wire spiral,[8] until the volume represented ~ 0·05% of the original wine, and no further solvent could be removed. 200 μl portions of this extract were treated further by shaking for 2 min with 2 ml propylene glycol and 2 ml trichlorofluoromethane. The trichlorofluoromethane layer was passed through a dry filter paper to remove traces of propylene glycol and concentrated as before. The final concentration thus obtained was approximately 10,000-fold. Samples (2 μl) of concentrated volatiles with and without propylene glycol treatment were used for analysis.

Analysis of wine extract

The details of the gas chromatography column and operating conditions are given in the legends to Figs 1 and 2. The column was operated in a combined gas chromatography–mass spectrometry apparatus[9] with the column effluent split 4 ml/min to a hydrogen flame ionisation detector and 3 ml/min to the mass spectrometer. Identification of components was made by comparison of mass spectral fragmentation patterns with published spectra[10] and confirmed by comparison of retention times with those of synthetic compounds.

Preparation of esters

A series of alkanoates from acetate to decanoate for methyl-, ethyl-, propyl-, butyl-, isobutyl-, 3-methylbutyl-, 2-methylbutyl-, pentyl-, hexyl- and 2-phenethyl-alcohols was prepared by incubating 10 μl of each acid, 0·2 ml of the alcohol and 0·1 ml Dowex 50 ion-exchange resin (in the hydrogen form) at 30° for 24 h. About 50 μl of each reaction mixture was then shaken with 2 ml trichlorofluoromethane and 2 ml propylene glycol to remove unreacted acid or alcohol. Succinate esters were prepared by incubating 0·1 g succinic acid with 0·1 ml 3-methylbutyl alcohol and 0·1 ml ethyl alcohol in the presence of Dowex 50 resin. After the esters had been shaken with propylene glycol and trichlorofluoromethane, analysis by gas chromatography showed three peaks, two of which had the same retention times as di-3-methylbutyl succinate and di-ethyl succinate and an intermediate peak which was assumed to be 3-methylbutyl ethyl succinate. Each series of esters was chromatographed together with the C_{2-10} alkanols, isobutyl-, 3-methylbutyl- and 2-phenethyl-alcohols in order to obtain retention data for each ester relative to each alcohol.

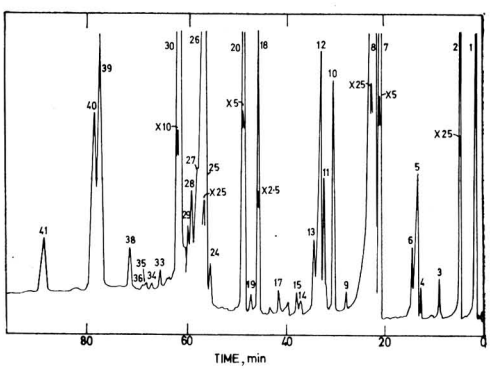

FIG. 1. *Chromatogram of extract of Riesling wine volatiles*

Column: Perkin-Elmer support-coated open tubular (50 ft × 0·02 in i.d.) coated with Ucon Oil LB 550X stationary phase. Helium flow rate 7 ml/min; temp 32°c for 3 min, then programmed at 2°c/min to 150°c

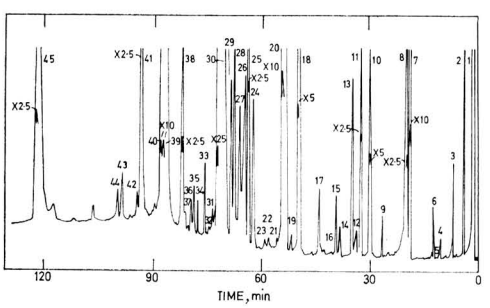

FIG. 2. *Chromatogram of Riesling wine volatiles after treatment with propylene glycol*

Column and operating conditions as for Fig. 1 except temp. 32°c at injection, programmed at 1·5°c/min to 165°c

Results and Discussion

Gas chromatograms of the volatiles in the trichlorofluoromethane extracts before and after propylene glycol treatment are shown in Figs 1 and 2 respectively. The identities of the components are given in Table I. Propylene glycol removed most of the isobutyl-, 3-methylbutyl-, hexyl- and 2-phenethyl-alcohols, allowing the remaining components to be concentrated a further 5-fold. About 20 compounds were identified by gas chromatography–mass spectrometry without propylene glycol treatment and this number was doubled after treatment.

The gas chromatogram of volatiles without propylene glycol treatment (Fig. 1) represents the approximate relative concentrations of compounds above C_5 in the original wine.[5] 3-Methylbutyl alcohol and 2-phenethyl alcohol were by far the major volatile compounds extracted. The predominant esters present were those of 3-methylbutyl-, 2-phenethyl-, ethyl-, hexyl- and isobutyl-alcohols, in descending order. In each case a series of esters was present including the octanoate,

TABLE I

Identities of peaks numbered 1–45 in Figs 1 and 2

Peak No.	Identity
1	Solvent
2	Ethyl acetate
3	Unknown, apparent mol. wt. 88, base peak 87
4	Isobutyl acetate
5	Isobutyl alcohol
6	Ethyl butyrate
7	3-Methylbutyl acetate
8	3-Methylbutyl alcohol
9	3-Methylbutyl propionate
10	Ethyl hexanoate
11	Hexyl acetate
12	Hexyl alcohol
13	3-Methylbutyl butyrate
14	Valerate ester
15	Butyl valerate
16	Propyl hexanoate
17	Isobutyl hexanoate, 3-methylbutyl valerate
18	Ethyl octanoate
19	Unknown, apparent mol. wt. 138, base peak 82
20	3-Methylbutyl hexanoate
21	Di-ethyl succinate
22	Propyl octanoate
23	Unknown
24	Isobutyl octanoate
25	2-Phenethyl acetate
26	2-Phenethyl alcohol
27	Hexyl hexanoate
28	Ethyl decanoate
29	Ethyl decenoate*
30	3-Methylbutyl octanoate
32	Heptyl hexanoate
33	3-Methylbutyl ethyl succinate
34	2-Phenethyl butyrate
35	Isobutyl decanoate
36	Isobutyl decenoate*
37	2-Phenethyl ester
38	Hexyl octanoate
39	3-Methylbutyl decanoate
40	3-Methylbutyl decenoate*
41	2-Phenethyl hexanoate
42	Di-3-methylbutyl succinate**
43	Hexyl decanoate
44	Hexyl decenoate*
45	2-Phenethyl octanoate

* From mass spectrum data only
** From retention data only

hexanoate, acetate, decanoate, decenoate and butyrate, in descending order of concentration. Some 2-methylbutyl alcohol and esters were probably also present but these have the same retention times as the corresponding 3-methylbutyl derivatives on the Ucon Oil column. The mass spectra of the esters isolated from the wine were, however, similar to published spectra of 3-methylbutyl alcohol and esters.[4,10] The identities of all the esters were confirmed by retention data except in the case of the decenoates where no decenoic acid was available. 9-Decenoic acid and esters have been found in wines[2,11,12] and the decenoate esters found here are therefore presumably 9-decenoates.

Many of the esters identified here have been reported in wines, albeit in many instances on the basis of retention data only. Di-ethyl succinate has been observed several times[2] but the mixed ester, 3-methylbutyl ethyl succinate has not been reported previously. This ester had a mass spectrum with a base peak at *m/e*: 101, and major peaks at *m/e*: 129 (71% of base peak), 71 (61%), 70 (57%), 43 (42%), 55 (40%). Other diagnostic peaks occurred at *m/e* 147 and 171. In view of the large numbers of alcohols in wine there is considerable scope for the formation of different mixed esters of succinic and other dicarboxylic acids. Some of the higher esters of isobutyl- and hexyl-alcohols are also reported here for the first time.

An advantage of trichlorofluoromethane in the analysis of esters in alcoholic beverages is the low efficiency of extraction of the lower organic acids, which reduces the likelihood of esterification in the extracts. Extracts of esters from wines, containing about 90% trichlorofluoromethane have been stored in sealed ampoules for periods of up to one year without alteration in composition as judged from the gas chromatographic traces. The methods described in this paper are now being used to study the volatiles in wines from different grape varieties and also the changes in volatiles which occur during wine maturation.

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References

1. Drawert, F., & Rapp, A., *Vitis*, 1966, **5**, 351
2. Webb, A. D., in 'Symposium on Foods – Chemistry and Physiology of Flavors' (eds Shultz, H. W., Day, E. A., & Libbey, L. M.) 1967 (Westport: Avi Publ. Co.)
3. Wagener, W. W. D., & Wagener, G. W. W., *S. Afr. J. agric. Sci.*, 1968, **11**, 469
4. Teranishi, R., Flath, R. A., Guadagni, D. G., Lundin, R. E., Mon, T. R., & Stevens, K. L., *J. agric. Fd Chem.*, 1966, **14**, 253
5. Hardy, P. J., *J. agric. Fd Chem.*, 1969, **17**, 656
6. Stevens, K. L., Bomben, J. L., & McFadden, W. H., *J. agric. Fd Chem.*, 1967, **15**, 378
7. Rankine, B. C., *J. Sci. Fd Agric.*, 1967, **18**, 583
8. Murray, K. E., & Stanley, G., *J. Chromat.*, 1968, **34**, 174
9. Stark, W., Smith, J. F., & Forss, D. A., *J. Dairy Res.*, 1967, **34**, 123
10. Cornu, A., & Massot, R., 'Compilation of Mass Spectral Data' (London: Heyden & Son Ltd.)
11. van Wyk, C. J., Kepner, R. E., & Webb, A. D., *J. Fd Sci.*, 1968, **32**, 664
12. van Wyk, C. J., Kepner, R. E., & Webb, A. D., *J. Fd Sci.*, 1968, **32**, 669

A ROLE FOR ACETATE IN THE DEVELOPMENT OF LOW-TEMPERATURE BREAKDOWN IN APPLES

By R. B. H. WILLS, K. J. SCOTT* and W. B. McGLASSON

Increased rates of water loss, which reduced susceptibility of Jonathan apples to breakdown, caused a reduction in the level of acetic acid in the fruit. Increasing the level of acid, by the injection of acetic acid into the fruit, increased the incidence of breakdown.

It is suggested that acetic acid could promote breakdown, and its removal as acetate esters or free acid would reduce the disorder.

Introduction

Low-temperature breakdown is a physiological disorder affecting many varieties of apples. Its development has been attributed to an accumulation of a toxic volatile compound in the fruit.[1-3]

Scott & Roberts[4] found that the effectiveness of many treatments which reduce breakdown could be explained in terms of increased water loss during storage. Wills[5] examined the volatiles given off by apples during cool storage, and found that, as the rate of water loss increased, the rates of loss of n-butyl, iso-amyl and n-hexyl acetates increased and the loss of the corresponding alcohols decreased. He suggested that the loss of acetate esters removes a factor which causes the disorder, and, although the levels of acetic acid were not measured, he further suggested that acetic acid was involved rather than the esters themselves.

This paper reports measurements of the rate of loss of acetic acid and the amount remaining in Jonathan apples which were evaporating water at different rates during storage at −1°, and the effect of injecting acetic acid into the fruit on the incidence of breakdown. Changes in esters and alcohols were also measured in the first study.

Experimental

Treatments

Jonathan apples were obtained from commercial orchards in New South Wales. Fruit from each orchard were systematically distributed into units of 20 fruit. All units were weighed before and after storage.

Fruit for the studies on changes in acid levels with different rates of water loss were obtained from two orchards (A and B). 20 units of fruit from orchard A were stored at −1° in air at about 40% relative humidity, and 20 units in air at about 95% relative humidity; two units were analysed for volatiles in the tissue before storage. Every two weeks the volatiles given off by two units from each storage treatment were measured. The fruit in these units were then cored and cut longitudinally into 12 sectors. About half a sector was taken from each apple, and these were combined to form a 100 g sample which was frozen in liquid nitrogen, enclosed in a sealed jar, and stored in dry ice until the tissue volatiles were extracted and analysed.

There were 22 units of fruit from orchard B, of which two units were examined for volatiles in the tissue before storage and 20 units were each stored at −1° in a box lined with a sheet of polyethylene film. In 12 units, a mixture of anhydrous calcium chloride (200 g) and vermiculite (500 g) was packed under the fruit to increase water loss. Every four weeks two units from each treatment were examined for the amount of acetic acid given off and the levels of tissue volatiles.

The fruit used in the injection studies were obtained from two orchards (C and D) with 20 units from orchard C and 16 units from orchard D. After the fruit had been stored at −1° for 1 week, four units from each orchard were injected with aqueous solutions (0·2 ml) containing 0, 16, 32 or 48 μmole of acetic acid. The solutions were injected into the core of the fruit with a hypodermic syringe. The remaining four units from orchard C were used as controls. All fruit were held at −1° for a further 20 weeks, when they were transferred to 20° and examined for breakdown after a further seven days.

Analysis of volatiles

The method described by Wills[5] was used to collect the volatiles given off by the fruit and to determine the esters, alcohols, and acetaldehyde. This involved collecting the volatiles in a cold trap and analysing the collected material by flame-ionisation gas chromatography using an FFAP column. Acetic acid was determined by injecting a 10 μl sample of the collected material onto a 3 ft column of phosphoric acid (4%) on Porapak Q (150–200 mesh).[6] The column temperature was 160° and the injector temperature was 205°. The carrier gas was nitrogen saturated with water at room temperature. The gas flow rates were: nitrogen 40 ml/min, hydrogen 20 ml/min and air 400 ml/min.

The volatiles in the frozen tissue were extracted by vacuum sublimation. The frozen fruit tissue was placed in a flask which was connected to a cold trap immersed in liquid nitrogen. The system was evacuated to a pressure less than 5 μm. After 24 h the vacuum was released, the volatile material collected in the cold trap was thawed, and an aliquot was analysed directly as described above.

Results

Effect of water loss on acetic acid concentrations

Changes in the rate of loss of acetic acid and the concentration in the fruit with respect to the rate of water loss and the time in store are shown in Fig. 1.

The concentration of acid in the fruit increased markedly to a maximum during the first few weeks the fruit was in cool storage. The loss of acid also showed a maximum value early in storage. Differences between the water loss treatments were apparent only in the early storage period when the level of acid was near its maximum. Fruit with the higher rate of water loss had a higher rate of loss of acetic acid and a lower concentration of acid in the fruit.

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Effect of water loss on ester and alcohol concentrations

The effect of water loss on the loss of esters and alcohols and the amounts remaining in the fruit are shown in Table I. The level of all compounds was lower in fruit with the higher water loss and, as expected from previous work,[5] the loss of ester was higher and the loss of alcohol was lower in fruit with the higher water loss.

Comparable fruit from orchards A and B were examined for breakdown after 20 weeks at −1°. Fruit from orchard B had 8% and 22% affected in the high and low water loss treatments respectively while no breakdown had yet developed in fruit from orchard A.

Effect of added acetic acid on the incidence of breakdown

The incidence of breakdown (percentage of affected fruits) in fruit injected with acetic acid was always significantly higher ($P < 0{\cdot}001$) than in fruit injected with water only (Fig. 2). The incidence of breakdown in fruit from orchard C that had been injected with water only was not significantly different from that in control fruit (5·3 and 6·0 ± 1·13%). The time-course development of the disorder in water- and acid-injected fruit is shown in Table II.

The appearance of the breakdown in fruit injected with acid was indistinguishable from that of control fruit stored at −1°. The concentrations of acid used in this study did not produce any visible damage other than typical breakdown, which appeared in the flesh away from the site of injection. There was no significant difference in weight loss between treatments.

Discussion

Treatments which reduce the incidence of breakdown are effective only when applied early in storage before the onset of the disorder, e.g. step-wise cooling[7] and warming during storage.[2] A reduction in the levels of acetic acid in the fruit by increased water loss was found only early in storage when the concentration of acid was near its maximum. During this period, the amount of acid in the fruit with high water loss was 20–25% less than that present in the fruit with low water loss.

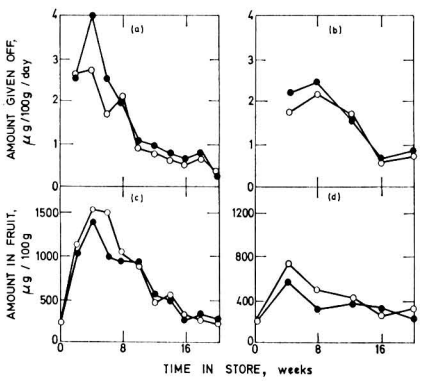

FIG. 1. *Effect of rates of water loss on changes in acetic acid levels of Jonathan apples during storage*
○ Low water loss; ● High water loss
(a) Orchard A, S.E. = ± 0·05 (n = 20); (b) Orchard B, S.E. = ± 0·08 (n = 10)
(c) S.E. = ± 64·0; (d) S.E. = ± 52·0

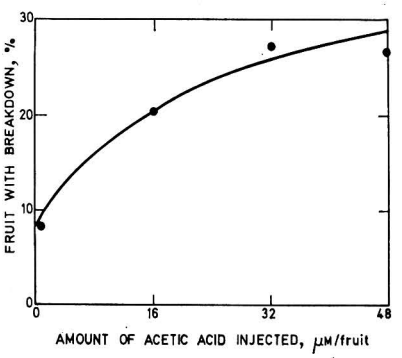

FIG. 2. *Effect of injections of acetic acid on the incidence of breakdown in Jonathan apples*
Points are means of eight units (two orchards × four units)

TABLE I
Levels of volatiles in Jonathan apples during storage

Compound	Amount in fruit*, μg/100 g tissue						Amount given off† μg/100 g fruit		
	Orchard A			Orchard B			Orchard A		
Water loss: low / high Rate of loss: (% wk.) 0·08 / 0·30	low	high	S.E.	low	high	S.E.	low	high	S.E.
n-Butyl acetate	205	172	± 5·7	925	846	±185·0	16·7	18·2	±0·79
Iso-amyl acetate	71	69	± 2·8	75	73	± 10·8	20·3	23·1	±1·02
n-Hexyl acetate	45	41	± 1·9	168	154	± 26·1	45·5	46·9	±1·97
n-Butanol	1820	1450	±51·6	1029	807	±124·8	40·6	33·6	±2·48
Iso-amyl alcohol	454	424	±18·7	60	46	± 6·1	44·8	39·5	±2·53
n-Hexanol	329	293	±11·0	147	127	± 14·6	82·0	41·3	±3·58
Ethanol	291	226	±23·2	500	281	±141·8	9·8	10·5	±1·94
Acetaldehyde	17	13	± 2·1	14	12	± 1·2	<1	<1	—

Standard errors were determined from pooled variances
* Means of all readings
† Total amount given off during storage was calculated from fortnightly readings

TABLE II

Changes in the level of breakdown during storage in Jonathan apples injected with water and acetic acid

Each value is from one unit of fruit from Orchard D

Time in store, weeks	Breakdown, %	
	Injected with water	Injected with 32 μM acid/fruit
5	0	0
11	5	5
16	10	15
19	5	30

Evidence that acetic acid is involved in the development of breakdown is supported by the observed increase in the disorder following injection of fruit with the acid. The amounts of acid injected were of the same order as those naturally present in stored fruit; e.g. the maximum concentration observed in these studies was 34 μmole/150 g fruit found in fruit from orchard A after four weeks in storage.

Acetic acid could be involved in the production of breakdown by direct injury or via further reactions. The latter alternative seems more likely, since the decrease in acid level occurred well before the appearance of the disorder. Also, fruit which were injected with acid showed no signs of breakdown until 11 weeks after injection.

The importance of acetic acid could be that, while it promotes breakdown, its concentration can be reduced by evolution of acetate ester or free acid and treatments which reduce the disorder could all act by increasing the loss of volatile acetate derivatives from the fruit.

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References

1. Overholser, E. L., Winkler, A. J., & Jacob, H. E., *Bull. Calif. agric. Exp. Stn, No. 370*, 1923, p. 28
2. Smith, W. H., *Nature, Lond.*, 1958, **181**, 275
3. Scott, K. J., & Roberts, E. A., *Aust. J. exp. Agric. Anim. Husb.*, 1967, **7**, 87
4. Scott, K. J., & Roberts, E. A., *Aust. J. exp. Agric. Anim. Husb.*, 1968, **8**, 377
5. Wills, R. B. H., *J. Sci. Fd Agric.*, 1968, **19**, 354
6. Mahadevan, V., & Stenroos, L., *Analyt. Chem.*, 1967, **39**, 1652
7. Trout, S. A., Tindale, G. B., & Huelin, F. E., *Bull. Coun. scient. ind. Res., Melb., No. 135*, 1940, p. 68

SUPERFICIAL SCALD, A FUNCTIONAL DISORDER OF STORED APPLES

V.*—Oxidation of α-farnesene and its inhibition by diphenylamine

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

α-Farnesene was oxidised to conjugated trienes with an absorbance maximum at 269 nm and the oxidation was inhibited by diphenylamine both in hexane solution and in the natural coating of stored apples. The results suggest that superficial scald is caused by the oxidation of α-farnesene and that the control of scald by diphenylamine is due to its antioxidant action. The mechanism of scald induction is discussed.

Introduction

In Part IV of this series[1] evidence was presented for a rôle of α-farnesene in superficial scald. More α-farnesene was found in apples picked early, which are more susceptible to scald than those picked later, and more in the scald-liable Granny Smith than in the scald-resistant Crofton variety. The movement of α-farnesene from the fruit to the oiled wraps provided further evidence. Since then Murray[2] has published the full account of his isolation and identification of α-farnesene from the natural coating of apples.

In this paper an account is given of the oxidation of α-farnesene both in hexane solution and in the natural coating of the fruit, and the inhibition of this oxidation by diphenylamine. This suggests oxidation products as the direct cause of scald.

Experimental

Solutions of (a) 6·48 mg α-farnesene, (b) 1·28 mg diphenylamine, and (c) 6·42 mg α-farnesene and 1·28 mg diphenylamine per litre were prepared in hexane (purified as previously described[1]). Of these solutions 10 ml aliquots were pipetted into tubes which were sealed to leave a 10 ml headspace above the solution. In half the tubes the headspace contained air and in the other half carbon dioxide. A second set of tubes was prepared containing solutions of slightly different concentration: (a) 5·92 mg α-farnesene, (b) 1·18 mg diphenylamine, and (c) 5·86 mg α-farnesene and 1·18 mg diphenylamine per litre. The tubes were stored without agitation at 20° and 37° and opened at intervals for analysis. The absorbance† was recorded in a 1 cm cell from 215–360 nm.

* Part IV: *J. Sci. Fd Agric.*, 1968, **19**, 297

† 'Absorbance' is now generally preferred to 'extinction', which was used in Part IV

The absorbance of the α-farnesene solution containing diphenylamine was corrected by subtracting the absorbance of the diphenylamine.

The Granny Smith and Crofton apples were picked, treated, stored, and analysed in 1964 as previously described.[1] The concentrations of α-farnesene reported earlier[1] were calculated from the absorbances of the Florisil-treated extracts. Evidence for oxidation is derived from the absorbance curves of the untreated extracts. The absorbance of extracts containing diphenylamine was corrected by subtracting the absorbance due to diphenylamine.

Results

Oxidation of α-farnesene in hexane solution

The absorbance curves of two α-farnesene solutions after 66 weeks at 20° in air are given in Fig. 1. The α-farnesene peaks at 232 nm were reduced from the original values of 0·74 and 0·70 to 0·53 and 0·36 respectively, and new peaks appeared at 269 nm with absorbances of 0·26 and 0·32. Freshly prepared α-farnesene solution gives a single peak at 232 nm with negligible absorbance above 260 nm. Associated with the 269 nm peak were shoulders at 260 and 280 nm. Anet (unpublished results) has isolated conjugated triene hydroperoxides from oxidation of α-farnesene with the major peak at 268–269 nm and subsidiary peaks at 259–260 and 279–281 nm.

The absorbance curves of α-farnesene solutions containing diphenylamine after 66 weeks at 20° in air are given in Fig. 2. The oxidation was largely inhibited by diphenylamine, as the absorbance at 232 nm had changed little from the original value and the absorbance above 260 nm was negligible and devoid of peaks.

The results for the whole experiment are summarised in Tables I and II, in which the absorbances at 232 and 269 nm are given during storage. The bottom half of each table gives the results for the second set of tubes with slightly lower initial concentrations of α-farnesene and diphenylamine. The α-farnesene solutions in air showed considerable falls in absorbance at 232 nm and increases at 269 nm. These changes were less in carbon dioxide but still appreciable. In view of other evidence that the changes involve oxidation of α-farnesene, it is probable that the carbon dioxide was not completely free of oxygen. The reaction of all the α-farnesene (0·29–0·32 μmole) in 10 ml of solution with an equimolar quantity of oxygen would require only 6·5–7·2 μl, about 0·07% of the headspace. The changes both in air and in carbon dioxide became negligible in the presence of diphenylamine.

These observations in hexane solution indicate that α-farnesene is oxidised to give a peak at 269 nm with shoulders or subsidiary peaks at 260 and 280 nm, and that the oxidation is inhibited by the antioxidant diphenylamine.

Oxidation of α-farnesene in the apple coating

The absorbance curves of coating extracts (each diluted 25 times) of the first picking of Granny Smith apples are given in Figs 3–5. In each figure the upper curve is derived from the untreated extract and the lower curve from the Florisil-treated extract. The lower curves are the same as those for pure solutions of α-farnesene.

The curves for extracts of untreated apples which were stored at 1° for 7 weeks are given in Fig. 3. Both the upper and lower curves show the α-farnesene peak at 232 nm but the upper curve exhibits a slight peak at 269 nm. After 22 weeks at 1° (Fig. 4) the peak at 269 nm with subsidiary peaks at 260 and 280 nm has become quite prominent. In contrast the diphenylamine-treated apples after 22 weeks at 1° (Fig. 5) showed no peaks in this region. These results suggest that the oxidation of α-farnesene and its inhibition by diphenylamine proceed in the natural coating of the apple as well as in hexane solution.

An estimate of conjugated triene oxidation products during storage was obtained by calculating the rise in absorbance from 262 to 269 nm for the extract of 24 apples or wraps in 500 ml and adjusting the values to a surface area of 3000 cm². The surface area of the samples varied from 2710 to 3050 cm². The values of this rise in absorbance and the scald scores for the first picking of Granny Smith apples are given in Table III.

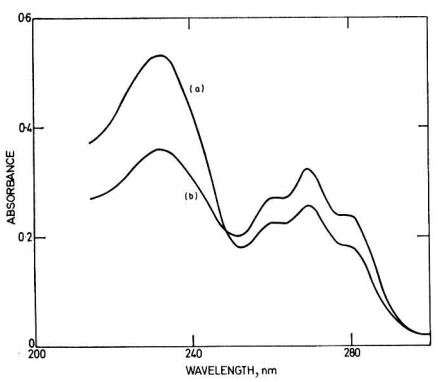

FIG. 1. *Absorbance curves of two α-farnesene solutions after 66 weeks at 20° C in air*
(a) From first set; (b) from second set

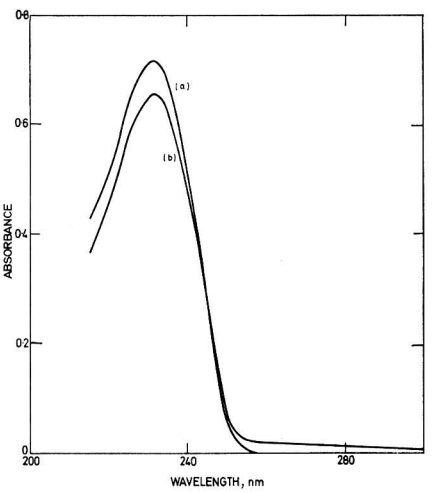

FIG. 2. *Absorbance curves of two α-farnesene solutions containing diphenylamine (corrected for absorbance of diphenylamine) after 66 weeks at 20°C in air*
(a) From first set; (b) from second set

TABLE I

Absorbance of α-farnesene solutions stored at 20°C

Weeks at 20°C	Absorbance after storage in							
	Air				Carbon dioxide			
	α-Farnesene		α-Farnesene, diphenylamine*		α-Farnesene		α-Farnesene, diphenylamine*	
	232 nm	269 nm	232 nm	269 nm	232 nm	269 nm	232 nm	269 nm
0					0·74	0·03	0·73	0·00
1	0·73	0·04	0·72	0·00	0·73	0·03	0·73	0·00
8	0·68	0·05	0·72	0·04	0·72	0·02	0·73	0·00
16	0·67	0·11	0·71	0·00	0·67	0·08	0·72	0·02
26	0·61	0·13	0·69	0·03	0·68	0·06	0·72	0·00
36	0·55	0·23	0·74	0·01	0·61	0·19	0·72	0·01
46	0·51	0·23	0·69	0·02	0·63	0·11	0·73	0·00
56	0·50	0·29	0·72	0·03	0·57	0·18	0·74	0·02
66	0·53	0·26	0·72	0·00	0·54	0·19	0·73	0·01
0					0·70	0·02	0·66	0·00
1	0·66	0·05	0·66	0·01	0·67	0·03	0·67	0·01
8	0·62	0·05	0·66	0·04	0·66	0·01	0·67	0·01
16	0·59	0·09	0·67	0·01	0·65	0·05	0·66	0·01
26	0·57	0·13			0·60	0·04	0·69	0·02
36	0·55	0·16	0·68	0·01	0·57	0·07	0·70	0·00
46	0·33	0·40	0·65	0·00	0·62	0·07	0·65	0·00
56	0·42	0·29	0·66	0·01	0·56	0·17	0·71	0·00
66	0·36	0·32	0·66	0·02	0·52	0·14	0·68	0·02

* Absorbance corrected by subtracting absorbance of diphenylamine

TABLE II

Absorbance of α-farnesene solutions stored at 37°C

Weeks at 37°C	Absorbance after storage in							
	Air				Carbon dioxide			
	α-Farnesene		α-Farnesene, diphenylamine*		α-Farnesene		α-Farnesene, diphenylamine*	
	232 nm	269 nm	232 nm	269 nm	232 nm	269 nm	232 nm	269 nm
0	0·74	0·03	0·73	0·00	0·71	0·03	0·72	0·00
5	0·60	0·13	0·71	0·01	0·67	0·05	0·71	0·01
10	0·54	0·17	0·70	0·01	0·61	0·10	0·71	0·01
15	0·40	0·27	0·69	0·02	0·62	0·09	0·70	0·01
20	0·31	0·28	0·65	0·05	0·55	0·14	0·72	0·00
25	0·27	0·22	0·68	0·02	0·40	0·22	0·71	0·01
30	0·27	0·20	0·66	0·03	0·33	0·23	0·71	0·02
0	0·69	0·04	0·67	0·00	0·67	0·03	0·68	0·01
5	0·60	0·08	0·64	0·01	0·63	0·04	0·65	0·00
10	0·51	0·15	0·65	0·01	0·61	0·05	0·64	0·00
15	0·35	0·26	0·65	0·01	0·54	0·10	0·65	0·01
20	0·32	0·26	0·61	0·05	0·52	0·11	0·67	0·01
25	0·24	0·21	0·64	0·01	0·50	0·12	0·66	0·01
30	0·24	0·19	0·66	0·01	0·41	0·17	0·65	0·02

* Absorbance corrected by subtracting absorbance of diphenylamine

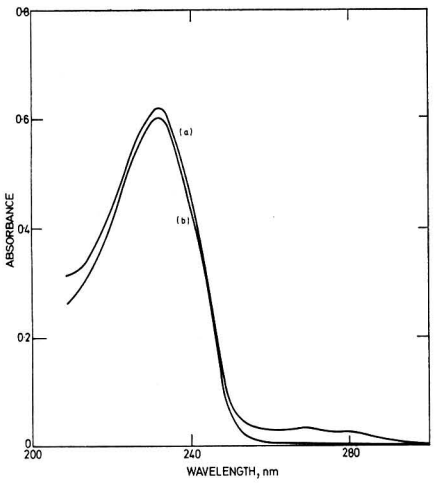

FIG. 3. *Absorbance curves of coating extract (diluted 25 times) of untreated Granny Smith apples, first pick, after 7 weeks at 1°C*
(a) Unpurified extract; (b) extract purified with Florisil

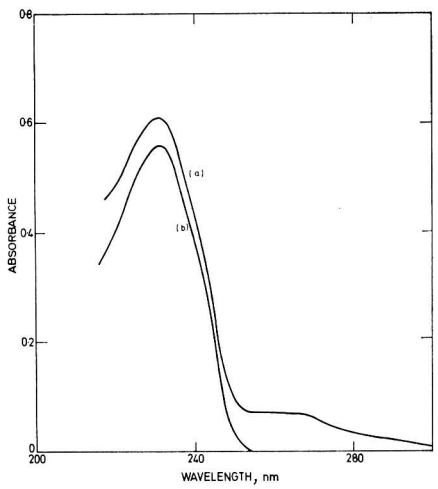

FIG. 4. *Absorbance curves of coating extract (diluted 25 times) of untreated Granny Smith apples, first pick, after 22 weeks at 1°C*
(a) Unpurified extract; (b) extract purified with Florisil

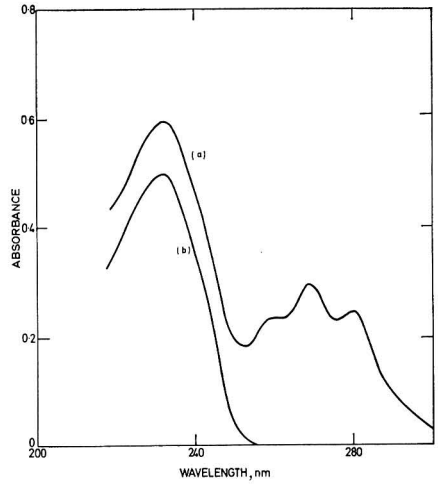

FIG. 5. *Absorbance curves of coating extract (diluted 25 times) of diphenylamine-treated Granny Smith apples, first pick, after 22 weeks at 1°C*
(a) Unpurified extract; (b) extract purified with Florisil

In both untreated and oil-wrapped apples the values reached a maximum after 17 weeks at 1° and then declined, indicating that the conjugated triene oxidation products undergo further reaction. In the oil wraps the conjugated trienes reached a maximum only after 27 weeks at 1° but earlier after removal to 20°. The data do not suggest how much conjugated triene in the oil wraps arose from oxidation *in situ* and how much from transfer from the fruit.

The conjugated triene oxidation products reached lower levels in the oil-wrapped fruit than in the untreated fruit. Their virtual absence from the diphenylamine-treated fruit is indicated by the low negative values, the peak at 269 nm being replaced by a gradual fall in absorbance with increasing wave length in this region. The incidence of superficial scald follows the same order, being less in the oil-wrapped than in the untreated apples and negligible in the diphenylamine-treated apples. These results provide evidence that the oxidation of α-farnesene is involved in scald. In the untreated and oil-wrapped apples scald first appeared just after the conjugated triene maximum during storage at 1° and before the maximum after removal to 20°.

The values for rise in absorbance from 262 to 269 nm as a measure of conjugated triene oxidation are only given for the natural coating and wraps of the 1st picking of Granny Smith apples. The values for the natural coating of the 2nd picking of Granny Smith apples and both pickings of Crofton apples were all negative, i.e. the rise was replaced by a fall in absorbance in this region. In most cases the fall was accentuated by a peak at about 258 nm, which was probably not due to a product of α-farnesene, as it was present in freshly picked Crofton apples with little α-farnesene. Scald was absent or only slight in the Crofton apples and the second picking of Granny Smith apples. The peak at 258 nm was also observed in extracts of the underlying cells, and interfered with the conjugated triene oxidation peaks even for the first picking of Granny Smith apples.

Discussion

Evidence has been obtained that α-farnesene is oxidised to conjugated triene products and that this oxidation is inhibited by diphenylamine both in hexane solution and in the natural coating of apples during storage. The severity of scald was positively correlated with the degree of conjugated triene oxidation and was negligible after treatment with diphenylamine. These results suggest that oxidation of α-farnesene is the cause of scald and that the control of scald by diphenylamine is due to its antioxidant action. This conclusion is supported by the fact that ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline), another inhibitor of scald,[3] is also an antioxidant.

TABLE III

Rise in absorbance from 262 to 269 nm* and scald score for stored Granny Smith apples, first picking

Weeks at 1°c	Weeks at 20°c	Rise in absorbance for				Scald score for		
		Untreated (coating)	Oil-wrapped (coating)	Oil-wrapped (wraps)	Diphenylamine-treated (coating)	Untreated	Oil-wrapped	Diphenylamine-treated
1	0	−0·05	−0·09	−0·02	−0·03	0	0	0
7		0·10	0·08	0·00	−0·08	0	0	0
12		1·01, 1·06	0·61	0·32	−0·07	0,0	0	0
17		1·83	0·97	0·62	−0·13, −0·10	0·04	0	0,0
22		1·66	0·89	0·83	−0·13	0·17	0·21	0
27		1·29	0·69	1·13	−0·03	1·12	0·58	0
32		0·98	0·41	0·97	−0·13	1·71	1·25	0
1	1	−0·07	−0·08	0·00	−0·08	0	0	0
7		0·20	0·15	1·02	−0·13	0	0	0
12		1·12	0·65, 0·82	1·37, 1·38	−0·17	0·87	0·37, 0·21	0
17		1·19	0·64	1·61	−0·08	2·67	1·21	0
22		0·92	0·38	1·23	−0·23	3·79	1·87	0·08
27		0·79	0·32	1·01	−0·22	4·33	2·21	0·08
32		0·63	0·16	0·73	−0·24	4·57	2·50	0

* Values for the whole sample of 24 apples or wraps in 500 ml. calculated from the values for the diluted extracts and adjusted to a surface area of 3000 cm^2

The oxidation of α-farnesene in the natural apple coating proceeds much more slowly than in a pure film, in which considerable oxidation was apparent in 4 hours at 20°.[2] The occurrence of natural antioxidants in the apple coating is indicated. The oxidation in hexane solution was also comparatively slow, and was probably limited by the solubility of oxygen and the diffusion of oxygen and α-farnesene in the unshaken solution.

Superficial scald has been shown by Bain[4] to involve the collapse and discoloration of hypodermal cells. Injury to the cells may occur as a result of the following sequence. The secretion of α-farnesene by the epidermis and hypodermis may be followed by oxidation, from which it is protected while in the living cell. The products of oxidation may then cause injury either by entering the cells or by polymerising to impermeable films which prevent gas exchange. The process would be accentuated if the oxidation of α-farnesene promoted simultaneous oxidation of the unsaturated fatty acids[5] of the coating.

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References

1. Huelin, F. E., & Coggiola, I. M., *J. Sci. Fd Agric.*, 1968, **19,** 297
2. Murray, K. E., *Aust. J. Chem.*, 1969, **22,** 197
3. Smock, R. M., *Proc. Am. Soc. hort. Sci.*, 1957, **69,** 91
4. Bain, J. M., *J. hort. Sci.*, 1956, **31,** 234
5. Davenport, J. B., *Aust. J. Chem.*, 1960, **13,** 411

NEW TROPICAL SEED OILS
III.*—Component acids of leguminous and other seed oils (continued)

By J. A. CORNELIUS, T. W. HAMMONDS, J. B. LEICESTER, J. K. NDABAHWEJI, D. A. ROSIE and G. G. SHONE

The fatty acid compositions are reported for seed oils of tropical origin.

Introduction

In previous papers,[1,2] the fatty acid composition of a number of tropical seed oils was reported. The fatty acid composition of a further 26 seed oils is now presented. They were examined in a collaborative programme undertaken to investigate the potential of little-known tropical seeds.

Experimental and Results

The seed oils were extracted at room temperature by grinding the seeds with light petroleum (b.p. 40–60°) and removing the solvent under reduced pressure. Methyl esters were prepared from the oils using 0·4 N sodium methoxide in methanol[3] or from the fatty acids using boron trifluoride in methanol reagent.[4]

In general the esters or the oils were examined by silica gel and silica gel–silver nitrate[5] thin-layer chromatography prior to gas chromatographic analysis. Infra-red spectra of the oils or their methyl esters were also obtained in some instances.

The methyl esters were separated by gas chromatography using packed columns coated with polyethylene glycol adipate and Apiezon L stationary phases, and identifications were tentatively made from the equivalent chain lengths obtained.

The presence of cyclopropenoid material was detected by the Halphen test[6] and cyclopropenoid contents were determined as sterculic acid by hydrobromic acid titration of the oil. For this determination, the oil was dissolved in benzene and titrated with a standard solution of hydrogen bromide in either acetic acid in benzene or glacial acetic acid alone.[7,8] Crystal violet in acetic acid was used as indicator. The percentage total fatty acids in oils containing cyclopropenoids was then derived from the composition of the methyl esters of the oil, determined by gas chromatography, excluding peaks due to cyclopropenoids and associated breakdown products. In the case of one cyclopropenoid-containing oil (from *Sterculia africana*), gas chromatography of mercaptan derivatives was used.[9]

The results are summarised in Table I.

Discussion

Where practicable, oil contents were determined on the separated kernels and, in the case of *Sacoglottis gabonensis*, on the seed coat, in preference to the whole seed. More than half the species yielded oil contents greater than 15%, but most of the Leguminosae seeds examined gave oil contents of less than 5%.

The presence of small amounts of higher fatty acids in *Glycine javanica*, *Kerstingiella geocarpa* and *Vigna dekindtiana* is characteristic of members of the Leguminosae; in common with other members of the Papilionaceae, *Pueraria phaseoloides*, *Centrosema pubescens*, *Glycine javanica*, and *Vigna dekindtiana* gave oils with high contents of linolenic acid.

Cyclopropenoid fatty acids were present in oils from all the Malvaceae, Bombacaceae and Sterculaceae species examined, from 34% in *Sterculia africana* down to trace amounts in *Urena lobata*.

Thespesia populnea seeds were found to possess pigment glands comparable to those present in cottonseed, and from which was isolated a substance giving a melting point (170°) and infra-red spectrum similar to gossypol (m.p. 178°). The presence of gossypol in the bark and flowers of this species has been reported recently by King & de Silva.[10]

The fat from *Rhopaloblaste hexandra* seed contains the varied range of saturated acids characteristic of the Palmae, but with a higher myristic acid content than usually found.

The presence of elaeostearic acid in the fat from *Parinari excelsa* is typical of other *Parinari* species.

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References

1. Gunstone, F. D., & Subbaro R., *Chem. Phys. Lipids*, 1967, **1**, 349
2. Gunstone, F. D., Taylor, G. M., Cornelius, J. A., & Hammonds, T. W., *J. Sci. Fd Agric.*, 1968, **19**, 706
3. Luddy, F. E., Barford, R. A., & Riemenechneider, R. W., *J. Am. Oil Chem. Soc.*, 1960, **37**, 447
5. Morris, L. J., *Chemy Ind.*, 1962, p. 1238
6. 'Cottonseed Oil Test', B.S. 684, 1958, p. 95 (London: British Standards Institution)
7. Feuge, R. O , Zarins, Z., White, J. L., & Holmes, R. L., *J. Am. Oil Chem. Soc.*, 1967, **44**, 548
8. Durbetaki, A. J., *Analyt. Chem.*, 1956, **28**, 2000
9. Raju, P. K., & Reiser, R., *Lipids*, 1966, **1**, 10
10. King, T. J., & de Silva, L. B., *Tetrahedron Lett.*, 1968, **3**, 261

* Part II: *J. Sci. Fd Agric.*, 1968, **19**, 706

TABLE I

Acid composition of seed oils

No.	Name	Source (see footnotes)	Oil content of seeds, %	Unsaponifiable content %	Component fatty acid composition, %								Other fatty acids
					16:0	16:1	18:0	18:1	18:2	18:3	20:0	Cyclopropenoid acids (as sterculic)	
	Palmae												
1.	*Rhopaloblaste hexandra*	S	8		20		2	6	13				12:0, 17%; 14:0, 42%
	Liliaceae												
2.	*Dracaena usambarensis*	R	3	<0·5	27	2	3	24	40	2			14:0, 2%
	Rosaceae												
3.	*Parinari excelsa*	Z	62*		8		6	28	11		tr		18:3, conj, 47%
	Leguminosae												
4.	*Acacia farnesiana*	T	2	2·9	12		3	21	64	tr	tr		12:0, 14:0, tr
5.	*A. nilotica*	R	3	2·5	17		9	26	48	tr	tr		14:0, tr
6.	*Bauhinia galpini*	R	21*	14·7	15		6	22	57				14:0, tr
7.	*B. violacea*	S	19		27		12	11	50	tr			
8.	*Hymenaea courbaril*	S	4	2·1	11		4	20	64		tr		14:0, tr
9.	*Centrosema pubescens*	T	3		8	tr	7	16	10	57	tr		12:1, 14:0, 20:1, 1%
10.	*Glycine javanica*	T	4		16		6	11	32	30	1		24:0, 3%, 14:0, 22:0, 1%
11.	*Kerstingiella geocarpa*	N	7		18		4	20	42	9	4		22:0, 3%
12.	*Pueraria phaseoloides*	T	1		10	1	2	28	21	33	tr		20:1, 1% 12:0, 12:1, 14:0, 22:0, 3%
13.	*Vigna dekindtiana*	T	1		22	tr	5	8	29	24	2		22:0 5%, 22:1 4%, 12:0,
	Humiriaceae												14:0, tr, post 20:0 to 24:0, tr
14.	*Sacoglottis gabonensis*		31†		50	1	2	25	22				
			21*		26	1	4	35	32	2			
	Simaroubaceae												
15.	*Soulamea soulameoides*	F	51*		13		13	41	32		1		
	Euphorbiaceae												
16.	*Uapaca kirkiana*	Z	22*		20	tr	8	18	47	5	tr		12:0, 14:0. tr
17.	*U. nitida*	Z	19*		13	tr	7	17	53	8	tr		12:0, 14:0, tr
	Malvaceae												
18.	*Hibiscus sabdariffa*	Th	16		19	1	2	27	46			5[a]	14:0. tr
	var. *altissima*												
19.	*Thespesia populnea*	F	15		29		2	16	44			8[a]	14:0, 1%
20.	*Urena lobata*	F	12	1·7	13	4	3	11	68			tr	14:0, tr
	Bombacaceae												
21.	*Adansonia digitata*	N	15		26		5	33	29			7[a]	14:0, tr
22.	*Ceiba pentandra*	F	25		22		tr	27	38			11[b]	
	Sterculiaceae												
23.	*Sterculia africana*	R	32*		17		3	17	27			34[b]	14:0, tr
	Passifloraceae												
24.	*Passiflora edulis*	F	25	2	11		2	14	73				14:0, tr
	Sapotaceae												
25.	*Chrysosphyllum albidum*	G	3*		14		4	52	27	2	1		14:0, tr
26.	*C. perpulchrum*	G	4*		20		9	39	31	1	tr		12:0, 14:0, tr

[a] HBr–acetic acid in benzene; [b] HBr in acetic acid
* Kernel
† seed coat
F = Fiji; G = Ghana; N = Nigeria; R = Rhodesia; S = Singapore; T = Tanzania; Th = Thailand; Z = Zambia

Common names of species listed in Table I

3 Rough skinned plum
4 Cassie flower, sweet acacia
5 Gum acacia
6 Lowveld bauhinia
11 Groundnut bean
14 Bitter bark tree
16 Wild loquat
18 Roselle
19 Portia
21 Baobab
22 Kapok
24 Passion fruit
25 White star apple
26 Monkey star apple

ANALYSIS OF FRUIT JUICE BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

II.*—Direct determination of several major and trace metals

By J. T. H. ROOS and W. J. PRICE

A method has been developed for the determination, by atomic absorption spectrophotometry, of calcium, magnesium, sodium, potassium, manganese and zinc in fruit juices. The metals may be determined by direct aspiration of the sample, suitably diluted with water, after removal of suspended matter by centrifugation. It is not necessary to ash the samples prior to analysis. Results are given for the analysis of different fruit juices, and figures are presented for the accuracy and reproducibility of the recommended procedures.

Introduction

The determination of iron and tin in fruit juice by atomic absorption spectrophotometry has been described in a previous paper.[1] The present paper presents results for the determination of calcium, magnesium, manganese, potassium, sodium and zinc by a procedure which is essentially an extension of that described previously.

Experimental

Apparatus and Reagents

This work was performed using a Unicam SP90 Atomic Absorption Spectrophotometer equipped with a lamp turret holding three hollow cathode lamps and a Unicam SP22 ln/log recorder. Air was supplied from a Unicam SP93 air compressor. Hollow cathode lamps were supplied by Pye Unicam Ltd., and a Griffin–Christ 'Universal Junior III' centrifuge was used throughout this investigation.

Metal ion stock solutions (100 mg/l) were prepared from analytical reagent grade materials dissolved, where necessary, in the minimum volume of 1 : 1 hydrochloric acid, and diluted to 1 l with de-ionised water.

Lanthanum chloride solution (5% wt./vol. of La^{3+}) was prepared by dissolving 58·6 g of atomic absorption grade lanthanum oxide in 500 ml of hydrochloric acid (20%) and making up to 1 l with de-ionised water.

Hydrochloric, nitric and perchloric acids were all of analytical reagent grade, and were used without further purification.

Preliminary investigation

Initially, a wet-oxidation procedure involving perchloric and nitric acids was used to prepare a solution of the sample for analysis. This procedure, although time-consuming, proceeded smoothly and resulted in a sample solution suitable for atomic absorption analysis. However, it was then established that wet oxidation of the sample was unnecessary because no significant differences in the atomic absorption response was obtained for any of the elements investigated, whether the organic matter in the sample was destroyed by ashing or whether a simple dilution of the centrifuged juice was aspirated directly (Table I).

It has already been shown that because of the relatively high viscosity of fruit juice, the sample should be diluted at least by a factor of five before being aspirated into the atomic absorption spectrophotometer.[1] The determination of manganese and zinc may be performed directly on such a solution provided that the original samples before dilution contained at least 2 ppm of these ions. For levels below about 2 ppm, these metals should be concentrated by extraction into an organic solvent before determination. Greater dilutions of fruit juice are normally required for the determination of calcium, magnesium, potassium and sodium. Table II shows the optimum concentration ranges for the determination of these elements by atomic absorption spectrophotometry, together with the dilutions of the original samples used for each determination.

In order to test the validity of the proposed method, extensive measurements were made of the recovery of added amounts of each of the elements to be determined. Recoveries obtained for calcium were found to be low by about 35% owing to interference from aluminium, phosphate, and/or silicate ions. Addition of lanthanum chloride solution to give an overall concentration of 0·5% La^{3+} (wt./vol.) was found to eliminate this interference completely.

TABLE I

Analysis of ashed and non-ashed samples

Element determined	Concentration found, ppm			
	Pineapple juice		Orange juice	
	Ashed	Non-ashed	Ashed	Non-ashed
Manganese	2·20, 2·20	2·09, 2·09	~0·3	~0·3
Zinc	3·2, 3·3	3·2, 3·1	5·8, 5·8	5·5, 5·5
Calcium	173, 171	173, 171	76, 76	76, 76
Sodium	8·6, 9·1	8·2, 8·4	28·4, 32·3	28·0, 29·5
Magnesium	238, 235	238, 239	148, 149	151, 153
Potassium	1310, 1290	1280, 1290	1090, 1080	1090, 1080

TABLE II

Optimum concentration ranges for the elements in an air–acetylene flame

Element	Optimum range, ppm	Approximate, levels in juice, ppm	Juice dilution
Manganese	1·6–16	5	5×
Zinc	1·2–12	5	5×
Calcium	3–30	100	10×
Sodium	1–10	20	10×
Magnesium	0·25–2·5	200	200×
Potassium	2–20	1000	200×

* Part I: *J. Sci. Fd Agric.*, 1969, **20**, 437

TABLE III
Recommended operating conditions

	Manganese	Zinc	Calcium	Magnesium	Sodium	Potassium
Wavelength, nm	279·5	213·9	422·7	285·2	589·0	766·5
Slitwidth, mm	0·15	0·1	0·08	0·1	0·08	0·08
Observation height, cm	1·0	1·0	0·8	1·0	1·0	1·0
Oxidant	Air	Air	Air	Air	Air	Air
Acetylene flow rate, ml/min	1000	1000	1200	1000	800	800
Scale expansion	None	None	None	None	None	× 2
Standard range, ppm	0–10	0–10	0–25	0–2·5	0–10	0–20

TABLE IV
Results for the analysis of fruit juices

Element	Pineapple juice				Orange juice			
	Mean natural concn, ppm	Added, ppm	Recovered, ppm	Coeff. var., %	Mean natural concn, ppm	Added, ppm	Recovered, ppm	Coeff. var., %
Manganese	12·0	2·5 5·0	2·5, 2·4 4·8, 4·8	3·8	0·3	2·5 5·0	2·5, 2·4 4·85, 4·95	3·5
Zinc	3·12	1·25 2·50	1·13, 1·18 2·38, 2·23	3·1	5·45	1·25 2·50	1·20, 1·20 2·45, 2·45	3·3
Calcium	82	100 200	98, 99 195, 190	0·8	85	100 200	101, 101 201, 201	1·4
Sodium	19·8	20 40	20·0, 20·0 40·2, 40·2	2·9	26·2	20 40	20·1, 19·1 39·4, 40·0	1·7
Magnesium	119	100 200	104, 102 206, 203	0·8	73·2	100 200	104, 105 206, 208	0·5
Potassium	1652	500 1000	462, 496 1014, 982	0·8	1238	500 1000	514, 510 980, 960	1·0

Procedure

Calibration standards were prepared for calcium (0–25 ppm in 0·5% lanthanum solution), magnesium (0–2·5 ppm), manganese (0–10 ppm), potassium (0–20 ppm), sodium (0–10 ppm) and zinc (0–10 ppm) by appropriately diluting the respective stock solutions. Sufficient hydrochloric acid was added to each solution to give 5% (by vol.) overall before diluting to the mark with water. Suitable dilutions of the fruit juice samples were prepared and the same amount of hydrochloric acid (and lanthanum solution in the case of calcium) was added as for the standard solutions. Portions of the sample solutions were centrifuged (filtering was less satisfactory) and the clear liquid was used for analysis.

The standard solutions and prepared sample solutions were aspirated using the instrumental settings shown in Table III, and the concentrations of the metals in the original samples were calculated.

Results and Discussion

The results obtained for the analysis of two different fruit juices (pineapple and orange) are given in Table IV. This table also includes recoveries obtained for the different elements and coefficients of variation calculated from the results of eleven replicate analyses, representing eleven different dilutions of the original sample, for each element in each fruit juice sample.

With few exceptions, individual recoveries lie between 96% and 104% of the expected values, indicating that the accuracy of the proposed procedure is equal to that normally expected from atomic absorption analysis. From Table IV it can be seen that the precision, too, is satisfactory. Except for the trace elements, zinc and manganese, coefficients of variation are of the order of 2% or better. It is therefore concluded that the accuracy of atomic absorption analysis for the determination of these elements in fruit juices has been proved.

The use of lanthanum (added as chloride) was found perfectly satisfactory for overcoming the interference (presumably because of phosphate ion in the samples) on calcium. This contrasts with the findings of Temperli *et al.*[2] who did not obtain satisfactory results with lanthanum or strontium.

Since prior ashing of the samples is not required, the proposed method is extremely rapid, needing only straight dilution and centrifugation of the sample material. This is of considerable importance when large numbers of samples are involved. The atomic absorption method thus exhibits a clear advantage over most colorimetric and spectrophotometric techniques as applied to this analysis.

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References

1. Price, W. J., & Roos, J. T. H., *J. Sci. Fd Agric.*, 1969, **20**, 437
2. Temperli, A. T., Joss, C., & Misteli, H., *Lebensm.-Wiss. Technol.*, 1968, **1**, 44

CHEMOTHERAPY OF FASCIOLIASIS

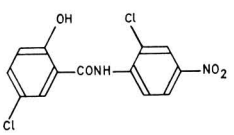
III.*—Amides derived from 4-cyano-2-iodo-6-nitrophenol (nitroxynil) and 2,6-di-iodo-4-nitrophenol (disophenol)

By BARBARA J. BROUGHTON, M. DAVIS and D. E. WRIGHT

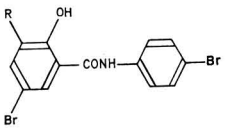
Amides have been prepared utilising 4-hydroxy-3-iodo-5-nitrobenzoic acid or 5-cyano-2-hydroxy-3-iodoaniline, the acid and amine respectively corresponding to 4-cyano-2-iodo-6-nitrophenol (nitroxynil). Analogous anilides were made from 3,5-di-iodo-4-hydroxybenzoic acid or 4-amino-2,6-di-iodophenol which bear a similar relationship to 2,6-di-iodo-4-nitrophenol (disophenol). None of the products was useful against *Fasciola hepatica* infections.

Introduction

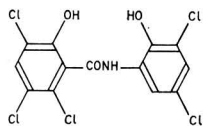
Many fasciolicides and cestodicides are benzanilides containing hydroxy groups in one or both rings *ortho* to the amide linkage. Among the most effective of these are yomesan[1] (I), the mixture[2] of the brominated salicylanilides (II and III), oxyclozanide[3] (IV), and clioxanide[4] (V).



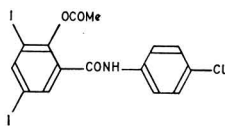
(I)



(II, R = Br)
(III, R = H)



(IV)



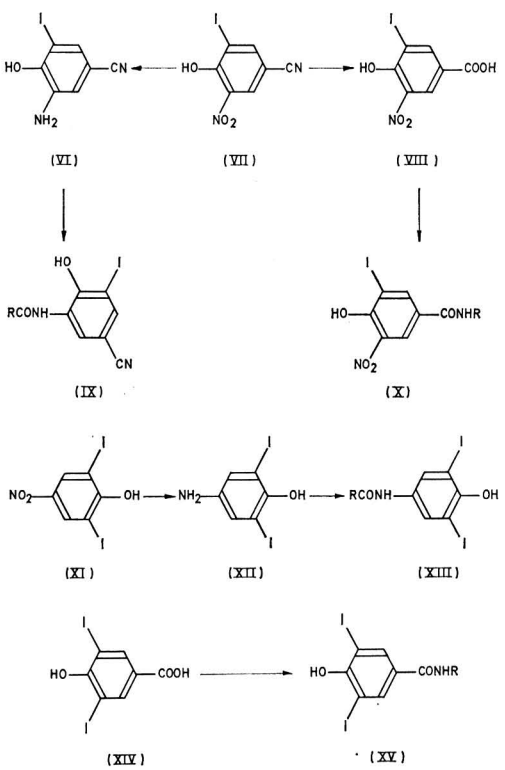
(V)

The high activity found for 4-cyano-2-iodo-6-nitrophenol[5] (nitroxynil) (VII) was encouraging and it was decided to incorporate structural features of this molecule into a series of benzanilides and certain related amides.

4-Hydroxy-3-iodo-5-nitrobenzoic acid[5] (VIII), obtained by hydrolysis of nitroxynil (VII), was condensed with a number of amines, representative of those with inherent fasciolicidal activity,[6] giving the amides (X) listed in Table I.

5-Cyano-2-hydroxy-3-iodoaniline[5] (VI), prepared by reduction of nitroxynil, was used as the amine moiety of similar anilides (Table II) formed from several chloro-, bromo-, and iodo-salicylic acids and related hydroxy aromatic acids. The corresponding anilide from 4-hydroxy-3-iodo-5-nitrobenzoic acid (VIII) is shown in Table I.

Since the anthelmintic 2,6-di-iodo-4-nitrophenol (disophenol) (XI) is also known to have some activity against liver fluke,[5] analogous anilides (XV) (Table III) and (XIII) (Table IV) were prepared using respectively 3,5-di-iodo-4-hydroxybenzoic acid (XIV) and 4-amino-2,6-di-iodophenol (XII).



(VI) (VII) (VIII)

(IX) (X)

(XI) (XII) (XIII)

(XIV) (XV)

Experimental

Condensations of the acid chlorides with the amines were carried out in benzene by the general methods given below. Certain condensations were effected with the acid in the presence of phosphorus trichloride and aluminium trichloride, a method previously used in the synthesis of polyhalogenosalicylanilides.[7]

The amides listed in Tables I and III were prepared by method A, and those in Tables II and IV by method A, B, or C as indicated.

* Part II: *J. Sci. Fd Agric.*, 1969, **20**, 748

TABLE I

Amides derived from 4-hydroxy-3-iodo-5-nitrobenzoic acid (X)

R	Yield, %	Cryst. from	M.p.,°C	Formula	Found, %		Required, %	
					N	I	N	I
5-cyano-2-hydroxy-3-iodophenyl	96	MeOH	267–269*	$C_{14}H_7I_2N_3O_5$	7·7	46·4	7·6	46·1
4-hydroxy-3,5-di-iodophenyl	89	Aq. $COMe_2$	291–292*	$C_{13}H_7I_3N_2O_5$	4·3	58·8	4·3	58·4
C_6H_4Br-*p*	85	MeOH	241·5–242·5	$C_{13}H_8BrIN_2O_4$	6·1	27·8	6·1	27·4
3-methoxy-4-5′-phenylpentyloxyphenyl	75†	MeOH	145–146	$C_{25}H_{25}IN_2O_6$	5·1	21·7	4·9	22·0
$C_6H_4.CH_2.C_6H_4$-*pp′*	63	MeOH	266–268*	$C_{27}H_{18}I_2N_4O_8$	6·9	32·2	7·2	32·5
C_6H_4-*o*	38	Aq. $NMe_2.CHO$	298–299*	$C_{20}H_{12}I_2N_4O_8$	7·9	36·4	8·1	36·8
pyrid-2-yl	75	MeOH	218–219*	$C_{12}H_8IN_3O_4$	10·6	32·6	10·9	33·0
6-methylbenzthiazol-2-yl	79	MeOH	295*	$C_{15}H_{10}IN_3O_4S$	8·9	28·1	9·2	27·9
n-$C_{18}H_{37}$	60	MeOH	121	$C_{25}H_{41}IN_2O_4$	5·2	22·6	5·0	22·6
2-hydroxy-4-nitrophenyl	71**	MeOH	253–255*	$C_{13}H_8IN_3O_7$	9·3	28·3	9·4	28·5
C_6H_4-*p*	30	Aq. $NMe_2.CHO$	>330	$C_{20}H_{12}I_2N_4O_8$	8·1	36·4	8·1	36·8
pr^i	48	MeOH	174–175	$C_{10}H_{11}IN_2O_4$	7·9	36·5	8·0	36·2
cyclohexyl	47	EtOH	213–214	$C_{13}H_{15}IN_2O_4$	7·2	32·5	7·2	32·5

* With decomposition
† From 1-(4-amino-2-methoxyphenoxy)-5-phenylpentane[10] ** In acetonitrile

TABLE II

Amides derived from 2-amino-4-cyano-6-iodophenol (IX)

R	Method	Yield, %	Cryst. from	M.p.,°C	Formula	Found, %		Required, %	
						N	I	N	I
3,5-dibromo-2-hydroxyphenyl	A	55	MeOH	260–261*	$C_{14}H_7Br_2IN_2O_3$	5·4	23·5	5·2	23·6
3,5-dichloro-2-hydroxyphenyl	B	22	MeOH	243–245*	$C_{14}H_7Cl_2IN_2O_3$	6·3	28·3	6·2	28·3
3,5-di-iodo-2-hydroxyphenyl	A	65	MeOH	266*	$C_{14}H_7I_3N_2O_3$	4·4	60·1	4·4	60·3
3,5-dibromo-4-hydroxyphenyl	A	25	Aq. MeOH	260*	$C_{14}H_7Br_2IN_2O_3$	5·6	23·5	5·2	23·6
3,5-dichloro-4-hydroxyphenyl	B	1	Aq. MeOH	248–249*	$C_{14}H_7Cl_2IN_2O_3$	6·0	27·8	6·2	28·3
2,4-dibromo-5-hydroxyphenyl	B	57	MeOH	227–229 (clears at 240)	$C_{14}H_7Br_2IN_2O_3$	5·2	23·3	5·2	23·6
5-bromo-2-hydroxyphenyl	B	34	MeOH	273–275*	$C_{14}H_8BrIN_2O_3$	6·0	27·7	6·1	27·7
3,4-dihydroxyphenyl	B	29	Aq. $COMe_2$	240–241*	$C_{14}H_9IN_2O_4$	6·9	32·0	7·1	32·0
n-$C_{17}H_{35}$	A†	37	MeOH	106–107	$C_{25}H_{39}IN_2O_2$	5·4	24·5	5·3	24·1
2-hydroxynaphth-3-yl	A	65	MeOH	252–254*	$C_{18}H_{11}IN_2O_3$	6·7	29·7	6·5	29·5
3,5-dibromo-2-acetoxyphenyl	B	69	Ethyl acetate	219–221	$C_{16}H_9Br_2IN_2O_4$	4·8	21·9	4·8	21·9

* With decomposition † In acetonitrile

TABLE III

Amides derived from 4-hydroxy-3,5-di-iodobenzoic acid (XV)

R	Yield, %	Cryst. from	M.p.,°C	Formula	Found, %		Required, %	
					N	I	N	I
5-cyano-2-hydroxy-3-iodophenyl	70†	MeOH	270*	$C_{14}H_7I_3N_2O_3$	4·2	59·9	4·4	60·3
4-hydroxy-3,5-di-iodophenyl	83	2N-NaOH/2N-HCl	282–283*	$C_{13}H_7I_4NO_3$	1·7	69·4	1·9	69·3
2-hydroxy-4-nitrophenyl	70†	MeOH	270–271*	$C_{13}H_8I_2N_2O_5$	5·6	47·8	5·3	48·2
3-methoxy-4,5′-phenylpentyloxyphenyl	67	Aqueous $COMe_2$	162–163·5*	$C_{25}H_{25}I_2NO_4$**	2·1	38·8	2·1	38·6

* With decomposition
† In acetonitrile
** Found: C, 45·7%; H, 3·9%. Required: C, 45·7%; H, 3·8%

TABLE IV
Amides derived from 4-amino-2,6-di-iodophenol (XIII)

R	Method	Yield, %	Cryst. from	M.p.,°c	Formula	Found, % N	Found, % I	Required, % N	Required, % I
5-iodo-3-nitrosalicylic acid	C	32	Aqueous 2-ethoxyethanol	228–230*	$C_{13}H_7I_3N_2O_5$	4·1	58·7	4·3	58·4
3-iodo-5-nitrosalicylic acid	C	39	MeOH	298–300*	$C_{13}H_7I_3N_2O_5$†	4·1	58·6	4·3	58·4
2,4-di-iodosalicylic acid	B	43	Aqueous $COMe_2$	220·5–222·5*	$C_{13}H_7I_4NO_3$	1·8	69·0	1·9	69·3
4-bromosalicylic acid	C	43	Aqueous MeOH	248–249·5*	$C_{13}H_8BrI_2NO_3$	2·3	45·0	2·5	45·3
salicylic acid	B	32	MeOH	251*	$C_{13}H_9I_2NO_3$	3·1	53·0	2·9	52·8
3,5-dibromosalicyloyl chloride	A	46	Aqueous EtOH	234–235*	$C_{13}H_7Br_2I_2NO_3$**	2·1	39·7	2·2	39·7

* With decomposition. † Found: C, 24·1%; H, 1·1%. Required: C, 24·0%; H, 1·1%. ** Found: C, 24·8%; H, 1·2%. Required: C, 24·4%; H, 1·1%.

5-Iodo-3-nitrosalicylic acid

A mixture of 2-cyano-4-iodo-6-nitrophenol[5] (51 g), sodium hydroxide (62·1 g) and water (555 ml) was heated under reflux for 7½ h. Excess hydrochloric acid was added to the cooled solution, and the acid separated as a yellow solid. Crystallisation from aqueous ethanol gave the acid (88%), m.p. 203–205° (Hübner,[8] m.p. 204°).

3-Iodo-5-nitrosalicylic acid

This compound was similarly prepared (77%) from 2-cyano-6-iodo-4-nitrophenol[5] and crystallised from aqueous ethanol It had m.p. 236–237° (Brennans & Prost[9] m.p. 228°).

4-Hydroxy-3-iodo-5-nitrobenzoyl chloride

A mixture of 4-hydroxy-3-iodo-5-nitrobenzoic acid[5] (53 g), thionyl chloride (53 ml) and anhydrous benzene (600 ml) was heated at 100° for 19 h. The solution was evaporated to dryness *in vacuo* and the residue was crystallised from a mixture of light petroleum (b.p. 60–80°) and benzene giving the acid chloride (89%), m.p. 73–75°. (Found: I, 38·5%; N, 4·4%. $C_7H_3ClINO_4$ requires I, 38·8%; N, 4·3%.)

3,5-Dibromo-5′-cyano-2,2′-dihydroxy-3′-iodobenzanilide (Method A)

(a) A solution of 3,5-dibromosalicyloyl chloride (59·2 g) in anhydrous benzene (1 litre) was slowly added to a stirred suspension of 2-amino-4-cyano-6-iodophenol (104 g, 2 equiv.) in anhydrous benzene (1 litre). The stirred mixture was heated at 100° for 18 h, and filtered. The solid was treated with boiling methanol (2 × 1 litre) to remove amine hydrochloride and unreacted amine, and the product was obtained as a fawn insoluble solid (55%), m.p. 260–261° (decomp.). Concentration of the pooled methanol extracts gave a further crop (9·0 g). After crystallisation from methanol, the anilide had m.p. 262–264° (decomp.).

(b) A solution of dicyclohexylcarbodiimide (2·06 g) in anhydrous tetrahydrofuran (10 ml), was slowly added, with stirring, to a mixture of 3,5-dibromosalicylic acid (2·95 g), 2-amino-4-cyano-6-iodophenol (2·6 g) and anhydrous tetrahydrofuran (30 ml). An exothermic reaction occurred, accompanied by separation of *N,N′*-dicyclohexylurea. The mixture was stirred at room temperature for 2 h and the urea (1·75 g, m.p. 229–230°) was filtered off. The filtrate was evaporated to dryness *in vacuo* and the residual gum dissolved in boiling methanol (50 ml). The solution was diluted with water and the solid which separated was ground with methanol. The insoluble material (1·4 g) m.p. 242–245° (decomp.), was dissolved in 2 N-sodium hydroxide and an excess of 2 N hydrochloric acid was added to the solution. The benzanilide (0·4 g, 7%) separated and was filtered off and washed with water and methanol; it had m.p. 259–260° (decomp.)

3,5-Dichloro-5′-cyano-2,2′-dihydroxy-3′-iodobenzanilide (Method B)

A mixture of 3,5-dichlorosalicylic acid (14·2 g), thionyl chloride (13·7 ml), anhydrous benzene (150 ml) and anhydrous pyridine (0·5 ml) was heated at 100° for 30 min. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in anhydrous benzene (200 ml). The solution was added to a stirred suspension of 2-amino-4-cyano-6-iodophenol (35·9 g, 2 equiv.) in anhydrous benzene (300 ml) and the mixture was stirred and heated at 100° for 18 h, then filtered. The solid was ground with methanol (200 ml) and recrystallised from boiling methanol, giving the benzanilide as pink needles (21%), m.p. 243–245° (decomp.).

2,4′-Dihydroxy-3′,5,5′-tri-iodo-3-nitrobenzanilide (Method C)

A mixture of 5-iodo-3-nitrosalicyclic acid (33 g), 4 amino-2,6-di-iodophenol (45 g, 2 moles), phosphorus trichloride (5·8 ml), aluminium trichloride (2 g) and anhydrous benzene (1·5 litre) was stirred and heated at 100° for 18 h. The mixture was filtered and the solid was ground with methanol, then with a mixture of methanol and N hydrochloric acid. The insoluble material was recrystallised from aqueous 2-ethoxyethanol when the anilide (32%) was obtained as a reddish brown solid, m.p. 229–232° (decomp.) with previous softening. It was further purified by dissolution in 2 N sodium hydroxide followed by precipitation with 2 N hydrochloric acid and trituration of the solid with methanol, yielding a pale yellow solid (11·0 g), m.p. 228–230° (decomp.).

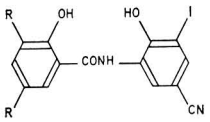
Biological Results and Discussion

Screening was carried out against *Fasciola hepatica* infections. The methods used have been previously reported.[6]*

3,5-Dibromo-5′-cyano-2,2′-dihydroxy-3′-iodobenzanilide (XVI*b*) was active at 0·5 g/kg post orally against mature flukes (2/2 infected rabbits cleared) and a similar result was obtained against the immature flukes. This compound was

*In the first paper in this series[6] dose levels were given erroneously as mg/kg. *They should have been g/kg.*

well tolerated in rabbits and further tests were carried out in sheep. However, at a dose of 0·2 g/kg administered either orally or subcutaneously, no worthwhile activity was demonstrated against the immature and mature infections.



(XVI *a*, R = Cl;
b, R = Br;
c, R = I)

Replacement of bromine by chlorine (**XVI*a***) caused no loss of activity against the mature infection in rabbits (2/2 cured at 0·37 g/kg) but the compound was inactive against the immature fluke (0/2 rabbits cured) at this dose level. The iodo-analogue (**XVIc**) cured 1/2 rabbits of the mature fluke at 0·5 g/kg but was also inactive against the immature form. The corresponding monobromo-analogue (**IX,** R = 5-bromo-2-hydroxyphenyl) was inactive against both the mature and immature infections in rabbits at a dose of 0·5 g/kg.

Two isomers of compound **XVI*b***, namely **IX** (R = 3,5-dibromo-4-hydroxyphenyl) and **IX** (R = 2,4-dibromo-5-hydroxyphenol) were inactive against both stages of the parasite at 0·5 g/kg.

None of the other compounds described had any activity against either form of the parasite.

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References

1. Gönnert, R., & Schraufstätter, E., *Arzneimittel-Forsch.*, 1960, **10,** 881
2. Boray, J. C., Happich, F. A., & Andrews, J. C., *Vet. Rec.*, 1965, **77,** 175
3. Froyd, G., *Br. vet. J.*, 1968, **124,** 116
4. Campbell, A., Martin, M. K., Farrington, K. J., Erdelyi, A., *et al.*, *Experientia*, 1967, **23,** 992
5. Davis, M., Rosenbaum, J., & Wright, D. E., *J. Sci. Fd Agric.*, 1969, **20,** 748
6. Collins, R. F., Davis, M., & Rosenbaum, J., *J. Sci. Fd Agric.*, 1969, **20,** 690
7. Lemaire, H., Schramm, C. H., & Cahn, A., *J. pharm. Sci.*, 1961, **50,** 831
8. Hübner, H., *Chem. Ber.*, 1879, **12,** 1347
9. Brennans, P., & Prost, C., *C. r. hebd. Séanc. Acad. Sci., Paris*, 1924, **178,** 1824
10. Collins, R. F., & Davis, M., *J. chem. Soc.*, 1961, p. 1863

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In the paper by Ssekaalo & Johnson, *J. Sci. Fd Agric.*, 1969, **20**

Page 582 left-hand column, line 10, *for* 15 ml *read* 1·5 ml

Fig. 2 caption *for* 5 µg/ml *read* 3 µg/ml

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

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ABSTRACTS

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1.—AGRICULTURE AND HORTICULTURE

Soils and Fertilisers

Soil Formation, Classification, Constituents

Peat formation. I. S. UTKINA and G. P. GRIGOR'EV (*Zh. prikl. Khim.*, 1969, **42** (1), 174–181. Russ., 23 ref.).—The pre-eminent rôle of microbiol. activity during the separation of peat-forming substances and the formation of humic acid does not fully explain the peat's behaviour under aerobic conditions. Self-oxidn. of org. substances by mol. O_2 according to the radical chain mechanism and the resulting processes occurring in the presence of phenol and aromatic amine inhibitors can explain the doubtful mechanism of peat formation. A series of reactions are suggested for the formation of humic acid. Haematomelanic acids are capable of changing to humic substances by reacting with the radicals of the oxidising compd. M. E. Traxton.

Quantitative determination of quartz in soils, sediments and rocks by pyrosulphate fusion and hydrofluosilicic acid treatment. S. L. CHAPMAN, J. K. SYERS and M. L. JACKSON (*Soil Sci.*, 1969, **107** (5), 348–355. 9 ref.).—Quartz and feldspar are separated from soil by fusion with $NaHSO_4$ followed by washing the residue with 3 N-HCl and boiling with 0·5 N-NaOH. The final residue is washed with 3 N-HCl and with water and treated with H_2SiF_6, stirring twice daily for 3 days to remove feldspars. After washing with water the residue is dried at 105° and checked for purity by *X*-ray diffusion. If feldspars or other minerals are present the H_2SiF_6 treatment is repeated before final weighing of the quartz.
A. G. Pollard.

Physical Properties of Soils

Relationship between different soil structure indices. B. S. SANDHU and D. R. BHUMBLA (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 129–133. 18 ref.).—Various amendments (gypsum, plant residues, including gums) were applied to a structurally deteriorated silty-clay loam. Air capacity (measured at 40 cm water tension) was highly positively correlated with hydraulic conductivity and with the % of water-stable aggregates. Clay was more effective than silt in forming aggregates. The summation (%) of aggregates > 0·25 mm was almost as reliable an index of soil aggregation as was the time consuming method of mean wt. dia.
A. G. Pollard.

Effects of two non-ionic surfactants on aggregate stability of soils. M. A. MUSTAFA and J. LETEY (*Soil Sci.*, 1969, **107** (5), 343–347. 3 ref.).—Two surfactants, 'Soil Penetrant' (alkyl polyoxyethylene ethanol and Aqua-gro (equal parts of polyoxyethylene ester and polyoxyethylene ether) were tested on two hydrophilic soils and one hydrophobic soil. As a measure of aggregate stability, a 50-g sample of soil (> 2 mm) was subjected to end-over-end shaking in surfactant soln. and the amount of clay + silt thus separated from the aggregates was determined; this test simulated the condition where surfactant is added to the irrigation water. In a second test soils were pretreated with surfactant and the sample was shaken with water. Data obtained indicate that the surfactants, whether applied before or in irrigation water, would lower the stability of aggregates in hydrophobic soils. The stability of the hydrophilic soils was either not affected or was increased by pretreatment with surfactants. A. G. Pollard.

One-dimensional, simultaneous motion of the aqueous phase and the solid phase of saturated and partially saturated porous media. P. A. C. RAATS and A. KLUTE (*Soil Sci.*, 1969, **107** (5), 329–333. 12 ref.).—Concepts of continuum mechanics of mixtures are used to develop a theory describing the transport of water in a deforming porous medium. Some applications to the movement of water in soil are discussed. A. G. Pollard.

Density effects in miscible displacement experiments. H. K. KRUPP and D. E. ELRICK (*Soil Sci.*, 1969, **107** (5), 372–380. 15 ref.).—Effects of small differences in density and viscosity on the displacement of one fluid by another in a porous medium, over a range of flow velocities typical of those in saturated soils, are examined, using the methods adopted by Elrick *et al.* (*Water Resources Res.*, 1966, **2**, 717). Displacement was influenced much more by differences in d than by differences in η. With decrease in flow rate the calc. dispersion coeff. afforded evidence of unstable effects. The need of a more comprehensive theoretical basis is indicated. A. G. Pollard.

Direction of water flow and potential difference. J. J. OERTLI (*Soil Sci.*, 1969, **107** (5), 315–322. 5 ref.).—A mathematical discussion leading to the view that water can move up the gradient of partial molal free energy and can gain free energy in the process. It is suggested that use of the term 'active water transport' for all 'up-hill' reactions with respect to free energy and with dependence on metabolism, would be a consistent and useful classification.
A. G. Pollard.

Preparation of sodium-saturated montmorillonites. I. BARSHAD (*Soil Sci.*, 1969, **107** (5), 337–342. 4 ref.).—Previous work has shown that to prepare an 'acid'-saturated montmorillonite, it is necessary first to saturate the clay with Na^+. Prepn. of Na-saturated bentonite by use of Calgon or of Na_2EDTA, or by preliminary saturation with Al^{3+} followed by titration with NaOH, is described. An appendix describes the methods used to determine exchangeable cations, total acidity and the Ca, Mg, Al, H, and OH contents of acid clays. A. G. Pollard.

Determination of soil cation exchange capacity by a simple semi-micro technique. D. R. KEENEY and J. M. BREMNER (*Soil Sci.*, 1969, **107** (5), 334–336. 11 ref.).—The soil sample is shaken with neutral N-NH_4OAc. The residual NH_4OAc is removed by leaching with N-NH_4NO_3, and the NH_4^+-N remaining in the soil sample is determined by distillation with 2 M-NaCl and MgO, after washing the soil with Pr^iOH to remove part of the NH_4NO_3. The NO_3^--N in the sample is then determined by distillation with Devarda's alloy and the salt retained in the sample is corrected by means of the NO_3^- value. Results obtained are comparable with those obtained by accepted methods, using NH_4^+ as the index cation.
A. G. Pollard.

Biological Aspects, Available Nutrients, Soil Analysis

Mathematical statistical methods for microbiological studies of soils. A. D. RAGUOTIS and YU. I. KRUOPIS (*Mikrobiologiya*, 1968, **37** (6), 1122–1127. Russ., 5 ref.).—It was shown that with variations in moisture content of woodland coverings and soils over the range 17–66%, their micro-organism distribution follows Poisson's law. Use of av. samples of soils or coverings, obtained by mixing separate samples taken from a single test area, was shown to be satisfactory for microbiological studies. The no. of main micro-organism groups indicated that considerable differences exist among the soils of the areas under study (Lithuanian fir and birch groves).
L. A. Haddock.

Correlation between microflora and the winter rye crop on peat-bog soils. F. P. VAVULO and E. N. VOROB'EVA (*Mikrobiologiya*, 1968, **37** (6), 1098–1103. Russ., 12 ref.).—Studies were made over 3 yr on 30 separate areas of peat-bog soils, and details are given of soil sampling for microbiological and chemical analysis. Data on crop yield and the no. of different micro-organism groups were mathematically evaluated by Rokitskii's method. Variations occurred between winter rye yield and the no. of groups of micro-organisms. The limits of rye yield were 15·57–43·35 centners/ha, while the total no. of micro-organisms lay between 41 and 1442 × 10^6 per g of dry soil. Correlation between groups of organisms and crop yield under identical culture conditions was determined primarily by the vegetative period conditions. When the latter were most favourable, there was a direct correlation between rye yield and bacteria assimilating available N, actinomycetes, oligonitrophyls and spore-forming bacteria. L. A. Haddock.

Antibacterial properties of actinomycetes from the soils of irrigated fields. A. M. BRYANSKAYA and L. D. KALYUZHNAYA (*Mikro-*

biologiya, 1968, **37** (6), 1091–1097. Russ., 12 ref.).—Samples of soils, irrigated for over 70 yr with sewage, were taken at various periods at a depth of 5–10 cm and were shown to contain a considerable number of actinomycetes antagonistic to Gram-positive and -negative, spore-forming, acid-stable, non-pathogenic and pathogenic organisms and yeast-like fungi. Irrigation with sewage increased almost twofold total actinomycetes content and the antagonist cultures. The most widely distributed were *A. lavendulae*, *A. olivaceous* and *A. griseus*. The antagonists were used to purify soil from pathogenic bacteria such as *Salmonella paratyphi* B, *S. typhimurium* and *Shigella paradysenteriae* Flex.

L. A. Haddock.

Biochemistry of the nitrifying micro-organisms. W. WALLACE and D. J. D. NICHOLAS (*Biol. Rev.*, 1969, **44** (3), 359–391. 145 ref.).— C. V.

Nitrogen in soil organo-mineral sedimentation fractions. F. W. CHICHESTER (*Soil Sci.*, 1969, **107** (5), 356–363. 14 ref.).—Loam surface soil and subsoil samples, ultrasonically dispersed in water, were submitted to particle size separation by centrifugal and wet sieving methods and the fractions analysed for total C and N and 'water'- (0·01 M-$CaCl_2$-), 6 N-H_2SO_4- and 0·5 N-Na pyrophosphate-extractable N. Total C and total N increased and the C/N ratios decreased with decrease in particle size. Mineralisation (%) of N under waterlogged conditions was greater in the finer fractions of both surface soil and subsoil. Differences in mineralisation among fractions are attributed to differences in the chemical forms of N compounds present. The amounts of N mineralised were highly correlated with the water-extractable portions of the total N.

A. G. Pollard.

Extraction of soil organic nitrogen by autoclaving in water. II. Kinetic approach to estimating the sodium hydroxide-distillable fraction. G. STANFORD (*Soil Sci.*, 1969, **107** (5), 323–328. 5 ref. Cf. *Idem. ibid.*, 1968, **106**, 345; 1969, **107**, 203).—Samples of soil devoid of NO_3^- or exchangeable NH_4^+-N were mixed with 0·01 M-$CaCl_2$ in centrifuge tubes and autoclaved (121° and 15 psi) for 16 h, and the extracts were separated; the process was repeated four times with the residual soil. Distillable N (NaOH) was determined in all extracts. The total N extracted in all the treatments represented 25–38% of the total N present in the soils and exceeded that obtained in an earlier study by 16-h extractions with boiling water. Distillable N was highly correlated with N mineralised anaerobically over a 13-week period. In all soils examined, the extraction of distillable N obeyed second-order kinetics. A. G. Pollard.

Effect of hydrolysis time and iron and aluminium removal on determination of amino compounds in soil. F. J. SOWDEN (*Soil Sci.*, 1969, **107** (5), 364–371. 20 ref.).—Well-decomposed muck soils were refluxed with 6 N-HCl (> 25 ml/g of soil) for various periods. The hydrolysates, after removal of HCl, were evaporated; the residues were wetted and dried several times and finally made up to vol. with 0·05 N-HCl for amino acid (I) determinations. Release of I by hydrolysis was generally complete in 16–24 h. With increase in the hydrolysis time, yields of threonine, serine and cystine diminished and those of valine, iso-leucine, lysine and ornithine increased; these changes were relatively small. Hexosamines diminished and free NH_3 increased as hydrolysis was prolonged. Most desalting methods (used prior to chromatographic analysis) caused losses of aspartic and glutamic acids, cystine and some basic I. Fe and Al interfered but could be extracted from hydrolysates by acetylacetone–chloroform with minimal loss of I.

A. G. Pollard.

Effect of flooding on soil reactions and mobilisation of various nutrients. I. C. MAHAPATRA (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 149–153. 14 ref.).—Samples of 20 cultivated surface soils were shaken with water (soil : water = 1 : 2) and changes in pH and water-sol. nutrients were determined. Nearly all the soils, initially acidic, showed pH $\geqslant$ 7·0 after waterlogging whereas in two alkaline soils the pH dropped to $\leqslant$ 7·0. Water-sol. Ca, Fe, Al and phosphate increased on waterlogging but citrate–dithionite-extractable Fe changed little. A. G. Pollard.

Measurement of ionic concentration gradient in soil near roots. E. FARR, L. V. VAIDYANATHAN and P. H. NYE (*Soil Sci.*, 1969, **107** (5), 385–391. 16 ref.).—Young straight-rooted onion seedlings were placed between two blocks of soil contained in Perspex cells; the parts of roots not actually between the blocks reached into distilled water. The plants were allowed to grow in moist air under standard conditions for 12 days, when the blocks of soil were separated and the plants were analysed for nutrients removed from the soil. The latter was frozen in liquid N_2 and sliced into thin sections in a low-temp. microtome; K^+, Ca^{2+} and H^+ exchangeable with Sr^{2+} were determined in successive slices, i.e., at increasing distances from the original positions of the plants. In an Upper Greensand soil a marked depletion of K^+ near the roots was shown; effects for H^+ and Ca^{2+} were less marked. Possible applications of the method are discussed. A. G. Pollard.

Studies on available phosphorus using the gradient elution method. P. SINGH and S. N. SAXENA (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 167–171. 7 ref.).—The uptake of P by pot-grown maize, after 75 days' growth in 21 different soils, is determined and compared with the amounts of P removed by various gradient elution methods from the original and from the rhizosphere soils.

A. G. Pollard.

Available phosphorus in relation to forms of soil phosphorus. O. P. SRIVASTAVA and A. N. PATHAK (*J. Indian Soc. Soil Sci.*, 1968. **16** (2) 103–109. 13 ref.).—A modification of the Chang and Jackson method (*Soil Sci.*, 1957, **84**, 133) for fractionating inorg. P was used to study the forms of P extracted by various reagents; correlation analyses were carried out between inorg. P fractions and the available P. The extractants used were: (*i*) water, (*ii*) 0·5 M-$NaHCO_3$ (pH 8·5), (*iii*) 0·03 N-NH_4F + 0·1 N-HCl, (*iv*) 0·03 N-NH_4F + 0·025 N-HCl and (*v*) 0·002 N-H_2SO_4 (pH 3). Besides the NH_4Cl-sol. P (adsorbed P), the most important fraction in controlling P extraction by methods (*ii*), (*iii*) and (*iv*) was the NH_4F-sol. P (Al-PO_4). Methods (*iv*) and (*v*) were highly correlated with Ca-PO_4 and (*ii*) with Fe-PO_4 also; all methods except (*iv*) were negatively correlated with clay content. A. G. Pollard.

Assessment of the phosphorus and sulphur status of subterranean clover pastures. I. Environmental and pasture responses. II. Soil tests. III. Plant tests. K. SPENCER and D. BOUMA, and D. V. MOYE (I, II) and E. J. DOWLING (I, III) (*Aust. J. exp. Agric. Anim. Husb.*, 1969, **9** (38), 310–319. 10 ref.; 320–328. 18 ref.; 329–340. 14 ref.).—I. Pasture growth responses were determined and relations between P and S status and environmental characteristics were investigated. P status was not related to rainfall, temp., site elevation or to soil type or pH. S status was related to rainfall for podzolic soils only, was poorly related to temp., and was not related to type of soil; there was a relationship with soil pH.

II. Pasture responses, measured in a large no. of field expt., were related to values obtained by several soil test procedures performed on soil samples from different depths. Bicarbonate-sol. P (Colwell) and P_2 phosphorus (Bray) tests gave results closely associated with response to P but Bray's P_1 values were not as good. Pasture on soils which had < 25 ppm of bicarbonate-extractable P responded best. Tests for S were not as reliable.

III. The relationships between several plant tests for P and S, and the yield responses to applied P and S for the clover component of the pasture, were studied. The results showed that the correlation between leaf area responses and the yield response to each element was not affected by differences in the supply of the other element. This is of possible advantage over ordinary plant analysis. M. T. Rawnsley.

Computer-oriented automation in chemical analysis of soils. J. KEAY (*Analyst, Lond.*, 1969, **94** (1121), 690–694. 1 ref.).—Equipment necessary to directly link the Technicon system of automated analysis to a small computer is discussed, and a system of commercial modules, permitting peak values to be sensed, digitised and transmitted to either a computer or a paper tape punch, is described. Regression methods are used to fit calibration graphs for accurate interpolation of unknown samples. Advantages are max. when several channels are used simultaneously for analyses, because one operator can supervise several spectrophotometers or flame photometers and the results from each channel can be processed in a few min. W. J. Baker.

Effect of organic matter on the determination of iron (II) in soils and rocks. G. PRUDEN and C. BLOOMFIELD (*Analyst. Lond.*, 1969, **94** (1121), 688–689. 7 ref.).—In the detn. of Fe^{II} in silicates, the Fe^{III} was reduced by org. matter when the sample was dissolved in aq. HF. When aq. $AlCl_3$ was used to extract labile Fe^{II} from waterlogged soils, there was partial dissoln. of Fe^{II} sulphide, so that yet more Fe was reduced by the liberated H_2S. This error is so serious that it is virtually impossible to determine Fe^{II} in rocks or labile Fe^{II} in soils when org. matter is present. W. J. Baker.

Use of *N,N,N',N'*-tetrakis(2-hydroxypropyl)ethylenediamine (THPED) for manganese determinations in soil extracts. S. S. KHANNA, M. L. MANCHANDA and S. L. CHOPRA (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 193–196. 7 ref.).—The THPED and the periodate methods are compared for determining available Mn in 22 soil samples. Data obtained by both methods were comparable

but the THPED method is faster and is not subject to interference by e.g., PO_4^{3-}. A. G. Pollard.

Zinc toxicity associated with galvanised wire netting. N. Collis-George and B. G. Davey (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 41–42. 14 ref.).—Retarded growth of wheat in two galvanised wire netting cages in N.S.W. was noticed. The Zn content of the soil was detd. at depths up to 6 in by atomic absorption spectrophotometry. The results showed much more Zn than in normal soils; atm. corrosion and electrochemical processes are thought to be responsible. Galvanised wire should be plastic painted in tests of this kind. M. T. Rawnsley.

Fertilisers

Effect of anions on the retention of manganese applied to soils. S. G. Misra and P. C. Mishra (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 173–178. 11 ref.).—Soil samples were equilibrated with aq. $MnSO_4$ (Mn 1100 ppm) and filtered. On the residual Mn (*R*) retained by the soil, the exchangeable Mn (*E*) was determined by N-NH_4OAc extraction. Reducible Mn still present in the soil, together with *E*, was taken to be available Mn in the soil residue. The effect of PO_4^{3-} was to lower retention of Mn; *E* was decreased by PO_4^{3-} in acidic soils but increased in an alkaline one. Slight increases in *R* occurred with oxalate in acidic soils; citrate decreased *R* in both acidic and alkaline soils. All these anions decreased reducible Mn. A. G. Pollard.

Losses of nitrogen from urine on soils from south-western Australia. E. R. Watson and P. Lapins (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 85–91. 16 ref.).—The N loss following urine application to soil in winter, spring and summer, the effect of rewetting the urine patch in summer, the effect of soil type on N loss and urine-N uptake by grass, and losses by volatilisation and leaching were studied, using two soils. Some expt. were carried out *in situ* and some in pots or with lysimeters. Results showed that grasses used nearly half the urine-N. Loss of N occurred much more on bare soil than on grass cover and the greatest loss occurred in high summer. Rewetting removed 80% of the urine after three wettings. The pH was noticeably increased over a long period. The losses involved partly explain the slow accumulation of N during grazing, and the value of grazing for this purpose is questioned. M. T. Rawnsley.

Correlation of soil test with response to the application to wheat. J. S. Grewal, N. S. Randhawa and D. R. Bhumbla (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 97–102. 12 ref.).—Comparison was made for 10 soils, of the amounts of Zn extractable by the acetate–dithizone (**I**) method, and those by other extractants, viz., 0·1 N-HCl, (**II**), N-NH_4OAc (pH 4·6), (**III**), 0·2% EDTA (**IV**) and 2 N-$MgCl_2$, (**V**); all were compared with the amounts of Zn taken up by wheat. Correlation coeff. with **I**-extractable Zn were significant at the 1% level whereas those with **III**, **IV** and **V**, were significant at the 5% level. Values for **II** were not significant. Soils containing < 0·55 ppm of **I**-extractable Zn, responded significantly to Zn application (optimum dose 10 ppm). A. G. Pollard.

Effect of association of organic matter with nitrogenous fertiliser on availability and uptake of plant nutrients and growth of the plant. I. Availability of plant nutrients. B. N. Naik and D. K. Ballal (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 155–160. 8 ref.).—To a medium black soil were added various amounts of org. matter (farmyard manure) (*FYM*) and $(NH_4)_2SO_4$, separately and in combination. Determinations of available nutrients, chemically and by Neubauer values, were made periodically. Addition of 0·5 and 1% of *FYM* increased available Fe, and that of 1 or 2% increased available P. Available K, Ca and Mg were increased by all applications of *FYM*. Neubauer values indicated that the association of mineral N and org. matter, restricted the uptake of mineral N but increased that of P, K, Ca and Mg by the plants at the 1 and 2% levels of *FYM*. A. G. Pollard.

Effect of lime on nitrogen availability in paddy soil. H. P. Borthakur and N. N. Mazumder (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 143–147. 7 ref.).—The av. mineral N content of these soils was increased by lime under low-moisture conditions; little change occurred in waterlogged soils. In general, the total N in waterlogged soils was lowered by liming but at low water content it was increased. Uptake of N by paddy seedlings was greater in limed soil regardless of moisture levels; it was significantly correlated with the mineral N level of soils, only under limed, waterlogged conditions. A. G. Pollard.

Building up soil structure by phosphate fertilisation of a legume in a crop rotation. II. Structure and organic carbon status of the soils after harvest of the succeeding non-leguminous crop (wheat). K. S. Pharande and T. D. Biswas (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 187–192. 6 ref.).—The org. C content and the structural condition of the soil produced by a legume crop, especially when a P fertiliser was used, deteriorated rapidly when the following crop was unmanured wheat. Comparison of a cowpea–wheat and a fallow–wheat rotation with treatments of farmyard manure (*FYM*) and/or superphosphate (*SP*) showed a greater build-up of org. matter with *FYM* but a more rapid decline after cropping. Application of *SP* restricted this loss of org. matter; this protective action seems to be associated with better maintenance of aggregate structure. Even when the amount of P supplied by *FYM* was the same as that provided by *SP*. the latter produced the higher wheat yields and better maintenance of soil structure. A. G. Pollard.

Returning wastes to the land. New rôle for agriculture. H. Bouwer (*J. Soil Wat. Conserv.*, 1968, **23** (5) 164–168. 22 ref.).—A review. C. V.

Production of ammonium nitrate including handling and safety. R. W. R. Carter and A. G. Roberts (*Fertil. Soc.*, 1969, 35 pp. 20 ref.).—The physical conditions for efficient prill formation, the prilling of NH_4NO_3, the design of prilling towers and optimum tower height are described. Attention is given to neutraliser, evaporator and prilling tower effluent and their disposal problems, and to NH_3 and NH_4NO_3 fume and tower top design. The safety factors in production, handling and storage are discussed. Product quality is discussed in terms of dust content, prill size and density, freedom from caking, and prill stability. J. C. T. N.

Responses of peach seedlings in sand culture to factorial combination of nitrogen, phosphorus and sheep manure. B. van den Ende and B. K. Taylor (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 234–238. 15 ref.).—Sheep manure was shown to be a good source of all essential elements for plant growth, although this was more pronounced when added P was omitted. Increasing N additions did not increase tree growth unless sheep manure was also present. M. T. Rawnsley.

Calcined rock phosphate as fertiliser for pasture and cereal production in Western Australia. M. G. Mason and W. J. Cox (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 99–104. 9 ref.).—'C' grade phosphate ore, when compared with superphosphate at equiv. levels, is not suitable, especially when a high initial P response is required. It could be used as a maintenance fertiliser where soil has a high S content or on loamy sands. M. T. Rawnsley.

Comparison of calcium/magnesium phosphate and superphosphate as phosphate fertilisers on acid soils. K. D. McLachlan and B. W. Norman (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 341–349. 19 ref.).—The effectiveness of thermal phosphate relative to superphosphate, in tests with subterranean clover, increased over the soil range, granitic, sedimentary and chocolate, with thermal phosphate being superior on krasnozem. This increase was associated with an increase in P sorption capacity, but this was not a completely direct relationship. Al, Fe and org. matter contents were also considered, free Fe oxide being most important. M. T. Rawnsley.

Determination of moisture in fertilisers and materials insoluble in methanol. A. M. Heslop, J. M. Skinner and A. C. Docherty (*Analyst, Lond.*, 1969, **94** (1121), 681–687. 1 ref.).—An apparatus is described that permits fine grinding of the sample (~ 20 g) and titration of the H_2O (0·3–0·7%) to be completed in one container. Grinding (with > 5% of original sample retained on No. 36 B.S.S.) was effected under MeOH by high speed cutter and the Fischer reagent was then added automatically, the end point being detd. by a sensing electrode. A compact and convenient assembly is achieved by use of transistorised components. Results for 0·4–1·9% of H_2O in oils, agree with those obtained by the Dean and Stark method. For 0·18–0·54% of H_2O in fertilisers, the coeff. of variation was 2·9% (42 detn.). W. J. Baker.

Alkalimetric quimociac method for phosphorus in fertilisers: collaborative study. P. R. Caudill (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 587–592. 4 ref.).—A modification to A.O.A.C. methods 2·023 and 2·025 is recommended. D. I. Rees.

Collaborative study on a modified sodium tetraphenylboron method for potassium in fertilisers. L. G. Hambleton (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 566–569. 5 ref.).— D. I. Rees.

Application of an automated coulometric titrator to the determination of water in phosphoric acids [and fertiliser solutions]. D. E. Jordan and J. L. Hoyt (*J. Ass. off. analyt. Chem.*, 1969, **52** (3),

569–577. 11 ref.).—The method involves the *in situ* coulometric generation of Karl Fischer reagent with automated end-point detection. It is applicable to systems which do not normally interfere with the Karl Fischer reagent. D. I. Rees.

Sampling bulk fertilisers in railroad cars and piles. C. W. Gehrke, W. L. Baker, G. F. Krause and C. H. Russell (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 592–599. 4 ref.).— D. I. Rees.

Composts. Fisons Fertilizers Ltd. (Inventor: S. B. Tree) (Br. Pat. 1,155,327, 21.9.66).—Compressed tablets of compost, containing peat, are obtained by mixing a peat, containing > 20% water by wt., with 5–20% (of the dry wt. of peat) of fertiliser and limestone (which may contain Mg), drying to a 3–20% water content and compressing the mixture in moulds. The product contains N 10–10,000, K (as K_2O) 10–10,000, and P 30–10,000 ppm. It swells on wetting and is suitable as a potting compost. S. D. Huggins.

Soil fertiliser. R. Bridwell Moxham (Br. Pat. 1,157,349, 12.12.66).—The fertiliser is obtained by anaerobic fermentation of a moist mixture of peat, soyabean, malt culms, seaweeds, dried blood, an inorg. Ca-containing material (lime or $CaCO_3$), *pulvis foenum-graeci* and gentian *pulv.* for 15 h to 4 days. The mixture contains 83–85% of peat; 3–4% S may be included prior to fermentation and a basic slag or granite dust before or after. S. D. Huggins.

Plant Physiology, Nutrition and Biochemistry

Light, Air and Water Relationships

Possible enhancement of photosynthesis by laser irradiation. C. Susskind and I. Garro (*Non-ioniz. Rad.*, 1969, **1** (1), 45–46. 9 ref.).—The effects of He–Ne laser irradiation and exposure to white light on photosynthesis in seaweed of *Ulva* sp., were compared. The ratio of O_2 evolution rate in laser radiation to that in white light was 2·75 at 0·06 mW and 32 at 0·6 mW. The reason for this greatly increased effect of laser radiation has not been explained. P. C. W.

Plant Nutrition and Metabolism

Effect of nitrogen, phosphorus and potassium nutrition levels on the drought resistance of ponderosa pine seedlings. D. L. Hauxwell (*Diss. Abstr. B*, 1967, **28** (3), 1746).—Seedlings grown in sand culture had lower drought resistance when 200–300 ppm N than when 25–200 ppm N was supplied. Highest rate of survival was achieved with N 56 and K 196 ppm. In the field, N, P and K fertilisers failed to stimulate growth. Examination of a N-P interaction for shoot wt. and max. root length, showed that N or K alone inhibited growth of these characteristics while fertilisation with both nutrients slightly stimulated shoot growth and root length growth. A. G. Pollard.

Mineral nutrition and metabolic processes in young plants. II. Effect of manganese nutrition on the levels of free amino acids in oats (*Avena sativa*) and wheat (*Triticium sativum*). F. Scheffer, E. Przemeck and V. K. Saolapurkar (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 135–141. 15 ref.).—Free amino acids in young wheat and oat plants, reacted very differently to variations in Mn nutrition and no definite relationships could be traced. In Mn-deficient oats, methionine synthesis was accelerated and simultaneous higher contents of glycine and total S corresponded with the conversion of serine to glycine, thus leaving one C-1 unit available for the methylation of homocysteine. A. G. Pollard.

Alkaloids in plant metabolism. K. Mothes (*Experientia*, 1969, **25** (3), 225–239. Ger., 69 ref.).—The biosynthesis of these compd., which usually serve no physiol. function, is discussed. P. P. R.

Rapid method for the selection of nodule bacteria capable of producing free pyridoxine. A. N. Pariĭskaya (*Mikrobiologiya*, 1968, **37** (6), 1128–1130. Russ., 3 ref.).—Full details are given for growing a culture of *Rhizobium meliloti* in a glucose–mineral medium on 10 cm plates. The amount of the vitamin pyridoxine was assessed by measuring the growth zones of *Saccharomycodes ludwigii*, used as an indicator (18–20 h, 28°, aeration conditions). The relative zone dia. for active strains of lucerne nodule bacteria were 24, 26, 28 and for inactive strains 16, 20 mm. Under optimum conditions, a zone of 30 mm dia. indicated a vitamin B_6 concn. of ~5 μg/ml. L. A. Haddock.

Introduction of *Rhizobium japonicum* to soil by seed inoculation of non-host legumes and cereals. A. Diatloff (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 357–360. 9 ref.).—The legumes and cereals were soyabean, lupin, field pea, oats, barley and wheat, and 2 g of culture were added to each 1 lb of seed. Encouraging results were obtained, esp. with soyabean, which supported earlier work. The difficulty of introducing rhizobia to soil when a fungicidal dressing is used, may be resolved by using this inoculation technique. M. T. Rawnsley.

Molybdenum as a necessary component of the nitrate reductase enzyme of the fodder beans *Vicia faba* L. Ya. V. Peïve and N. N. Ivanova (*Dokl. Akad. Nauk SSSR*, 1969, **184** (5), 1224–1227. Russ., 14 ref.).—The enzyme was prepared from the leaves of *Vicia faba* L. and dialysed against buffering soln. (pH 7·0) containing various metal-complexing agents such as KCN and 5-sulpho-8-mercaptoquinoline. The enzyme lost activity and sometimes became quite inactive. Addition of 1–50 μg of Mo did little to raise the activity. Mo was bound to protein in the enzyme and addition of Mo salts raised activity again only slightly; other metal additives were ineffective. Mo was dissociated completely from the protein only at high pH and ionic strength (0·1–0·2 M-NH_4OH, pH 11·0), conditions which led to dissociation of the quaternary structure of the protein. Mo probably participated in stabilising the quaternary structure of the apoenzyme nitrate reductase. L. A. Haddock.

Effect of phosphorus on symbiotic fixation of nitrogen by leguminous crops. N. K. Khare and M. M. Rai (*J. Indian Soc. Soil Sci.*, 1968, **16** (2), 111–114. 4 ref.).—Fixation of N by sunnhemp (**I**) soyabean (**II**), cowpea (**III**), dhaincha (*Sesbania aculeata*) (**IV**), moong (*Phaseolus aureus*) (**V**), and urd (*P. mungo*) (**VI**) was examined in relation to P supply. Excretion of N was highest in **IV** followed by **II**, **III** and **V**. In **I** and **VI** there was a negative N balance. The legumes, when treated with P, increased soil N content to extents decreasing in the order **II**, **IV**, **VI**, **III**, **V**, **I**. The total N fixed (plant and soil) symbiotically was more than double that of controls with no P applied. A. G. Pollard.

Radioactivation analysis of trace elements in rice: determination of halogen elements. Y. Kusaka H. Tsuji and M. Shinogi (*Radioisotopes, Tokyo*, 1968, **17** (3), 108–112. Jap.).—The technique used to study the distribution of halogen elements is described. Cl was concentrated in the seed, and Br and I in the leaf. The method used can be combined with one previously described (*ibid.*, 1967, **16** (10), 526) for the study of Mn, Mg, K, Na, Cu and Zn. (From Engl. summ.) C. V.

Colorimetric determination of protein in plant tissues. R. L. Mattoo (*Indian J. Biochem.*, 1969, **6** (2), 98–99. 5 ref.).—It was shown that the colorimetric phenol reagent method of estimating the protein content in lyophilised or acetonised prepn. of *Cuscuta campestris* Yunck., *C. indecora* Choisy and *Orobanche cernua* Loefl., gave results higher than those calculated on a total N basis. It was also shown that acetonisation or lyophilisation (and freezing in some cases) rendered the plant tissues unsuitable for protein determination with the phenol reagent. Enzyme inactivation or lack of solubility as a result of acetonisation may be due to the linkage of phenolics with enzyme protein. F. C. Sutton.

Fluorometric analysis of selenium in plants. O. E. Olson (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 627–634. 18 ref.).—A modification of Watkinson's method (*Analyt. Chem.*, 1966, **38**, 92) was sensitive to ~0·02 μg of Se but had a high reagent blank. D. I. Rees.

Germination, Growth Regulation, Senescence

Biogenisis of ethylene. L. W. Mapson (*Biol. Rev.*, 1969, **44** (2), 155–187. 96 ref.).—A review. C.V.

Effect of ethylene on tuber initiation in *Solanum tuberosum* L. A. H. Catchpole and J. Hillman (*Nature, Lond.*, 1969, **223** (5213), 1387. 3 ref.).—Treatment of sprouts with 50 vpm of C_2H_4 in moist air (250 ml/min) at 12° led to inhibition of extension growth of sprout and stolon, loss of positive geotropic response of the shoot and inhibition of root development. Most striking response was the swelling of all sub-apical regions of stolons, stem and axillary buds, but the C_2H_4-induced swellings gave no colour reaction with I_2 soln., suggesting that starch deposition may occur later. Similar responses were obtained with 2-chloroethylphosphonic acid. W. J. Baker.

Effects of polystyrene glycols on uptake of gibberellic acid and growth retardants by strawberry plants. C. G. Guttridge and H. M. Anderson (*Hort. Res.*, 1968, **8** (1), 83–88.)—Addition of

2–12·5% of polyethylene glycols (Carbowax grades 200–4000) to foliar sprays of the growth retardant, 'B-Nine' (Alar), on strawberry plants in the glasshouse, increased plant response in terms of no. of runners and petiole length. The response to gibberellic acid was slightly increased by Carbowax but that to the retardant CCC was unaffected. A. G. Pollard.

Growth stimulation of certain biological specimens subjected to vertical vibrations. N. L. DELONE, V. V. ANTIPOV, E. M. MOROZOVA *et al.* (*Cosmic Res.*, 1969, **6** (5), 663–666. Engl., 11 ref. [*Kosmich. Issled.*, 1969, **6** (5), 788–792. Russ.]).—The growth of the onion *Allium cepa* (var. Danilovskii) was stimulated by 10 h of exposure to vibrations of 70 Hz frequency. The gain in body wt. of mice was significantly increased (by max. 8%) after 1 h of exposure to 70 and 1500 Hz vibrations. P. C. W.

Other Aspects

No abstracts

Crops and Cropping

Field Crops

Grain quality in hybrids of *Avena sativa* L. and *A. byzantina* C. Koch. G. JENKINS (*J. agric. Sci., Camb.*, 1969, **72** (2), 311–317. 11 ref.).—Results of grain analyses for N, oil and husk are given for the F_1 and F_2 generations of four subject varieties, Condor (*A. sativa*), Sierra, Avon and Anita (*A. byzantina*), nine tester varieties and the 36 crosses between them. Relationships between improved grain quality and yielding capacity, and their significance for future breeding experiments are discussed. M. Long.

Comparison of the performance of homogeneous and heterogeneous barley populations. R. E. CLAY (*Diss. Abstr. B*, 1967, **28** (3), 1741).—The two types of barley varieties were compared mainly on the basis of stability of yield and disease resistance. A. G. Pollard.

Nutrio-physiology of crops grown in sand-dune soil. IV. Effect of nitrogen top-dressing at time of maximum tillering on growth of upland rice. M. YAMANOUCHI and S. SAWADA (*Sand-Dune Res.*, 1967–8, **14** (2), 22-31. Jap.).—Engl. summ. C. V.

Seed production from three annual species of Trifolium on sandy soils in the wheat belt of Western Australia. G. B. TAYLOR and R. C. ROSSITER (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 92–98. 8 ref.).—The varieties used were subterranean, cupped, and rose clovers. Subterranean was the earliest flowering but yields were not as high as with the others. The reason for the low yield was probably failure of inflorescences to set seed. Plant density did not generally affect seed yields. M. T. Rawnsley.

Effects of close grazing and cutting on the yield, persistence and nitrogen content of four tropical legumes with Rhodes grass at Samford, south-eastern Queensland. P. C. WHITEMAN (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 287–294. 7 ref.).—*Phaseolus atropurpureus* cv. Siratro, *Lotononis bainesii*, *Glycine javanica* cv. Cooper and *Desmodium uncinatum* cv. Silverleaf were grown with Rhodes grass (*Chloris gayana*), and the effects of defoliation by grazing sheep and of cutting at 2 in were studied. Yields of all the legumes steadily declined, and effects of grazing and cutting were the same. *L. bainesii* had a different growth pattern, having a peak early in the expt. then almost disappearing. Grazing reduced survival more than cutting, *G. javanica* surviving best. White clover settled in grazed and cut plots. The behaviour of *L. bainesii* is discussed. M. T. Rawnsley.

Horticultural Crops

Production of polyploidy lettuce by colchinine treatment. T. HIRAOKA (*Bull. Kanagawa Hort. Expt. Stn*, 1967, No. 15, 59-64. Jap., 16 ref.).—Seeds were treated with 0·05, 0·1 and 0·5% solutions at 10 and 25° for 24 h, then washed and planted; 10° treatment was more effective in inducing tetraploidy than at 25°. The characteristics of these tetraploids are described. (From Engl. summ.) C. V.

Relationship between seed exudation and field emergence in peas and French beans. S. MATTHEWS and W. T. BRADNOCK (*Hort. Res.*, 1968, **8** (1), 89-93. 12 ref.).—A negative correlation was established between field emergence of the seeds and the rate at which seed electrolytes were released to steep-water. In the case of peas, the exudation of sol. carbohydrates was similarly related to the rate of emergence. A. G. Pollard.

Methods of evaluating the ripening of pods of string beans. P. VERGNIAUD (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (5), 348–351. Fr., 3 ref.).—Two methods of detn. of optimum date for mechanical harvesting are (*i*) screening the pods; max. crop yield is not always coincident with max. % of pods passing an 8–10·5 mm screen (most favoured market quality); this max. % is usually reached when the % of 6–8 mm and > 10 mm pods are equal, and (*ii*) detn. of wt. of ovules/wt. of empty pod; this serves as control of ovule size during ripening. Results for varieties grown in the Lower Rhône valley are discussed briefly. W. J. Baker.

Plantation Crops

Nitrogen and phosphorus inter-relationship in the fertilisation of sugar-cane in some North-Indian soils. S. C. SRIVASTAVA and M. P. AGRAWAL (*J. Indian Soc. Soil Sci.*, 1968, **16**, 161–165. 3 ref.).—Yields of cane were only slightly increased by heavy applications of either N or P. With heavy N treatment, graduated supplements of P produced progressively increasing yields in most cases. An explanation is based on the P-fixing capacity of the soils. A. G. Pollard.

Effects of witholding water from the newly planted tobacco crop in north Queensland. J. M. HOPKINSON (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 221–227. 5 ref.).—Although previous workers have reported that the delay makes a stronger crop, this work shows that this is not so, and it could even be harmful. M. T. Rawnsley.

Forest Crops

Influence of calcium saturation of sphagnum peat on the rooting of five woody species. J. L. PAUL and A. T. LEISER (*Hort. Res.*, 1968, **8** (1), 41–50. 12 ref.).—Rooting of cuttings in samples of the peat, varying from 2·7 to 88·5% in exchangeable Ca saturation, was examined. In some cases the no. of roots per cutting was related to the Ca-level of the peat. A. G. Pollard.

Use of beta rays in determining wood properties. I. Measurement of wood density and interpretation of results. II. Measuring earlywood and latewood. III. Summarising radial variations in wood density. IV. Estimating the thickness of tracheid cell walls. V. Measuring resin content. J. M. HARRIS (*N.Z. Jl Sci.*, 1969, **12** (2), 395–408. 9 ref.; 409–418. 11 ref.; 419–431. 8 ref.; 432–442. 16 ref.; 443–451. 7 ref.).—I. A densitometer using a ^{90}Sr source was used to determine wood *d*, and a graphical solution which provided first order correction of mean *d*, estimated by linear integration of the densitometer records, is described. Allowance for the width of the beam of β particles and for when a growth layer is curved or not properly aligned in the plane of the beam is essential.

II. Mean *d*, plus some measure of *d* variation within the annual growth layers, and a record of max. contrast in *d* (e.g., between latewood and the succeeding earlywood in conifers) are sufficiently comprehensive to correlate with other wood properties.

III. Mean, min. (earlywood) and max. (latewood) *d* were plotted together with the mid-point. The relative positions of the mean and mid-point values indicate latewood development at any point and the total range of *d* within each growth layer is also easily seen.

IV. Thickness of tracheid cell walls in *Pinus radiata* was estimated from measurements of wood *d*, tracheid dia. and cell-wall *d*.

V. The densitometer enabled local concn. of resin, such as occur in latewood bands of pine heartwood, to be located and measured very accurately by measuring wood *d* before and after extraction with solvents. E. G. Brickell.

Method for controlling plant growth. ALLIED CHEMICAL CORP. (Br. Pat. 1,157,298, 24.4.67. U.S., 25.4.66).—The use of perhalogenated acetones (**I**) and their hydrates for the control of growth of sprouts on plants, e.g., potatoes, is claimed. **I** are of formula $X_3C{\cdot}CO{\cdot}C(X_2)F$ where X is F or Cl, e.g., $F_3C{\cdot}CO{\cdot}CF_3{\cdot}3H_2O$. They are applied to the upper parts of the crop plant before harvesting. S. S. Chissick.

Animal Husbandry

Feedstuffs

Possibility for increasing fodder yeast yield from molasses at the expense of adding growth substances. G. F. Soshko, M. A. Parkhomchuk and A. G. Zabrodsky (*Pishch. Tekhnol.*, 1968, [6 (67)], 56. Russ.).— P. P. R.

Evaluation of urea, biuret, urea phosphate and uric acid as NPN sources for cattle. R. R. Oltjen, L. L. Slyter, A. S. Kozak and E. E. Williams, jun. (*J. Nutr.*, 1968, **94** (2), 193–202. 29 ref.).— C. V.

Effects of grazing intensity on herbage consumption and animal production. III. Dairy cows grazed at two intensities on clean or contaminated pasture. J. F. D Greenhalgh and G. W. Reid (*J. agric. Sci., Camb.*, 1969, **72** (2), 223–228. 11 ref).—The fouling of pasture (by intensive grazing) leads to appreciable wastage of herbage and to reduced herbage consumption (*HC*) by cows. Although the relative affects on *HC* and milk production of varying grazing intensity (*GI*) appear to be the same for fouled and for clean pastures, the production per animal at high *GI* on clean pasture is equalled on fouled pasture only when the *GI* is reduced. M. Long.

Dry-matter intake and live-weight gain of cattle and sheep offered different grass varieties with and without clover. D. G. Miles, R. J. K. Walters and E. M. Evans (*Anim. Prod.*, 1969, **11** (1), 19–28. 28 ref.).—Single variety swards of S.24 perennial ryegrass, S.22 Italian ryegrass, S.37 cocksfoot and S.51 timothy were established and grazed intermittently by sheep. Wide differences were demonstrated in feed intake and live-wt. gain between swards. S.37 gave consistently good and S.51 consistently poor results when grazed at similar levels of digestibility. Clover supplementation often markedly improved feed intake and animal performance. M. Long.

Effect of level of feeding on digestibility of diets for sheep and cattle. J. D. Leaver, R. C. Campling and W. Holmes (*Anim. Prod.*, 1969, **11** (1), 11–18. 21 ref.).—The org. matter digestibility of a diet (hay and concentrate 1 : 1) to castrated male sheep fell linearly from 74·4 to 68·6% on increasing the dry matter intake from 600 to 1400 g. A curvilinear decline was obtained with a hay to concentrate ratio of 1 : 4. Crude fibre digestibility also decreased as food intake increased. On a metabolic live-wt. basis (kg $W^{0.73}$) lactating cows ate 34% more org. matter than did dry cows when fed long dried grass, offered *ad lib*. Sheep fed the same hay consumed only 54% of the dry-cow intake. The org. matter digestibility for the dry cows was 1·9 units higher than that of the lactating cows, and that for sheep at *ad lib* levels of intake was 5·6 units lower than that of the dry cows. M. Long.

Effect of chemical curing with paraquat on the intake and digestibility of Phalaris pasture. B. Romberg, G. R. Pearce and D. E. Tribe (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 71–73. 4 ref.).—The exptl. pasture comprised 70% *Phalaris tuberosa*, 20% *Bromus* spp., 5% *Lolium perenne* and 5% *Trifolium subterraneum*, and was sprayed with 40 fl. oz/acre at early head emergence in one case (spray 1) and at 50% flowering in another (spray 2). A control remained unsprayed. The mown, chaffed fodder was fed to caged sheep. The protein level was significantly higher with the sprayed pasture, but sheep lost more wt. when fed spray 2 fodder. The results agreed with previous work. M. T. Rawnsley.

Use and reliability of an *in vivo* nylon bag dry matter digestion technique. J. W. Lusk (*Diss. Abstr. B*, 1967, **28** (3), 1742–1743).—The retention of forage by a nylon bag suspended in the rumen of a cow fitted with a ruminal fistula is examined together with the relation between the *in vivo* dry-matter digestion of small samples and the conventional digestion data. It is concluded that correlations between data obtained by the nylon bag technique and those by the conventional method can be obtained only if certain restrictions are adopted. Forages of the same species should be used and the animal should be fed a forage similar to that being studied. Lucerne hay, grain sorghum and maize silage require a digestion period of 24 h only; annual grass hays need 48 h and perennial grass hays require at least 60 h. A. G. Pollard.

Comparison of cellulose and pentosan digestibilities in roughage feeds. T. L. Balwani, R. R. Johnson and B. A. Dehority (*J. Dairy Sci.*, 1969, **52** (8), 1290–1294. 22 ref.).—Stover and sorghum silages had the highest pentosan contents (19–20%) and lucerne the lowest (11%). Cellulose and pentosan digestibilities in 10 forages ranged from 37 to 78% and 46 to 78%, resp. The digestibility of lucerne pentosan was higher than that of lucerne cellulose, but digestibilities of pentosan and cellulose were similar in the grasses and sorghums. M. O'Leary.

Comparison of the digestibility by pigs of whole and rolled sorghum grain fed either in restricted amounts or *ad libitum*. R. M. Beames (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 127–130. 13 ref.).—Digestibility of org. matter and N-free extract differed for the two cereal types, and the difference for digestibility of protein and of crude fibre was greater when cereals were fed on a restricted basis. However, this method could result in uneven growth and adverse market value. M. T. Rawnsley.

Effect of dicalcium phosphate supplements on the intake and digestibility of Townsville lucerne and spear grass by sheep. M. J. Playne (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 192–195. 16 ref.).—Animals fed lucerne with addn. of 4·5 g of dicalcium phosphate for 77 days, with urea addition to maintain N balance, gained more wt. than those without the supplement. Sheep on spear grass lost wt., with or without the P supplement. M. T. Rawnsley.

Digestibility and nutritive value of *Pennisetum pedicellatum* (Dinanath) grass at flowering stage. P. N. Johri, J. P. Srivastava and S. K. Sinha (*Indian J. Dairy Sci.*, 1969, **22** (1), 1–4. 13 ref.).—The results of feeding trials with bullocks showed that the average digestibility coeff. for crude protein, ether extract, crude fibre, N-free-extract and total carbohydrates of Dinanath grass were 50, 63, 65, 62 and 65, resp. Figures for digestible crude protein, total digestible nutrients and starch equiv. were 3·34, 56·26 and 41·05 kg per 100 kg of dry matter, resp. M. O'Leary.

Chemical composition and nutritive value of Kikuyu grass (*Pennisetum clandestinum*) for sheep. R. C. Katiyar and S. K. Ranjhan (*Indian J. Dairy Sci.*, 1969, **22** (1), 42–45. 6 ref.).—The nutritive value of Kikuyu grass in terms of digestible crude protein, total digestible nutrients and starch equiv. was found to be 3·52, 18·68 and 12·85 kg per 100 kg of grass (30·3% dry matter), resp. The chemical composition in terms of crude protein, ether extract, crude fibre, N-free extract and ash was 14·29, 1·8, 37·83, 29·12 and 16·96%, resp. on a dry matter basis. M. O'Leary.

Yield and chemical composition of Dhaincha (*Sesbania aculeata*)–its nutritive value for sheep. R. C. Katiyar and S. K. Ranjhan (*Indian J. Dairy Sci.*, 1969, **22** (1), 33–36. 5 ref.).—Dhaincha fodder was found to be quite palatable to rams. Its nutritive value in terms of digestible crude protein, total digestible nutrients and starch equiv. was found to be 3·71, 12·17 and 10·54 kg per 100 kg of green fodder (18% dry matter), resp. M. O'Leary.

Comparative study on the nutritive value of jowar green, silage and hay. B. M. Patel, C. A. Patel and P. C. Shukla (*Indian J. Dairy Sci.*, 1968, **21** (4), 208–212. 10 ref.).—The nutritive value of jowar (*Sorghum vulgare*) in its green form was superior to that of either the silage or hay form. All three forms were deficient in P, and P supplementation is necessary when animals are fed mainly on this fodder. M. O'Leary.

Availability of phytate-phosphorus in soyabean meal before and after treatment with a mould phytase. T. S. Nelson, T. R. Shieh, R. J. Wodzinski and J. H. Ware (*Poult. Sci.*, 1968, **47** (6), 1842–1848. 14 ref.).—Phytase, produced by *Aspergillus ficuum* and other moulds, hydrolysed the phytate-P in soyabean meal. Chicks did not utilise phytate-P in untreated soyabean meal, but utilised hydrolysed phytate-P as efficiently as they did inorg. P. The balance of Ca to non-phytate P was important when estimating the relative utilisation of different P sources. A. H. Cornfield.

Availability of niacin in corn [maize], soyabean meal and wheat middlings for the hen. A. G. Manoukas, R. C. Ringrose and A. E. Teeri (*Poult. Sci.*, 1968, **47** (6), 1836–1842. 21 ref.).—Availability of the total niacin present was 30% for yellow maize, 36% for wheat middlings, and 100% for dehulled soyabeans. Tryptophan was converted into niacin by the hen (187 mg tryptophan ≡ 1 mg niacin). A. H. Cornfield.

Metabolisable energy value of dehydrated Coastal Bermudagrass and pearl millet for the growing chick. W. S. Wilkinson and C. Barbee (*Poult. Sci.*, 1968, **47** (6), 1901–1905. 17 ref.).—The Bermudagrass had an av. metabolisable energy, corrected to N equilibrium, of 1370 and pearl millet an av. value of 1420 kcal per kg (dry basis). These compare well with those for dehydrated lucerne. A. H. Cornfield.

Relation between the available essential amino acid patterns in four fish meals and their values in broiler rations. J. O. Anderson, K. Wisutharom and R. E. Warnick (*Poult. Sci.*, 1968, **47** (6),

1787–1796. 4 ref.).—The essential amino acid patterns of fish meals, prepared from herring, anchovy, hake and tuna, differed considerably and even meals prepared from different sources of the same fish species differed somewhat. Large differences were noted in chick performance when the meals were added to the diet to provide 3·6% protein in a 22%-protein maize–cottonseed meal diet. Lysine and methionine deficiencies appeared to be the main factors involved where a meal produced poor chick performance. When the meals were added to provide 5% protein in a 24%-protein maize–soyabean meal diet or 3·6% protein in a 24%-protein maize–cottonseed meal–soyabean meal diet, there were no differences in chick performance. A. H. Cornfield.

Effects of Diet and Environment on Livestock

Food intake and live-weight gain comparisons of *Bos indicus* and *Bos taurus* steers on a high plane of nutrition. A. Rogerson, H. P. Ledger and G. H. Freeman (*Anim. Prod.*, 1968, **10** (4), 373–380. 6 ref.).—At similar live-wt. *Bos taurus* (**I**) had a much higher feed intake relative to maintenance than *B. indicus* (**II**). Water intake was closely related to dry matter intake, irrespective of live-wt.; there was also little varietal difference. **I** were more than twice as efficient as **II** in converting feed into live-wt. gain. M. Long.

Factors affecting the voluntary intake of food by cattle. T. A. McCullough (*Anim. Prod.*, 1969, **11** (2), 145–153. 21 ref.).—Six diets, providing ratios of concentrate (*C*) to hay ranging from 100 : 0 to 60 : 40, were given to Friesian steers *ad lib.* The hay was offered separately, the ratio over a period being maintained by restriction of the item in short supply. At 42 weeks a digestibility trial was carried out. Daily dry matter intake was max. when the diet contained 80% *C*. The apparent digestibility and mean retention time of the diets increased with proportion of *C*. The *C*-only diet gave the lowest acetic acid and highest propionic and butyric acid concn. in the rumen. M. Long.

Effect of artificial lighting and cobalt supplementation on the performance and coat shedding of steers intensively finished in yards. J. G. Morris (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 278–281. 9 ref.).—No significant effects were noted, but the cattle may have had some reserves of Co. The results agreed with most previous work. M. T. Rawnsley.

Intensive finishing of steers on rations containing high levels of either wheat, barley or sorghum grain. Effect of level of roughage and sodium chloride. J. G. Morris, P. M. Pepper and R. J. W. Gartner (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 57–62. 13 ref.).—Steers were fed wheat, barley or sorghum *ad lib.* with 0, 1 or 2 kg oaten chaff/head/day. Some had access to NaCl. In some cases, steers were lame because of laminitis, but no significant effect on body wt. gain or carcass wt. gain was observed. All three grains were considered useful, but sorghum was thought to be 'safest' because fewer steers showed lameness, engorgement, etc. M. T. Rawnsley.

Beef production from nitrogen-fertilised pangola grass (*Digitaria decumbens*) on the coastal lowlands of southern Queensland. T. R. Evans (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 282–286. 14 ref.).—The expt. lasted 2 yr and the N was applied as 400 or 800 lb of Ca ammonium nitrate per yr in split applications Maintenance superphosphate and KCl were similarly applied. The highest rates of live-wt. gain occurred at the time when the grass had the highest crude protein and phosphate. Losses which occurred in winter were reduced by 50% in the 800 lb N/acre expt. The results indicated that less superphosphate could have been used, and that high levels of animal production could be obtained from pangola grass if the N supply was adequate. M. T. Rawnsley.

Propionate tolerance in the dairy cow. D. A. Corse (*Diss. Abstr. B*, 1969, **29** (11), 2983–2984).—Tolerance tests were carried out on cows during the dry period, at 3 weeks and 4 months after calving. Among the conclusions reached was that the rate of propionate (**I**) utilisation was significantly lower ($P < 0{\cdot}005$) in the dry period than during early or later lactation; the rate was reduced in ketotic and partially starved animals. The rates of glucose increase, ketone decrease, serum vitamin B_{12} levels and mean daily production in the ensuing lactation were not correlated with rate of disappearance of **I**. P. P. R.

Ammonium salts of fatty acids for milk production. I. Effect of feeding a salt solution containing ammonium acetate on yield and composition of milk produced by Jersey cows fed hay/concentrate diets. J. H. D. Prescott, A. S. El-Shobokshy and D. G. Armstrong. **II. Effect of feeding a salt solution containing ammonium acetate on yield and fatty acid composition of Jersey milk fat.** K. Hutton, J. H. D. Prescott, R. C. Seeley and D. G. Armstrong (*Anim. Prod.*, 1969, **11** (2), 195–207. 20 ref.; 209–218. 25 ref.).—I. The cows received hay *ad lib.* and concentrates according to milk yield. The control cows received water and a groundnut cake concentrate, and the treatment cows a dil. solution of NH_4^+ salts, supplying 30% of the digestible crude protein, and an all-cereal concentrate. The salt solution also supplied 270–310 g of acetate and 43–49 g of propionate daily. Cows yielding > 13 kg milk/day produced less when fed the salt solution but those producing ≤ 11 kg were unaffected. The fat content of the milk was increased as was total fat production.

II. In the trials reported above, the outputs of C_4-C_{14} acids and of palmitic acid were greater than the intakes and the output of C_{18} acids was less than the intake; treatment intensified these effects. M. Long.

Voluntary intake of acetate by dairy cows given ammonium salts of short-chain fatty acids in their drinking water. P. Jackson, J. Hodgson and J. A. F. Rook (*Anim. Prod.*, 1968, **10** (4), 473–481. 10 ref.).—Considerable variation was found in the amounts (0·5–8%) of these salts tolerated by lactating Jersey cows. Adjustment of pH of the drinking solution to 6·5–7·5 increased the animals' tolerance where previously low. A mean daily intake ≡ 480 g AcOH was achieved without decrease in water intake. Replacement of 50% of NH_4^+ by Ca^{2+} increased the intake of salts by most animals. Addition of saccharin, vanilla or aniseed had little effect, whereas Na cyclamate, ethyl acetate or, notably, molasses reduced animal variability and caused a small increase in salt intake. M. Long.

Relationship of pearl millet to milk fat depression in dairy cows. I. Cation fertilisation. J. P. Harner, R. W. Hemken, J. H. Vandersall, N. A. Clark and B. A. Schneider (*J. Dairy Sci.*, 1969, **52** (8), 1244–1248. 11 ref.).—The results of trials with dairy cows, extending over 3 yr, indicated that increased levels of Ca and K fertiliser and periods of low soil moisture are associated with the depression of milk fat caused by feeding pearl millet to cows. M. O'Leary.

Further evaluation of milk production responses from urea-treated corn [maize] silage. H. H. Van Horn, R. Hocraffer and C. F. Foreman (*J. Dairy Sci.*, 1969, **52** (8), 1249–1252. 6 ref.).—Substitution of soyabean meal by urea in concentrates and high dry matter maize silage caused a significant ($P < 0{\cdot}10$) depression in milk production of Holstein cows. Milk production of cows receiving 31·9% of dry matter maize silage containing 0·5% of urea was significantly higher ($P < 0{\cdot}10$) than that of animals receiving 46·2% of dry matter silage containing 0·5% of urea. M. O'Leary.

Effect of delaying the introduction of concentrates on the summer milk production of hay-fed dairy cows. J. C. Radcliffe (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 66–70. 18 ref.).—Cows in all stages of lactation from South Australian dryland dairy farms were fed concentrates (crushed oats 70, crushed barley 10, linseed meal 15, 50% crude protein meatmeal 7 lb) in five groups, beginning the trials with each group at 2-week intervals from the end of the pasture-growing period in spring. Introduction of concentrate feeding produced an increase in solids-not-fat (but not in protein), butterfat (very slight) and overall milk yield, but the earlier starting dates produced the best results. Cows approaching the end of their lactation did not respond as effectively to concentrate feeding when their intake had previously been restricted to hay and paddock feeding of low quality. The cost of feeding these concentrates at this stage is not feasible under present economic conditions. M. T. Rawnsley.

Effect of plane of nutrition during calfhood on the subsequent performance of Hereford × Ayrshire steers. P. J. Broadbent, C. Ball and T. L. Dodsworth (*Anim. Prod.*, 1969, **11** (2), 155–160. 3 ref.).—Restricted feeding for 12 weeks after weaning from liquid feed to the time the calves were put out to grass caused a difference of 52 lb per head in live-wt. gain, with 15·2 lb persisting at slaughter. Small differences were found in carcass conformation and composition. M. Long.

Cold exposure of Southdown and Welsh sheep. I. Effects of breed, plane of nutrition and acclimatisation to cold upon resistance to body cooling. II. Effects of breed, plane of nutrition and previous acclimatisation to cold upon skin temperature, heart rate, shivering and respiration rate. III. Changes in plasma-calcium, phosphorus, magnesium, sodium and potassium levels. A. R. Sykes, A. C. Field (III) and J. Slee (*Anim. Prod.*, 1969, **11** (1), 65–75. 6 ref.; 77–89. 8 ref.; 91–99. 18 ref.).—I. Sheep kept at

8° cooled more slowly than those kept at 30°. Sheep on high planes of nutrition cooled more slowly than those on low planes. Southdowns cooled more slowly than Welsh sheep.

II. Plane of nutrition influences cold resistance and some of the associated physiological responses. Acclimatisation induced by chronic cold exposure was associated with a temporary increase in basal metabolic rate. Breed differences were small.

III. Exposure to 8° reduced plasma-Mg by 12%; there was no effect on Ca, Na or K levels. Breed x temp. interactions were found with P. Acute cold (−20°, 4 mph wind) reduced plasma-Mg and -Ca and increased -P. Only for Ca was there evidence that prior exposure to 8° modified the response to acute cold.
M. Long.

Wool production and liveweight of wethers in relation to stocking rate and superphosphate application. D. J. CANNON (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 172–180. 11 ref.).—The land under test had already received a total of 1800 lb of superphosphate/acre and 2 oz of Na molybdate/acre; the expt. lasted 3 yr. Each autumn or winter 50, 150 or 250 lb of superphosphate/acre were applied to each of five plots and in one year molybdate was also applied. There were 2–8 sheep on one plot. In most cases wool production/acre increased with stocking rate but production/animal decreased. With 50 lb of superphosphate/acre the optimum was 4 sheep/acre, with 150 lb, 5 sheep/acre, and with 250 lb production was higher in all cases. Increases in wool production can be obtained, but not necessarily every year, with this type of application. M. T. Rawnsley.

Voluntary feed intake of lactating ewes, their milk yield and the growth rate of their lambs. J. M. FORBES (*Anim. Prod.*, 1969, **11** (2), 263–266. 5 ref.).—Voluntary hay intake and milk yield were measured in 11 single-suckling and 4 twin-suckling ewes for the first 7 weeks of lactation. Intakes did not differ significantly between the two groups and milk yield differed only in the 7th week. Lamb wt. gain was correlated with milk yield and intake of the dam. M. Long.

Effects of varying the quantity and distribution of liquid feed in lambs reared artificially. J. B. OWEN, D. A. R. DAVIES and W. J. RIDGMAN (*Anim. Prod.*, 1969, **11** (1), 1–9. 5 ref.).—The effects on growth rate of supplying lambs with 5, 7 or 9 kg milk replacer powder, given according to four systems of daily restriction varying from severe to *ad lib.*, were investigated. The age of weaning varied with treatment from 18 to 50 days but did not affect overall growth rate. Severe restriction affected early growth but had no effect on overall growth rate. It was found uneconomic to give > 5 kg milk powder and there was no advantage in adopting systems which delayed weaning much beyond 20 days.
M. Long.

Effect of replacing rolled barley with swedes or potatoes on the intake and rumen volatile fatty acid composition of lambs. E. R. ØRSKOV, R. P. ANDREWS and J. C. GILL (*Anim. Prod.*, 1969, **11** (2), 187–194. 14 ref.).—The effect of gradually replacing barley with swedes or potatoes was investigated using female and castrated 6-month old sheep. Digestible dry matter intake (*DDMI*) was greatest when the proportion of rolled oats was two-thirds of the estimated max. intake and roots were given *ad lib.* Replacement of barley entirely by swedes reduced *DDMI* to 80% and by potatoes to 70% of highest intake. Replacing barley with roots had little effect on volatile fatty acid composition. M. Long.

Effect of different dietary energy concentrations on voluntary intake and growth of intensively-fed lambs. R. P. ANDREWS, M. KAY and E. R. ØRSKOV (*Anim. Prod.*, 1969, **11** (2), 173–185. 22 ref.).—Five groups of Romney × Swaledale lambs were offered five pelleted diets *ad lib.* in which rolled barley was gradually replaced by an 80 : 20 mixture of rolled oats : oat husk such that the metabolisable energy (*ME*) fell from 2·9 to 2·5 Mcal/kg dry matter (*DM*). The effects on daily live-wt. gains, daily carcass gains, efficiency of utilisation of *ME*, and *DM* intake are discussed. Similar effects were found in a second experiment involving 2 of the diets and a third diet of chopped, dried grass. M. Long.

Wool characteristics as an indication of nutrient attributes in herbage varieties. B. D. PATIL, D. I. H. JONES and R. HUGHES (*Nature, Lond.*, 1969, **223** (5210), 1072–1073).—Relations between feed intake and staple length (*l*) and crimp of wool were detd. for lambs offered different grasses (optionally with 25% red clover) during 12 weeks. Max. *l* was attained by lambs offered tall fescue; crimp was generally decreased, especially when timothy was offered; supplementary clover increased crimp in lambs on ryegrass and timothy (especially the latter) and also increased *l* except in the tall fescue group. Wool colour was variable (e.g., complete lack of pigmentation in lambs offered timothy), and there was some degree of correlation between Cu intake and the differences in *l* and crimp. Differences in *l* did not correlate with contents of S and protein in the grasses. W. J. Baker.

Effects of pattern of feed distribution during the reproductive cycle on the performance of sows. G. A. LODGE (*Anim. Prod.*, 1969, **11** (2), 133–143. 14 ref.).—Three groups of Large White gilts were allotted to 3 feeding regimes for 3 successive pregnancies and lactations. The effects of these regimes on litter size, birth wt., combined litter wt., feed consumption of creep-feed (*ad lib.*), sow wt. -gain and -loss, and efficiencies of food conversion by sow and litter are discussed. M. Long.

Effect of level of feed intake in pregnancy and in lactation upon the productivity of sows. F. W. H. ELSLEY, M. BANNERMAN, E. V. J. BATHURST, *et al.* (*Anim. Prod.*, 1969, **11** (2), 225–241. 10 ref.).—A series of co-ordinated feeding trials carried out by eight centres are reported; six treatments, combining three levels in pregnancy and two in lactation, were used, and are related to sow live-wt.-gains and -losses, litter size, birth-wt. and wt. at weaning. M. Long.

Copper supplementation of pig diets. Effect of protein level and zinc supplementation on response to added copper. T. J. HANRAHAN and J. F. O'GRADY (*Anim. Prod.*, 1968, **10** (4), 423–432).—Groups of pigs were fed high- and low-protein diets, unsupplemented or supplemented with 0·1% $CuSO_4.5H_2O$ or with 0·1% $CuSO_4.5H_2O$ and 0·025% $ZnCO_3$. Pigs fed the low-protein ration with Cu supplementation had an inferior performance compared with control and deaths occurred due to Cu poisoning. When Zn was added, performance equalled control and no toxic symptoms were seen. Cu added to the high-protein ration slightly reduced performance and caused some deaths; the addition of Zn improved growth rate and eliminated Cu toxicity. Effects of treatment on haemoglobin and packed cell vol. were insignificant. M. Long.

Response surface approach to examining the use of separated milk and wheat by growing pigs. J. M. HOLDER, B. R. WILLIAMS and R. J. WILLIAMS (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 121–126. 20 ref.).—The 28 diets used varied from all wheat to all separated milk, and were fed to 128 pigs at levels from near maintenance requirements to near stomach capacity. High daily wt. gains gave earlier marketing, but the carcasses were fat and not as valuable as those of slower-grown pigs. Response surface analysis is an effective method for nutritional evaluation.
M. T. Rawnsley.

Use of distillers' by-products in diets for growing pigs. R. M. LIVINGSTONE and D. M. S. LIVINGSTON (*Anim. Prod.*, 1969, **11** (2), 259–261. 1 ref.).—Distillers' grains plus solubles (**I**), was evaluated with 48 growing pigs. The product was included in three diets at 0, 14·7 and 25%, the other constituents being adjusted to give similar total digestible nutrients, dry matter and crude protein. Performance and carcass quality were little affected by inclusion of 14·7% but with 25% of **I**, growth rate was significantly reduced from 632 (0% of **I**) to 578 g/day. M. Long.

Nutritional evaluation of diets containing meat meal for growing pigs. I. Effect of calcium level in wheat–animal protein diets. E. S. BATTERHAM and J. M. HOLDER (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 43–46. 14 ref.).—Diets contg. 1·4, 2·5 and 3·5% of total Ca were fed to selected piglets. The influence of the Ca content on live-wt. gain (*A*), feed conversion efficiency (*B*), carcass quality, dry matter digestibility (*C*) and N retention was studied. *A*, *B* and *C* were significantly depressed, but parakeratosis did not appear. The tolerance of pigs to Ca must be considered in relation to all other factors, including type of cereal grain, protein supplement, dietary phytic acid level, mineral and vitamin content and feeding rate. M. T. Rawnsley.

Rearing of colostrum-deprived piglets. G. C. PERRY and J. G. LECCE (*Anim. Prod.*, 1968, **10** (4), 433–444. 6 ref.).—Significantly improved growth rates were found in piglets receiving 22% cows' milk solids from birth, compared with a sow-suckled group, and with other, artificial treatments. Each of the artificial treatments led to fewer deaths than the sow-suckled group. M. Long.

Effect of phytate on calcium requirement of chicks. T. S. NELSON, J. J. MCGILLIVRAY, T. R. SHIEH, *et al.* (*Poult. Sci.*, 1968, **47** (6), 1985–1989. 14 ref.).—The Ca requirement of chicks to 3 weeks of age, fed a purified diet containing no phytate, was 0·5%. This was increased to 0·95% by feeding a diet composed of natural ingredients and containing 1·25% phytic acid or phytate. Hydrolysis of 90% of the phytic acid with phytase, reduced the Ca required in the latter diet by ~33%. A. H. Cornfield.

Effects of dietary protein intake on performance of the laying hen. S. M. TALLEY (*Diss. Abstr. B*, 1967, **28** (3), 1743–1744).—From the data obtained in the 2-year study described, it appears that high dietary protein is necessary to maintain optimum productive efficiency, especially when birds are subjected to the stress of senescence. A level of > 16% is recommended when operating under stresses of senescence and excessive heat.
A. G. Pollard.

Lack of interference between dietary acidulated soyabean soapstock and calcium in chicks and laying hens. B. LIPSTEIN and S. BORNSTEIN (*Poult. Sci.*, 1968, **47** (6), 1905–1911. 22 ref.).—Addition of 5–10% acidulated soyabean soapstock or refined soyabean oil to the chick diet had no effect on bone ash% or bone Ca% to 4 weeks of age irrespective of the Ca% of the diet (0·76–1·98% Ca). Calcification was max. with 1·34% Ca in the diet. Dietary Ca level had no effect on fat retention and metabolisable energy content of the diets for either chicks or hens receiving the supplementary oils. A. H. Cornfield.

Comparison of phosphorus assay techniques with chicks. III. Development of a calcium standard curve for soft phosphate, defluorinated phosphate, and calcium phosphate. B. L. DAMRON and R. H. HARMS (*Poult. Sci.*, 1968, **47** (6), 1878–1883. 5 ref.).—A diagram is given showing the total Ca% of the diet required for optimum growth and bone ash of chicks supplied with varying levels of total P, including supplemental P in three forms.
A. H. Cornfield.

Propylene glycol as an energy source in broiler diets. P. W. WALDROUP and T. E. BOWEN (*Poult. Sci.*, 1968, **47** (6), 1911–1917. 16 ref.).—Broilers tolerated up to 5% propylene glycol in their diets without adverse effects up to 28 days of age. Higher levels reduced wt. gains and feed efficiency and caused diarrhoea, and development of deformed toes. A. H. Cornfield.

Effect of varying levels of hydrolysed animal and vegetable fat on growth and carcass characteristics of broilers. C. L. QUARLES, T. W. BURR, J. H. MACNEIL and G. O. BRESSLER (*Poult. Sci.*, 1968, **47** (6), 1764–1767. 11 ref.).—Addition of 2–6% blended fat to the diet of broilers to 59 days of age had no effect on body wt., feed gain ratio, % skin fat or litter moisture. It increased no. of breast blisters and improved tenderness, but had no effect on the quality of meat stored for 0–16 weeks. A. H. Cornfield.

Growth rate inheritance in Japanese quail. III. Thyroid activity of lines selected under different nutritional environments. P. D. LEPORE and H. L. MARKS (*Poult. Sci.* 1968, **47** (6), 1774–1780. 8 ref.).—All three quail lines (selected for growth rate in presence and absence of a chronic goitrogenic challenge) had similar thyroid excretion rates (1·6–2·6 μg thyroxine per 100 g body wt. per day).
A. H. Cornfield.

Ovulation and longevity in the Japanese quail, *Coturnix coturnix japonica*, under constant illumination. G. L. DANIELS (*Poult. Sci.*, 1968, **47** (6), 1875–1878. 4 ref.).— A. H. Cornfield.

Reproductive performance of pheasant breeder hens. L. T. SMITH, R. S. HINKSON and L. E. OUSTERHOUT (*Poult. Sci.*, 1968, **47** (6), 1858–1862. 3 ref.).—The effects of male beak treatment, age of hen, antibiotic feeding and egg fumigation on hen performance were studied. A. H. Cornfield.

Linoleic acid requirement of the hen for reproduction. H. MENGE (*J. Nutr.*, 1968, **95** (4), 578–581).—After a 20-week experimental period, the data showed the hen to require ~2% of dietary linoleic acid for egg production and max. egg size, and 1% for hatchability of fertile eggs. Various oleate : linoleate ratios are considered. C. V.

Effect of ethylene dibromide (EDB) in feed on the growth, sexual development and fertility of chickens. E. ALUMOT, E. NACHTOMI, O. KEMPENICH-PINTO, *et al.* (*Poult. Sci.*, 1968, **47** (6), 1979–1985. 10 ref.).—When female chicks were fed 40 ppm EDB from hatch there was no delay in the onset of egg production, but egg size was decreased. Eggs obtained from EDB-treated hens were infertile. The sexual development of cockerels and their sperm characteristics were not affected by treatment, from 3 days of age, with 80–180 ppm EDB but comb wt. was significantly decreased. Treatment of mature cockerels had no effect on sperm characteristics or fertility.
A. H. Cornfield.

Electrical potential on the surface of fertilised hen's egg as an indicator of embryonic development. T. OOKAWA, J. BUREŠ and H. MYSLIVEČKOVÁ (*Poult. Sci.*, 1968, **47** (6), 1862–1870. 9 ref.).—The possibility of using this potential for distinguishing between fertilised and unfertilised eggs, for recognising abnormal development and for predicting the somatic features of the adult organism was studied. A. H. Cornfield.

Response of vitamin K-deficient chicks subjected to heat stress. O. W. CHARLES and E. J. DAY (*Poult. Sci.*, 1968, **47** (6), 1996–1999. 7 ref.).—Vitamin K-deficient chicks subjected to heat stress (42° for 48 min.) at 3 or 5 weeks of age showed a significant rise in prothrombin time, whilst those receiving supplementary vitamin K (0·00044 g per kg of diet) maintained normal prothrombin times. All birds showed increased body temp. after subjection to heat stress. A. H. Cornfield.

Influence of environmental temperature upon adrenal activity of bursectomised chicks. T. M. HUSTON and TATA SUBHAS (*Poult. Sci.*, 1968, **47** (6), 1760–1763. 15 ref.).— A. H. Cornfield.

Chemical nature of component I, a phosphoprotein isolated from the blood sera of diethylstilbestrol-treated cockerels. A. V. DE-GUZMAN and R. E. CLEGG (*Poult. Sci.*, 1968, **47** (6), 1890–1897. 30 ref.).—Component I was shown to be a glycoprotein, serine being the predominant amino acid. A. H. Cornfield.

Blood cells and chemistry of young chickens during daily administration of adrenocorticotropin and cortisol. H. S. SIEGEL (*Poult. Sci.*, 1968, **47** (6), 1811–1817. 24 ref.).— A. H. Cornfield.

Dietary antibiotics and absorption of ^{65}Zn and ^{131}I-labelled oleic acid. D. E. TURK (*Poult. Sci.*, 1968, **47** (6), 1768–1771. 7 ref.).—Addition of chlortetracycline (0·055–0·220 g), oxytetracycline (0·055 g), erythromycin (0·02 g) or bacitracin (0·055 g per kg of feed) to the diet of broilers increased wt. gains to 42 days of age. Tests with labelled Zn and oleic acid, administered orally, showed that the antibiotics did not affect the time required for absorption of either nutrient or the amount absorbed over an 8-h period, thus growth responses due to antibiotics are not due to improved absorption of Zn or oleic acid. A. H. Cornfield.

Thiol level in liver and kidneys of chickens. V. L. MILLER, G. E. BEARSE, T. S. RUSSELL and E. CSONKA (*Poult. Sci.*, 1968, **47** (6), 1884–1885. 10 ref.).—The liver-thiol level was higher in an avian leukosis-resistant line, which also retained larger amounts of Hg in both liver and kidney, than in a susceptible strain.
A. H. Cornfield.

Effect of thiouracil on free amino acids in the blood-plasma of chicks. TSANG-CHENG SHAO and D. C. HILL (*Poult. Sci.*, 1968, **47** (6), 1806–1810. 16 ref.).—Addition of 0·2% thiouracil to the diet (wheat–maize–soyabean meal) of chicks from 1 to 3 weeks of age, reduced wt. gains and levels of lysine, threonine, tryptophan, methionine and leucine in the plasma. The effects were not influenced by reduced protein intake or changes in blood-plasma vol. A. H. Cornfield.

Effects of iodocasein feeding on broiler thyroid. S. HOSHINO, H. DOIZAKI and K. MORIMOTO (*Poult. Sci.*, 1968, **47** (6), 1928–1933. 14 ref.).—Addition of 0·05% iodocasein to the diet of chickens to 7 weeks of age, increased body and thyroid wt. and reduced thyroidal ^{131}I uptake and the serum protein-bound iodine/iodide ratio. A. H. Cornfield.

Pacific oyster culture in British Columbia. D. B. QUAYLE (*Bull. Fish. Res. Bd Can.*, 1969, (169), 192 pp. 8 ref.).— P. C. W.

Analysis and Other Aspects

Simple method for the determination of 5-vinyloxazolidine-2-thione in rapeseed meal. A. SZEWCZUK, P. MASTALERZ and W. NADWYCZAWSKI (*Can. J. Biochem.*, 1969, **47** (18), 817–818. 4 ref.).—Cruciferae seeds contain a thioglucoside yielding 5-vinyloxazolidine-2-thione (VOT). The new sensitive titrimetric method of VOT determination is based on its ability to catalyse the reduction of I_2 by N_3^-; the amount of I_2 consumed is proportional to the amount of VOT used, and the need to extract the VOT from the aq. phase is removed. The titrimetric and spectrophotometric methods gave comparable results, and the method thus appears to be useful for the determination of VOT. It is possible to determine 10 μg of VOT per sample. J. C. T. N.

Gas chromatographic determination of 2-chloro-4-nitrobenzamide, 3,5-dinitrobenzamide and 3,5-dinitro-*o*-toluamide in animal feedingstuffs. R. A. HOODLESS and R. E. WESTON (*Analyst, Lond.*, 1969, **94** (1121), 670–673. 6 ref.).—The additives were extracted into MeOH and then converted into the Me esters by reaction with boiling MeOH–HCl. The esters were then detd. by g.c. with an electron-capture detector. A 60-cm column of Chromosorb G coated with 1% Apiezon L and 0·2% Epikote resin 1001 was used. Because retention times of the 3,5-dinitrobenzamide (I) and

zoalene (II) esters are similar, the MeOH extract was initially cleaned up by treatment on an Al_2O_3 column and then with $KMnO_4$; the Me ester of I then gave a response but the oxidn. product of (II) did not. Recoveries compared favourably with those obtained by alternative methods. Dimetridazole was the only other additive (at concn. of 500 ppm) found to interfere.
W. J. Baker.

Collaborative study on a potentiometric titration of soluble chlorides in feeds. D. L. SHARPE (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 607–609. 3 ref.).— D. I. Rees.

Estimation of non-epoxide xanthophyll in sun-cured and freeze-dried alfalfa [lucerne], clovers and grasses. A. L. LIVINGSTON, J. W. NELSON and G. O. KOHLER (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 617–622. 19 ref.).—After chromatog. separation, the xanthophyll fraction was acidified and the absorbance of the non-epoxide xanthophyll was measured at 475 nm. Values obtained were similar to those obtained by the t.l.c. method of Nelson and Livingston (*J. Chromat.*, 1967, **28**, 465). D. I. Rees.

[Analysis of] vitamin A in liquid feed supplements [for livestock]. D. B. PARRISH and D. AGUILAR (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 451–454. 18 ref.).—Difficulties in comparing bioassays of vitamin A in these feeds with results of biopotency estimates using maleic values are discussed. D. I. Rees.

Effect of soluble components on crude fat determination in grains and stock feed. G. I. DEBECZE (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 610–616. 3 ref.).— D. I. Rees.

Welfare of domestic animals. W. H. THORPE (*Nature, Lond.*, 1969, **224** (5214), 18–20. 21 ref.).—Some of the details in 'The Revised Draft Codes of Practice' (Agricultural Miscellaneous Provisions Act, 1968, governing animal welfare under intensive husbandry systems) are discussed and criticised, mainly in so far as they do not reflect all the findings of the Brambell Committee (1965) nor take into account the evidence of animal behaviour studies. These details concern the necessity for animals to have sufficient freedom of movement to be able easily to get up, lie down, groom normally, turn around and stretch their limbs. All conditions leading to physical deformity and highly abnormal nutritional physiology must also be excluded. The surgical operations permitted by the Codes are, it is thought, likely to prove a retrograde step in farming practice. The amendments required to the Codes, before they are passed, are listed. W. J. Baker.

Inhibition of mould growth in crops and animal feedstuffs. BP CHEMICALS (U.K.) LTD. (Br. Pat. 1,155,485, 18.8.67. Den., 15.9.66).—The crops etc., are treated with 0·1–10% by wt. of $EtCO_2H$ (I), optionally as an aq. soln. E.g., oats containing 20–30% of moisture are treated by tumbling them with 0·5% of I. After 9 months storage no mould growth is observed.
S. S. Chissick.

2.—FOODS

Cereals, Flours, Starches, Baking

Influence of heat treatment on the carbohydrate-amylase complex of maize. M. V. ROSHAK, N. V. ROMENSKY and V. A. YAKOVENKO (*Pishch. Tekhnol.*, 1968, [5 (66)], 69. Russ.).— P. P. R.

Chemical composition of rice lipids. Z. Y. SANDLER, Y. I. DENISENKO and A. P. NECHAYEV (*Pishch. Tekhnol.*, 1968, [6 (67)], 11. Russ.).— P. P. R.

Effect of drying and storage on yellow dent corn [maize] and its dry milled products. W. HEUSDENS (*Diss. Abstr. B*, 1967, **28** (3), 1741–1742).—Methods are sought for determining whole-maize properties which would correlate with the final quality and quantity of dry milled commercial products. Fat acidity, glutamic acid decarboxylase activity and viability were used to study commercial maize, while degree of gelatinisation, and fat acidity values were obtained for the finished products. These data were correlated with a standard commercial evaluation (crack and overhang) of the finished product. It appeared that milling technique and other chemical analyses could to some extent predict the suitability of maize for dry milling. A. G. Pollard.

Influence of lipids on properties of gluten. II. V. G. BAIKOV, A. P. NECHAYEV and L. I. PUCHKOVA (*Pishch. Tekhnol.*, 1968, [5 (66)], 24. Russ.).— P. P. R.

Characteristics of oat flour [assessed] by amino acid composition and digestibility of proteins. I. P. SALUN and L. V. SMIRNOVA (*Pishch. Tekhnol.*, 1968, [5 (66)], 27. Russ.).— P. P. R.

Functional (breadmaking) and biochemical properties of wheat flour components. R. C. HOSENEY (*Diss. Abstr. B*, 1968, **29** (6), 1958).—Partial solubilisation of the gluten protein fraction in 0·002 N lactic acid gave a sol. gliadin-rich fraction, responsible for loaf vol. potential, and an insol. glutenin-rich fraction, responsible for mixing time. Reconstituted flour was prepared by extraction with light petroleum, washing out and solubilising the gluten in 0·005 N lactic acid and centrifuging at 100,000 *g* for 5 h; the centrifugate was extracted with water–satd. butanol. The reconstituted flour, after premixing, was used to study the rôles of free polar, free non-polar and bound polar lipids. Addition of large amounts of bound or free polar lipids restored loaf vol., though more bound polar lipids were required than free polar lipids. C. J. R.

Isolation and characterisation of water-soluble gums in wheat flours. FANG MING LIN (*Diss. Abstr. B*, 1968, **29** (6), 2079).—Six flours milled from various wheat samples were used in the isolation of starch-free pentosans. The effects of these on bread-making were studied at different stages of isolation. Amylase modification of crude pentosan increased loaf vol. while treatment with xylanases, glucuronidase and pronase decreased loaf vol. Pentosan prepn. at initial stages of isolation were more effective in improving loaf vol. and crust colour than purified prepn.
C. J. R.

Change in free radical concentration in starch with irradiation dose. K. A. KOROTCHENKO, K. ADAMICH, M. SHARA and P. TSEVS (*Pishch. Tekhnol.*, 1968, [5 (66)], 31. Russ.).— P. P. R.

Development of a method for determination of starch damage. L. C. ROSE (*Diss. Abstr. B*, 1968, **29** (6), 2080).—A modification of the ferricyanide reduction method for determining reducing sugars was used for the determination of starch damage in flour.
C. J. R.

Dimethylsulphoxide solution of starch. S. TOMITA and K. TERAJIMA (*Rep. Govt chem. ind. Res. Inst. Tokyo*, 1969, **64** (6), 249–259. Jap., 9 ref.).—A study by paper chromatog. of the starch dispersive ability of various aq. salt soln. and org. solvents, correlated with examination of the ultracentrifugal sedimentation patterns of the starch soln., indicated that the most effective solvent for starch (dissolving both the amylose and the amylopectin components) is DMSO in which the starch granules dissolve endothermally, the η–temp. curve having a peak similar to that of the gelatinisation of starch pastes in water. A series of photographs show the sedimentation patterns of soln. of starch in various solvents and of soln. of various types of starch in DMSO. The ratio of sedimentation coeff. of amylopectin to that of amylose depends on the type of starch, and the relative magnitude of this ratio is generally greater for starch from grain crops than for starch from root crops. (From Engl. summ.) J. M. Jacobs.

Influence of [individual] factors on composition of non-volatile organic acids of wheat dough and bread. G. V. KOROL'KOVA, R. G. RAKHMANKULOVA and L. I. LOSHKAR'YOVA (*Pishch. Tekhnol.*, 1968, [5 (66)], 22. Russ.).— P. P. R.

Structural alteration of macaroni dough proteins in screw chamber. B. M. AZAROV, L. N. KONCHAYEVA, N. A. MANKEYEVA, *et al.* (*Pishch. Tekhnol.*, 1968, [5 (66)], 73. Russ.).— P. P. R.

Effect of *cis-trans* isomers and related physical properties of monounsaturated lipids on shortening power. A. J. GOEBEL OSTRANDER (*Diss. Abstr. B*, 1969, **29** (11), 4219).—The lipids studied include a vegetable oil, oleic acid, triolein, elaidinised oleic acid, elaidinised triolein and mixtures of oleic acid and of triolein with their elaidinised counterparts. A multiple regression equation has been developed for predicting breaking strength values in pastry wafers made with the various shortenings. P. P. R.

Recirculation method of aeration of baker's yeast. S. E. KHARIN, L. K. GROMKOVSKAYA and A. I. GROMKOVSKY (*Pishch. Tekhnol.*, 1968, [6 (67)], 49. Russ.).— P. P. R.

Sugars, Syrups, Confectionery

Thermal stability of products of beet sugar manufacture. L. I. TREBIN and K. D. ZHURA (*Pishch. Tekhnol.*, 1968, [5 (66)], 79. Russ.).— P. P. R.

Regeneration of anion exchanger after decolorisation of sugar-beet syrup. I. F. ZELIKMAN, N. M. KODENKO and D. M. LEIBOVICH (*Pishch. Tekhnol.*, 1968, [6 (67)], 32. Russ.).— P. P. R.

Determination of granulometric composition of sugar crystals in massecuite. Y. D. KOT and L. G. BELOSTOTSKY (*Pishch. Tekhnol.*, 1968, [5 (66)], 168. Russ.).— P. P. R.

Enzymatic glucose determination in corn [maize] starch hydrolysates. J. T. BRADY and J. A. ZAGORSKI (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 556–559. 11 ref.).—The method involves catalytic oxidation of glucose (**I**) with I-oxidase to form H_2O_2 which, in presence of peroxidase and *o*-dianisidine, gives a product which when acidified yields a stable, coloured soln. whose intensity is proportional to [**I**]. D. I. Rees.

Modified method for determining ash in maple syrup. A. S. WENDT and C. FLYNN (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 554–555).— D. I. Rees.

Separation of fixed organic acids in table syrups. A. R. JOHNSON and E. FERNANDEZ-FLORES (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 559–564. 19 ref.).—Org., non-nitrogenous acids were isolated from syrups and identified by gradient elution chromatography and by programmed temp. g.l.c. of their trisilyl deriv. Aconitic acid predominates in sugar-cane and sorghum-cane syrups, while malic acid is found in measurable amounts in maple sugar and syrup. D. I. Rees.

Relationship between the thermodynamic stability of cocoa butter and the storage stability of chocolate. H. WITZEL and R. HEISS (*Fette Seifen AnstrMittel*, 1969, **71** (8), 663–671. Ger., 18 ref.).—The *X*-ray powder diagrams of the unstable pre-β-form of cocoa butter glycerides are compared with the more stable β-form. Although this technique cannot be used for a quant. determination of these forms, it can be used to determine amounts of the unstable form and its effect on the bloom in chocolate. Measurements of the intensity of two *X*-ray reflexions at 002 and 005 can be used to indicate directly the tendency for bloom formation during the cooling of chocolate mixes. G. R. Whalley.

Modern emulsifiers for retarding the formation of fat bloom in chocolate. K.-G. LUDWIG (*Fette Seifen AnstrMittel*, 1969, **71** (8), 672–678. Ger., 35 ref.).—The five polymorphic glyceride forms of cocoa butter are described, including the conditions for their existence, with particular reference to the β-form and its effect on fat bloom in chocolate compositions. A rotational viscometer was used to determine the % of solid fats in a chocolate mix during the cooling process and its relationship to the % of β-form present. The effects of the inclusion of 1% of nonionic emulsifiers in chocolate showed that both sorbitol monostearate and polysorbitol 60 were effective in reducing bloom formation, with optimum effect in a 60 : 40 mixture. G. R. Whalley.

Malting, Brewing and Alcoholic Beverages

Analysis of hop resin components by countercurrent distribution. A. M. HUMPHREY (*Chemy Ind.*, 1969, (35), 1235–1236. 5 ref.).—Based on the method of Verzele *et al.* (*J. Inst. Brew.*, 1967, **73**, 298), an improved and more rapid quant. separation of components is obtained by use of five pH groups each occupying 5 tubes and with iso-octane as mobile phase. After 25 transfers, the tubes are each acidified with N-H_2SO_4, the separated components are transferred to the mobile phase, and the absorbances of these soln. at 275 nm are plotted vs. tube no. Concn. of components in a specific band are calc. from total absorbances for that band. Humulinic acids (**I**) separate at pH 4·5, isohumulones (**II**) at pH 6·8, humulones plus 4-deoxyhumulones at pH 10 and lupulones at pH 11. Results for **II** agreed with those obtained by countercurrent distribution with pH 3·25 citrate-buffer. Among the merits of the method are the accurate quant. detn. of **I** and the applicability to a wide range of hop extracts. W. J. Baker.

Nitrous composition and fermentation activity of autolysates of beer yeast under different conditions of their autolysis. M. V. ZAZIRNAYA, P. M. MAL'TSEV, A. E. MELET'YEV and A. P. CHIST'YAKOVA (*Pishch. Tekhnol.*, 1968, [6 (67)], 21. Russ.).— P. P. R.

Methods for disinfection of water extracts from malt germs. G. I. FERTMAN (*Pishch. Tekhnol.*, 1968, [6 (67)], 59. Russ.).— P. P. R.

Anthocyanogens of barley and their rôle in malt and beer technology. P. M. MAL'TSEV and L. K. SKRIPCHENKO (*Pishch. Tekhnol.*, 1968, [5 (66)], 101. Russ.).— P. P. R.

Influence of ultra-violet rays on wine yeast. A. P. MARCHENKO (*Pishch. Tekhnol.*, 1968, [5 (66)], 98. Russ.).— P. P. R.

Influence of sugar content of wine on thermal autolysis of wine yeast. V. M. LOZA and E. M. SOBOLEV (*Pishch. Tekhnol.*, 1968, [5 (66)], 96. Russ.).— P. P. R.

Spectrophotometric determination of tanning and colouring substances in cognacs and wines. Y. E. FAL'KOVICH and L. T. GRIGOR'YAN (*Pishch. Tekhnol.*, 1968, [5 (66)], 170. Russ.).— P. P. R.

Evaporation and rectification coefficients of some volatile impurities of wine. Z. N. KISHKOVSKY and A. E. SHEIN (*Pishch. Tekhnol.*, 1968, [6 (67)], 150. Russ.).— P. P. R.

A chemiluminescent method for control of the extent of thermal treatment of fortified wines. E. M. SOBOLEV (*Pishch. Tekhnol.*, 1968, [6 (67)], 147. Russ.).— P. P. R.

Fruits, Vegetables and Their Products

Determination of chlorcholine chloride residues in strawberries. G. PETROSINI, M. BUSINELLI, F. TAFURI and L. SCARPONI (*Analyst, Lond.*, 1969, **94** (1121), 674–677. 13 ref.).—Residues were extracted with H_2O and red anthocyanin was removed with Et_2O. This was followed by purification on a 2-column chromatographic system, with cation exchanger Dowex 50WX4 in the first column (to retain quaternary NH_4 compd.) and Al_2O_3 in the second (to remove other choline deriv.). Finally, the extinction of the dipicrylamine–chlorcholine chloride complex was measured at 415 nm. Av. recovery was ~80% for 0·4–4 ppm. Field trials showed that residues, from plants treated for control of runnering activity, were 5–9 ppm (first crop) and 0·5–3 ppm (2nd crop). Lyophilisation does not interfere with the extraction, so that detn. can be postponed until convenient. W. J. Baker.

Chemical composition of fresh elderberries. J. R. TAYLOR and E. FERNANDEZ-FLORES (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 643–646).—Data are tabulated (12 samples) for amino acids, insol. and sol. solids, ash, K_2O, P_2O_5, invert sugar, *l*-malic acid, total polyphenolics, protein and titratable acidity. D. I. Rees.

Chemical and physico-chemical characteristics of residue in grape juice. T. F. ZYKINA (*Pishch. Tekhnol.*, 1968, [5 (66)], 44. Russ.).— P. P. R.

Cryoscopic determination of grape juice characteristics. J. L. OWADES and J. M. DONO (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 651–653. 3 ref.).—The f.p. of a 2-ml sample is determined by first cooling below the f.p. and noting the temp. rise, using a thermocouple–galvanometer system. The addition of 10% of sugar (water being added to the same °Brix as the original juice) can be detected by this means if an authentic sample of juice is available. D. I. Rees.

Paper chromatographic detection of major organic acids in fruit juices. J. FITELSON (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 646–649. 3 ref.).—The acids are pptd. with $Pb(OAc)_2$ and then regenerated with H_2S. The soln. is analysed by paper chromatography with Et_2O–HCO_2H–H_2O (20 : 5 : 3) as solvent and the dry paper is treated with aniline–furfural reagent. The R_F values of common fruit acids and the major org. acids of some fruit juices are tabulated. The method was used to detect added citric acid in cherry and blackberry juices. D. I. Rees.

Paper chromatographic detection of adulteration in dark coloured fruit juices. J. FITELSON (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 649–651. 2 ref.).—The method involves comparison of the anthocyanin and, after acid hydrolysis, anthocyanidin patterns from suspected adulterated samples with those of authentic samples. It was used to detect foreign natural colouring in blackberry and cherry juices. D. I. Rees.

Chemistry of potato chip flavour. R. E. DECK (*Diss. Abstr. B*, 1968, **29** (6), 2078).—Isolated flavour compounds of fresh potato chips were separated into 2 fractions by extraction with 10% Na_2CO_3. Eight acids were identified in the acid fraction. The non-acidic fraction on g.c. yielded 45 compounds; 7 of the most important were identified, by i.r. comparison with synthesised compounds, as alkyl-substituted pyrazines. C. J. R.

Non-alcoholic Beverages

Chromatographic determination of caffeine in instant tea. J. M. NEWTON (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 653–656. 6 ref.).—The chromatographic extraction and clean-up procedure of Yeransian *et al.* (*ibid.*, 1963, **46**, 315) was used. The extracted caffeine was determined by g.l.c. with use of a KCl thermionic

detector which was 100 times more sensitive than the flame ionisation detector and could detect 1 mg of caffeine. Analysis of samples containing ~ 5% of caffeine gave results similar to those obtained by the spectrophotometric method. D. I. Rees.

World coffee survey. C. A. KRUG and R. A. DE POERCK (*FAO agric. Stud.*, 1968, (76), 476 pp., including 3 Appendices and 8 pp. of ref.).— P. P. R.

Methylated xanthines of coffee. I. Theophyllins. S. TABAK, A. DEL'ACQUA, M. L. RIBEIRO and L. P. DIAS (*Anais Acad. bras. Cienc.*, 1969, **41** (1), 59–62. Port., 5 ref.).—Coffee was analysed and caffeine (**I**), theobromine (**II**) and theophylline (**III**) were found, together with three other unidentified compounds. In purified and impure commercial **I** obtained from coffee, **I**, **II** and **III** were found. From these results combined with previous work on biosynthesis, it is suggested that **II** and **III** could result from demethylation of **I** and not from direct biosynthesis. (From Engl. summ.) J. C. T. N.

Gas chromatographic determination of methyl salicylate, safrole and related compounds in non-alcoholic beverages. D. LARRY (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 481–485. 4 ref.).—The steam distillate from the sample containing methyl salicylate, safrole, dihydrosafrole, anethole, dihydroanethole and estragole) is extracted with $CHCl_3$ and the extract analysed by g.l.c. Recoveries of ~ 95% for methyl salicylate and ~ 50% for the other compounds were obtained. D. I. Rees.

Milk, Butter, Other Dairy Products, Eggs

Amperometric method for the estimation of calcium and magnesium in milk. D. SETHU RAO, C. S. SUDHEENDRANATH, S. KRISHNA RAO, M. BHIMASENA RAO and C. P. ANANTAKRISHNAN (*Indian J. Dairy Sci.*, 1969, **22** (1), 37–41. 20 ref.).—A description is given of a method for the successive complexometric determination of Ca and Mg in the same sample of milk, without the elimination of phosphate, employing the technique of constant potential amperometry. M. O'Leary.

Precipitation of milk proteins by sodium carboxymethylcellulose. F. J. CLUSKEY, E. L. THOMAS and S. T. COULTER (*J. Dairy Sci.*, 1969, **52** (8), 1181–1185. 16 ref.).—Carboxymethylcellulose (CMC) was shown to be capable of pptg. 90–96% of the casein from a simplified casein system and about 71% of the total protein from skim-milk. Max. pptn. occurred at pH 7·5. The reaction was Ca-dependent and protein–Ca–CMC bridging is considered to be involved at pH > 4·6. At pH 4·6, CMC stabilised casein. The typical cellulose–protein complex was shown to contain 51·7% protein, 7·3% ash, 0·44% Ca and 10–17% CMC. M. O'Leary.

Protein aggregation in conventional and ultra-high temperature heated skim-milk. C. V. MORR (*J. Dairy Sci.*, 1969, **52** (8), 1174–1180. 10 ref.).—Sephadex G-100 gel filtration and zonal electrophoresis were used to demonstrate that none of the ultracentrifugate supernatant fractions from skim-milk heated by conventional or *UHT* treatments contained higher levels of non-sedimenting N components ($\leqslant$ 75–100 S and excluded from Sephadex G-100) than those from raw skim-milk. Evidence was obtained, however, that ultracentrifugate supernatant fractions from skim-milk heated at *UHT* treatments of $\geqslant$ 146° contained slightly more of the non-sedimenting N components than did those from lower temp. *UHT* treatments. M. O'Leary.

Effect of sterilisation on the proteose-peptone level in milk. I. Effect of temperature and time of sterilisation. II. Effect of dialysable milk constituents. B. S. GUPTA, N. C. GANGULI and V. R. BHALERAO (*Indian J. Dairy Sci.*, 1969, **22** (1), 46–51, 15 ref.; 52–56, 11 ref.).—I. Sterilisation at 115–125° for 10–20 min resulted in a 50% decrease in the proteose-peptone content of cow and buffalo milk. The proteose content of cow milk increased by 62% on sterilisation while that of buffalo milk increased by 34%. Proteose-peptone solutions were stable on sterilisation, though the proteose component increased slightly. Removal of micellar casein from milk resulted in higher levels of proteose-peptone remaining intact after sterilisation than in normal milk. A similar effect was observed with dialysed milk.

II. The addition of galactose (10 g/100 ml) to milk increased the proteose-peptone content reduction resulting from sterilisation at 115° for 10 min, while the addition of lactose had the opposite effect. Glucose and sucrose had no effect. Ca and citric acid increased and phosphates decreased the reduction of milk proteose-peptone on sterilisation. Thiamine, riboflavin and pantothenic acid had no effect. M. O'Leary.

Influence of temperature and pH on the proteolytic activity of rennet extract. P. F. FOX (*J. Dairy Sci.*, 1969, **52** (8), 1214–1218. 25 ref.).—When changes in the electrophoretic pattern of casein were used as the criterion, the pH optimum for rennin proteolysis was shown to be 5·8, whereas when changes in non-protein N were used as the criterion, a much lower pH optimum was indicated. The nature of proteolysis products was not temp.-dependent but at low temp. (4°), β-casein was more susceptible to proteolysis than α_{S1}-casein, whereas the reverse applied at higher temp. (32°). M. O'Leary.

Flavour retention during drying. G. A. REINECCIUS and S. T. COULTER (*J. Dairy Sci.*, 1969, **52** (8), 1219–1223. 14 ref.).—Optimum flavour retention during spray-drying of skim-milk was obtained by drying a high solids extract (about 50% total solids) using an exit air temp. of ~ 100° and an inlet air temp. of ~ 160°. Particle size had no effect on flavour retention. M. O'Leary.

Peculiarities of milk fat crystallisation under the influence of low frequency vibrations. S. S. GUL'YAYEV-ZAITASEV and G. A. YERES'KO (*Pishch. Tekhnol.*, 1968, [5 (66)], 88. Russ.).— P. P. R.

Preparation of milk fat methyl esters by alcoholysis in an essentially non-alcoholic solution. S. W. CHRISTOPHERSON and R. L. GLASS (*J. Dairy Sci.*, 1969, **52** (8), 1289–1290. 5 ref.).—The rapid prepn. of the Me esters of milk fat, by treating 19 vol. of a 10% soln. of milk fat in petroleum ether with 1 vol. of either 2 N methanolic KOH or NaOMe at room temp., is described. M. O'Leary.

Comparative biochemical studies of milks. V. Triglyceride composition of milk fats. R. L. GLASS, R. JENNESS and L. W. LOHSE (*Comp. Biochem. Physiol.*, 1969, **28** (2), 783–786).—The milk fats used were those described in the previous paper of this series (*ibid.*, 1967, **22**, 415). On t.l.c., milk fats of fifteen species of ruminants exhibited two distinct triglyceride (*TG*) spots while those of 40 species of non-ruminants showed only a single spot. The faster moving *TG* in the ruminant group milk fats had higher content of 18 C acids and was devoid of butyric acid. The palmitic acid contents of the two groups of *TG* were almost identical. C. V.

Study of lipid oxidation in model systems of copper–proteins, milk lipids and milk dialysate. J. SINGH AULAKH (*Diss. Abstr. B*, 1968, **29** (6), 2078).—Model systems of copper–micellar caseinate, copper–fat globule membrane protein (**I**), copper–sodium α-caseinate, and copper–β-lactoglobulin (**II**) were incorporated into systems of washed fat-globules (**III**) and milk dialysate (**IV**). A system without copper served as control. The systems were heated at 145° F for 30 min and at 190° F for 10 min. Those of **I**, **II** and **III**, **IV** were also heated separately and combined. Samples were stored at 35° F for 10 days. At regular periods, oxidn. was followed by determination of thiobarbituric acid and peroxide values. Results showed that heated systems had less tendency to oxidise than unheated systems; this suggests that physical or physico-chemical changes at the fat-globule surface are associated with the beneficial effects of high-heat treatment of milk. C. J. R.

Moisture in butter in relation to dielectric constant measurements. S. PARKASH and J. G. ARMSTRONG (*J. Dairy Sci.*, 1969, **52** (8), 1224–1228. 12 ref.).—In a particular butter, the dielectric const. (*D*) increased with increase of moisture or salt content. In butters worked for the same time, *D* increased almost linearly with increase in moisture content. Additional working caused *D* to decrease to a const. value which depended on the moisture content of the butter. In 33 samples of commercial butter, a correlation coeff. of + 0·554 was observed between *D* and the no. of droplets per cm^2. *D* of solutions of butter in dioxane was not an indicator of the moisture content of the butter. M. O'Leary.

Extraction of plant sterols from adulterated butter oil using a digitonin-impregnated Celite column. D. E. LACROIX (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 600–602. 6 ref.).—As little as 3 mg of β-sitosterol per 100 g of butter oil can be detected and the method used as an index of adulteration of butter oil with > 5% of vegetable oil. D. I. Rees.

Controlling oxidised flavours in high-fat sterilised creams. H. K. WILSON and E. O. HERREID (*J. Dairy Sci.*, 1969, **52** (8), 1229–1232. 8 ref.).—A description is given of the prepn. and sterilisation (at 155°) of a 50% fat cream capable of being stored for six months at 4°. Sodium polyphosphate was used to inhibit gelation and ascorbic acid and α-tocopherol were used to prevent oxidised flavour defects. Ice cream, capable of being stored in a frozen condition for a year without ill-effect, was made from the high-fat sterilised cream. M. O'Leary.

Rôle of ghee residues as antioxidants in ghee. M. K. RAMA MURTHY, K. M. NARAYANAN and V. R. BHALERAO (*Indian J. Dairy Sci.*, 1969, **22** (1), 57–58. 7 ref.).—The residues obtained on filtration of cow and buffalo ghee through Whatman No. 4 filter paper at 100° were shown to have good antioxidant properties, possibly due to their relatively high phospholipid contents.
M. O'Leary.

Free fatty acids and the flavour of dairy products. D. D. BILLS, R. A. SCANLAN, R. C. LINDSAY and L. SATHER (*J. Dairy Sci.*, 1969, **52** (8), 1340–1345. 28 ref.).—The relationship between free fatty acids and the flavour of dairy products is reviewed. Problems involved in the analysis and sensory evaluation of the fatty acids are discussed. M. O'Leary.

Ester production by *Pseudomonas fragi*. III. Synergistic flavour interaction of esters at subthreshold concentrations. M. C. REDDY, R. C. LINDSAY and D. D. BILLS (*J. Dairy Sci.*, 1969, **52** (8), 1198–1201. 11 ref.).—A synergistic flavour interaction between various esters, capable of influencing the flavour of dairy products, was demonstrated. M. O'Leary.

Microstructure of loose cheese and accumulation of calcium salts in it. G. G. TIN'YAKOV and V. I. KULIKOVA (*Pishch. Tekhnol.*, 1968, [5 (66)], 92. Russ.).— P. P. R.

Variation in initial quality of chicken eggs. III. Interior quality decline under various storage conditions as related to initial quality. J. H. SKALA (*Poult. Sci.*, 1968, **47** (6), 1849–1858. 24 ref.).—Eggs of high initial quality ($\geqslant$ 80 Haugh units) tended to show similar declines in quality and changes in albumen pH and moisture loss when stored under a variety of conditions. Eggs with initial quality of $\leqslant$ 74 Haugh units showed slightly less quality loss than did those of high initial quality during short storage (5–10 days at 13–16°); differences became more marked with longer storage (33 days at 13–16° and 12 weeks at 3°). A. H. Cornfield.

Suspension weighing technique for the rapid determination of specific gravity of eggs. R. A. E. PYM (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (37), 131–134. 13 ref.).—A suspension weighing apparatus, accurate to 0·1 g, with a water-levelling device, is described. The sp. gr. of new-laid eggs were determined by weighing in air and then in water, and this method was much more rapid than a method involving immersion in salt soln. of various sp. gr. An impact apparatus for measuring shell strength was also devised. Eggs from White Leghorns, Australorps and Synthetics were tested, the last having the lowest sp. gr. and shell strength. There was a high correlation between shell strength and sp. gr. and in general shell thickness increased with egg size, sp. gr. being independent of egg wt. M. T. Rawnsley.

Quantitative gas chromatographic determination of lactic and succinic acids in eggs. W. F. STARUSZKIEWICZ, JUN. (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 471–476. 5 ref.).—Egg extracts (containing 1–86 mg of lactic and 0·4–50 mg of succinic acids) were cleaned-up and analysed as their Pr esters. Av. recoveries were 105 and 102% for the two acids, respectively. D. I. Rees.

Whey product. FOREMOST DAIRIES INC. (Br. Pat. 1,155,654, 13.4.67. U.S., 14.4.66).—Liquid whey (e.g., from Cheddar cheese manufacture) containing $\leqslant$ 80% by wt. of heat denaturable protein in undenatured form, is concentrated, without denaturing, adjusted to pH 6·2–6·4, clarified, electrodialysed (15% reduction, by wt., of the ash content) and some lactose removed, so that 35–70% by wt. lactose (dry solids basis) remains in the final whey. The product which can be used in, e.g., ice cream, is spray-dryable if required in powdered form. S. D. Huggins.

[A] Preparation of milk coagulating enzyme. [B] Method of making cheese. GODO SHUSEI K.K. (Br. Pat. 1,156,387–8, [B] div. out of [A], 24.2.67. Jap., 26.2.66).—[A] The enzyme (**I**) is prep. by aerobic cultivation of *Bacillus polymyxa* (**II**) or *B. cereus* var. *mycoides* in an aq. nutrient medium at 25–37° and at pH 5–8 and isolated by salting out. [B] The use of **I** in making cheese is claimed. **I** is added to a whole milk/$CaCl_2.2H_2O$/starter mixture and after 25 min an elastic curd is obtained, which after cutting and cooking is cast and compressed. After 4 months ripening there is no detectable difference between cheese prep. by this method and that prep. using conventional rennet. S. S. Chissick.

Edible Oils and Fats

Neutralisation and bleaching of maize oil in the laboratory. R. DE CASTRO RAMOS (*Grasas Aceit., Seville*, 1969, **20** (4), 183–188. Span., 45 ref.).—The effect of the amount of alkali soln. on losses during refining of maize oil was studied. The colour of the neutralised oils decreased as the amount of alkali increased and it increased as alkali concn. increased. Oils neutralised with Na_2CO_3 were darker in colour. After bleaching, the colour of the oils followed that after the neutralisation. L. A. O'Neill.

Volumetric determination of groundnut oil in seeds by indirect complexometry with Mg(II). R. GARCÍA-VILLANOVA and M. T. MARÍN AZNAR (*Grasas Aceit., Seville*, 1969, **20** (4), 180–183. Span., 6 ref.).—The ground seeds are refluxed with 8% ethanolic KOH, filtered, the soln. is made up to vol. with aq. EtOH and an aliquot (25 ml) is pptd. with 0·05 M-$MgSO_4$ in presence of an NH_3/NH_4Cl buffer. After cooling and filtering, the excess Mg(II) in the filtrate is measured by titration with 0·05 M-EDTA soln. with Eriochrome Black T indicator. The most accurate results were obtained with $\leqslant$ 0·5 g of oil. Results obtained directly on the seed agreed well with those obtained on the material produced by Soxhlet extraction, but results obtained gravimetrically by Soxhlet extraction were much higher (by ~10%). L. A. O'Neill.

Differential thermal analysis of fats. A. J. HAIGHTON and L. VERMAAS (*Fette Seifen AnstrMittel*, 1969, **71** (8), 614–618. Ger., 19 ref.).—Use of a Differential Scanning Calorimeter showed that the characteristic points on cooling and m.p. curves were directly proportional to the rates of heating and cooling. Difficulties were encountered when using the cooling curves because of supercooling, and the m.p. curves were often difficult to interpret when crystals formed during cooling were present in their metastable state. With water-in-oil systems, a precise crystallisation point could be obtained for the pure water at −43°, and this ice melted again at 0°. A similar effect was obtained with butter and margarine.
G. R. Whalley.

Emulsifiers. III. Per-iodic acid analysis for 1-monoglycerides. E. DISTLER and F. J. BAUR (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 602–607. 4 ref.).—A comparison of 2 methods for the analysis of monoglycerides in shortenings. D. I. Rees.

Change in sunflower seed lipids on thermal drying. V. G. SHCHERBAKOV and A. M. MALYSHEV (*Pishch. Tekhnol.*, 1968, [5 (66)], 34. Russ.).— P. P. R.

Kinetics of fat hydrogenation. II. N. L. MELAMUD, G. M. PAVLOV and O. A. PROTSENKO (*Pishch. Tekhnol.*, 1968, [5 (66)], 37. Russ.).— P. P. R.

Meat, Poultry, Fish

Nature of boiled beef flavour. K. O. HERZ (*Diss. Abstr. B*, 1968, **29** (4), 1398).—Volatile flavour compounds from semitendinosus muscles of 22-month-old prime and choice Hereford steers were isolated and identified by i.r. and mass spectrometry and by g.c. of authentic compounds. The 4 acidic and 49 non-acidic compounds identified in the flavour isolate from fresh boiled beef are listed. Non-carbonyls giving a meaty aroma were found, in contrast to previous theories that meat flavour is due to carbonyls in particular, or to groups of compounds that individually have no meaty odour.
P. R. G.

Proposed revision for preparation of meat samples for total phosphorus determination. M. OKAMOTO, J. W. SHAFER and C. L. ETTINGER (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 634–637. 2 ref.).—The proposed method involves drying the sample at 125° for 30 min followed by ashing at 550° and dissolving the cooled residue in HNO_3 by heating on a steam bath for 30 min. The extract thus obtained and that obtained by the A.O.A.C. method 2·017(c) were analysed for total P by the gravimetric quinoline molybdate method 2·025(b). Similar results for total P were obtained by this and by the A.O.A.C. method 2·017(c) on six ham samples. D. I. Rees.

A test of bacterial cultures utilisation in sausage manufacture. I. I. KARGAL'TSEV and G. V. TIMINA (*Pishch. Tekhnol.*, 1968, [6 (67)], 67. Russ.).— P. P. R.

Moisture effect on the nitric oxide pigments in freeze-dried beef. R. J. BRADDOCK and L. R. DUGAN, JUN. (*Fd Technol., Champaign*, 1969, **23** (8), 1085–1086. 13 ref.).—Spectrophotometric measurements on treated beef showed that there was no difference between NO haemochromes extracted from nitrite-treated and gas-treated meat. The amt. of moisture significantly affected the amt. of pigment formed, and pigment concn. increased with longer exposure times. Rehydration of NO-treated meat gave a pink colour,

untreated meat turning brown. Some of the results verified previous work, and may be useful to producers of dried soup mixes, etc. M. T. Rawnsley.

Cure diffusion through pre- and post-chilled porcine muscles. F. C. Arganosa and R. L. Henrickson (*Fd Technol., Champaign*, 1969, **23** (8), 1061–1065. 18 ref.).—Pre-chilling gave significant effects on nitroso-pigment content, cure diffusion distance, moisture, NaCl content and pH of different muscles from Hampshire barrows. Muscle type affected total pigment, myoglobin, moisture, NaCl, ether extract, cure diffusion and pH. No correlation was found between muscle type and treatment. M. T. Rawnsley.

Effect of freezing rate on soluble proteins of frozen and freeze-dried muscle tissue. C. S. Huber (*Diss. Abstr. B*, 1969, **29** (11), 4219).—The relative solubilities of chicken, turkey (raw and cooked) and porcine (raw) muscles were studied; in the raw state, porcine muscle was the least sol. Differences in protein solubility between frozen and freeze-dried muscle varied considerably among the 3 species. Except for chicken, raw freeze-dried muscle was more sol. than frozen muscle. P. P. R.

Characterisation of the chicken broiler as a function of sex and age: live performance, processing, grade and cooking yields. E. T. Moran, jun. and H. L. Orr (*Fd Technol., Champaign*, 1969, **23** (8), 1077–1084. 13 ref.).—Live performance, holding, dressing and evisceration losses, moisture absorbed on chilling, grade, gross chemical analysis of carcass, yield of various commercial cuts, cooking losses, and proportions of meat, skin and bone in the cooked parts were determined using Cornish male × White Rock female broiler chicks. While the two sexes developed at different rates, the optimum age for slaughter for both was 8 weeks. M. T. Rawnsley.

Colour and myoglobin concentration in turkey meat as affected by age, sex and strain. G. W. Froning, J. Daddario and T. E. Hartung (*Poult. Sci.*, 1968, **47** (6), 1827–1835. 13 ref.).—A study was made of the colour and myoglobin content of the meat of five strains of turkeys of both sexes. A. H. Cornfield.

Amino acid composition of meat removed from boned turkey carcasses by use of a commercial boning machine. E. O. Essary and S. J. Ritchey (*Poult. Sci.*, 1968, **47** (6), 1953–1955. 4 ref.).—Amino acid composition was determined on ground meat from turkey carcasses after breast meat, wings, drumsticks, thighs and neck were removed; it was very similar to that for turkey (breasts and legs), chicken, beef, pork, milk and eggs and would be satisfactory for use in processed meat products. A. H. Cornfield.

Estimating breading content of battered and breaded poultry products. K. N. May, A. J. Farr and J. P. Hudspeth (*Fd Technol., Champaign*, 1969, **23** (8), 1087–1090. 2 ref.).—Products were weighed, agitated by compressed air in water to remove the coating and reweighed to determine the original amount of coating present. M. T. Rawnsley.

Relationship between ion and water content of cod (*Gadus morhua* L.) muscle. A. H. Sutton (*Comp. Biochem. Physiol.*, 1968, **24** (1), 149–161. 15 ref.).—Na and Cl contents increased and K, Mg and P decreased with muscle water content, showing an increasing extracellular and decreasing intracellular fraction in the muscle. Increase in water content is a characteristic of malnutrition and ionic changes have been interpreted to show components and phases of the degeneration processes. C. V.

Chemical composition and commercial utilisation of shrimps. N. E. Nikolayeva (*Pishch. Tekhnol.*, 1968, [5 (66)], 56. Russ.).— P. P. R.

Oxidation of fish fat by oxygen. G. B. Chizhov and E. M. Rodin (*Pishch. Tekhnol.*, 1968, [5 (66)], 52. Russ.).— P. P. R.

Separation of lipids of sturgeon roe by absorption chromatography in thin layers. E. B. Ost'yakova and A. P. Chernogortsev (*Pishch. Tekhnol.*, 1968, [6 (67)], 24. Russ.).— P. P. R.

Determination of boron as boric acid in caviar: a collaborative study. J. W. Brunstad (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 485–487. 4 ref.).— D. I. Rees.

Making fermented meat products. Dairy Technics, Inc. (Inventors: H. Rothchild and R. H. Olsen) (Br. Pat. 1,157,423, 20.2.68. U.S., 6.3.67).—The title products are made by mixing into a comminuted meat mix a thawed bacterial culture (*Pediococcus cerevisiae*) (**I**) (≮ 10^9 cells/ml), a stabilising agent, e.g., glycerol, and a nutrient medium and then heating at 110–125°F until the pH is 4·4–5·4 (8–15 h), when the temp. is raised to ≮ 138°F. E.g., frozen **I** is mixed into a boneless meat mix at 55°F and the product stuffed into cases and showered with water (140°F, 15 sec). The casings are transferred to a smoke house at 115°F for 12 h and smoked during the final 8 h. Prior to leaving the smoke house the temp. is raised to 160°F and the process terminated when the sausage meat is at 142°F. S. S. Chissick.

Fish concentrate and method of preparation. K. Chung Lum, jun. (Br. Pat. 1,157,415, 4.4.68. U.S., 12.4.67).—A protein concentrate is prep. by digesting fish by enzymic proteolysis, screening to remove bone, etc., and extracting the oils and fats with an org. solvent, e.g., hexane and Pr^iOH (9 : 1). The residue is dried to produce the fish flour concentrate. S. S. Chissick.

Food Additives

Preservatives, Colouring Matter

Application of colouring matter from cranberries pressed skins in non-alcoholic beverage manufacture. E. M. Usova, E. G. Red'ko and L. P. Dubkova (*Pishch. Tekhnol.*, 1968, [5 (66)], 105. Russ.).— P. P. R.

Spices, Flavours, Other Additives

Composition of pepper oil. Y. S. Lewis, E. S. Nambudiri and N. Krishnamurthy (*Perfum. essent. Oil Rec.*, 1969, **60** (7/8), 259–262. 10 ref.).—The compositions of 17 samples of black pepper oils were compared using column chromatog., t.l.c. and g.l.c. The oils varied widely in odour quality, and samples showing a strong peppery note contained high proportions of monoterpenes. A high pinene content gave a turpentine odour to the oil. Oxygenated compounds, probably occurring as the oxides, were present (< 4%). G. R. Whalley.

Gas chromatographic determination of methylene chloride, ethylene dichloride and trichloroethylene residues in spice oleoresins. L. A. Roberts (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 477–481. 23 ref.).—Quant. recoveries were obtained except for methylene chloride (73%). D. I. Rees.

Total luminescence of coumarin derivatives isolated from expressed lime oil. H. W. Latz and B. C. Madsen (*Analyt. Chem.*, 1969, **41** (10), 1180–1185. 15 ref.).—11 crystalline components (including two not previously reported) responsible for the room- and low-temp. fluorescence and low temp. phosphorescence of expressed lime oil (**I**) were separated by chromatography, characterised by their R_F values, m.p., mass and u.v. spectra, and their individual luminescence characteristics were measured and compared with those of samples of **I** of different geographical origin. All luminescence emission characteristics showed wide variations according to origin, but owing to the small no. of samples involved, no definite correlation between sample origin and emission intensity (coumarin deriv. content) could be established. The coumarin and psoralen deriv. responsible for the various emission characteristics of the whole oil were identified. S. S. Chissick.

T.l.c. identification of four artificial sweeteners in beverages. T. Korbelak (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 487–491. 3 ref.).—The sweeteners are extracted into EtOAc from the acidified sample and analysed by t.l.c. Concn. as low as 0·004% of saccharin, 0·05% of cyclamate, 0·03% of dulcin and 0·002% of P-4000 (5-nitro-2-propoxyaniline) in beverages can be detected. D. I. Rees.

Sodium cyclamate, saccharin and food efficiency. L. M. Dalderup (*Nature, Lond.*, 1969, **223** (5213), 1368–1369. 6 ref.).—Addn. of Na cyclamate and saccharin (each in concn. of 0·43%) to Ran-1961 food supplied to male and female rats during 5 weeks increased their food efficiency and improved their growth. These effects were only slightly enhanced when the two compd. were added to stock food; the effects were more convincing in males than in females. No explanation for the action is offered. W. J. Baker.

Determination of cyclohexylamine in artificially sweetened foods and artificial sweeteners. J. W. Howard, T. Fazio, B. A. Klimeck and R. H. White (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 492–500. 15 ref.).—Procedures for determination of cyclohexylamine in carbonated beverages, dry beverage bases, canned fruits, fruit juice drinks, liquid and dry weight control prepn., food sweetener prepn.

and Na and Ca cyclamates, are described. Recoveries were ~ 90–100% at the 0·2 ppm and 2 ppm levels, respectively, depending on the product. D. I. Rees.

[Preservative for] mayonnaise and mayonnaise-based food products. KYOWA HAKKO KOGYO CO. LTD. (Br. Pat. 1,155,490, 11.9.67 Jap., 26.9.66).—The products contain < 0·001% by wt. of tryptophan or a deriv. thereof, e.g., 5-hydroxytryptophan, as an antioxidant and preservative. S. S. Chissick.

Basic amino acid succinates and their use in seasonings. KYOWA HAKKO KOGYO K.K. (Br. Pat. 1,154,926, 29.9.67. Jap., 30.9.66).—A seasoning of use to patients suffering with kidney and other diseases has a salty taste and consists of a Na^+-free, non-salty seasoning, e.g., a glutamate (80–95%) mixed with at least one basic amino acid salt of succinic acid (5–20%), e.g., lysine, arginine or histidine succinate, and a binding agent, e.g., polyvinyl alcohol. S. S. Chissick.

Food Processing, Refrigeration, Packaging and Storage

Estimating thermal death time characteristics of thermally vulnerable factors by programmed heating of sample solution or suspension. K. HAYAKAWA, P. G. SCHNELL and D. H. KLEYN (*Fd Technol., Champaign*, 1969, **23** (8), 1090–1094. 20 ref.).—A principle similar to that used for determining thermal processes in canned foods was developed. Thermal death time characteristics are detd. from (*i*) a temp. history curve of the sample during heat treatment and (*ii*) values for the survival ratio of vulnerable factor at four or more heating time periods. Fewer values are required than in present procedures. Use of the equations, etc., is illustrated by thermal death time characteristics of alkaline phosphatase in raw milk. M. T. Rawnsley.

Improved storage of garlic bulbs by gamma irradiation. C. M. MESSIAEN and P. PEREAU-LEROY (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (7), 485–490. Fr.).—Keeping quality was considerably improved by irradiation of bulbs immediately after harvesting, especially for those bulbs which had been kept at temp. (5–10°) conducive to emergence from dormancy. Infection by mildew was minimised by inoculation of irradiated bulbs with *Penicillium* about 5 months later, i.e., during December. The method ensures a much higher % of marketable bulbs the following June, at an estimated cost of only 1–3 centimes/kg. W. J. Baker.

Process criteria for producing radiation-sterilised fish products. R. L. LEARSON, L. J. RONSIVALLI, B. W. SPRACKLIN and F. HEILIGMAN (*Fd Technol., Champaign*, 1969, **23** (8), 1071–1077. 11 ref.).—Steps prior to radiosterilisation were: addn. of preservatives, coating, enzyme inactivation, packaging, bringing the packages to −78° or 0·6° and packing the cans with ice. Immediately after treatment, the fillets obtained were very palatable, but storage at room temp. caused browning. This factor must be eradicated before acceptable fish fillets can be produced. M. T. Rawnsley.

Two revolutionary systems for the sterile preservation of foods. G. NUGHES (*Materie plast. Elast.*, 1969, **35** (8), 1111–1113. It.).—Flexible containers for foods are based on polypropylene (I), which is completely non-toxic, contains no plasticisers and does not affect flavour, but which is permeable to O_2 and light. This is overcome by coupling the I on the outside with a layer of epoxide varnish or, in special cases, depositing a thin layer of Al by metallisation *in vacuo* after first treating with a primer to promote adhesion. A third layer of polyester film then protects the outside. The containers withstand the pressures developed during sterilisation and may be sealed in the usual manner and printed by lithography, offset, etc. Rigid containers are injection moulded. Advantages claimed are lower cost than with tins, lower operation costs with less skilled and fewer personnel; the organoleptic characteristics of the packed product are preserved unchanged by time and unaffected by the material of the container. There is greater guarantee of hermeticity and resistance to sterilisation stresses, and no rusting of containers on prolonged storage. The inertness of the material makes it suitable for mineral waters and infant feeds. J. I. M. Jones.

Food sterilisation by microwave radiation. J. BILBROUGH (*Non-ioniz. Rad.*, 1969, **1** (2), 70–72. 1 ref.).—Normal methods of sterilisation are briefly compared with the effect of microwave processing whose main objective is to kill mould spores on the inside of wrappers. A number of design features of suitable equipment are described with particular reference to the prevention of stray radiation. P. C. W.

Influence of salt concentration and microflora on proteolysis of protein in salting and storage of fish. V. V. BAL' and A. A. KONNOVA (*Pishch. Tekhnol.*, 1968, [6 (67)], 17. Russ.).— P. P. R.

Investigation into the accuracy of volumetric dosing of meat in mechanical prepackaging. A. I. POPOVICH (*Pishch. Tekhnol.*, 1968, [6 (67)], 90. Russ.).— P. P. R.

Materials, machines and equipment for economical packaging. W. K. STERLING (*Fette Seifen AnstrMittel*, 1969, **71** (8), 678–685. Ger.).—The construction, dimensions, materials of construction and methods of sealing transportation containers are compared, together with a discussion and description of commercial machines for cartoning, shrink-wrapping, producing blister-packs, vac. packing, heat sealing, carton making and banding. G. R. Whalley.

Stability of fats and fat-containing products packed in plastic foils. G. HARTMANN and W. HUBIG (*Fette Seifen AnstrMittel*, 1969, **71** (8), 685–695. Ger., 17 ref.).—The methods used and the general concept of fat deterioration are discussed, with special reference to fat storage in commercial PVC and polystyrene films. The physical data relating to these films are also presented. Studies with packed mayonnaise, margarine and cooking fats showed that O_2 penetration through films rapidly and adversely affected the stability of fatty materials. Light had a similar adverse effect. It is suggested that more attention should be directed to the selection of the optimum foil, in order to give max. stability to fat-containing foodstuffs. G. R. Whalley.

Low temperature injury of Starking Delicious peaches in relation to weight lost during cool storage. K. J. SCOTT, R. B. H. WILLS and E. A. ROBERTS (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 364–366. 9 ref.).—Storage treatments used were initial storage at low r.h.; hydrocooling; delayed storage (2 days at 30°F); stepwise cooling (41°F for 3 weeks then 68°F at 70% r.h. for 3 days, then 30°F); K_2CO_3 dip; control at 30°F. Low temp. injury increased as wt. loss increased for all treatments. Warming during storage, stepwise cooling and delayed storage were most effective in reducing low temp. injury. M. T. Rawnsley.

Storage and ripening for banana quality. A. TSALPATOUROS (*C.r. hebd. Séanc. Acad. Agric. Fr.*, 1969, **55** (5), 351–357. Fr., 2 ref.).—The problem of controlling storage-ripening conditions so as to ensure prolonged keeping quality and favourable organoleptic properties of bananas after removal from the warehouse is discussed. The rôle of exogenous and endogenous C_2H_4 during fruit metabolism, the need for genuine colour (unripe fruit often turns yellow through some specific action of C_2H_4), the control of ambient ripening atm. by detn. of C_2H_4 concn., and the need for uniformity in quality of collected batches of unripe bananas are among the topics considered. W. J. Baker.

Nutrition, Proteins, Amino Acids, Vitamins

Present knowledge of methyl groups in nutrition. W. H. GRIFFITH and H. M. DYER (*Nutr. Rev.*, 1968, **28** (1), 1–4).— C. V.

Enzymic processing of vegetable protein foods. K. R. SREEKANTIAH, H. EBINE, T. OHTA and M. NAKANO (*Fd Technol., Champaign*, 1969, **23** (8), 1055–1061. 21 ref.).—Defatted meals of groundnut and sesame, and decuticled chickpea, green gram, black gram and field bean, were soaked, cooked and processed with 0·15–0·25% of proteolytic enzymes. These were Molsin (from *Aspergillus saitoi*), Proctase (*A. niger*), Protamylase (*A. oryzae*) and Q-12-C (*Trametes sanguina*). The optimum concn. was a 10% suspension for cooking, and 15–20 min were required. Hydrolysis by enzymes at 45° and pH 3·0 was complete in 5 h. The protein content of sesame meal increased from 7·21 to 64·14% and of chickpea from 16·68 to 86·12%. The most useful enzyme was that from *T. sanguina*. M. T. Rawnsley.

Production of microbial food from low-cost starch materials and purification of industry's waste starch effluents through the Symba yeast process. K. JARL (*Fd Technol., Champaign*, 1969, **23** (8), 1009–1012. 2 ref.).—The process is intended for conversion of starch into yeast cell mass by growing torula yeast (*Candida utilis*) in symbiosis with an amylase-producing yeast sp. *Endomycopsis fibuliger*. The *E. fibuliger* : *C. utilis* initial ratio is critical and cooling, prevention of frothing and careful recovery and drying are essential. With whole potatoes or tapioca as raw material, yields of 40 and 50% of protein were obtained in a batch process. Con-

tinuous processes are in hand. Waste water contg. conc. starches can be purified to removal of 80–95% of the B.O.D., depending on flow rate, etc. M. T. Rawnsley.

Protein quality of wheat and soyabeans after *Rhizopus oligosporus* fermentation. H. L. WANG, D. I. RUTTLE and C. W. HESSELTINE (*J. Nutr.*, 1968, **96**, 109–114. 17 ref.).—The fermentation process did not significantly change the essential amino acid composition of wheat (*W*) or mixture of *W* + soyabeans (*SB*). Growth of rats fed on fermented *W* improved significantly over those fed on unfermented *W*. Protein efficiency ratio (*PER*) of *W* was increased by fermentation. These improvements were partly attributed to the increased availability of lysine in *W* by fermentation. A mixture of *W* and *SB* (1 : 1) gave a good pattern of amino acids and the mixture as a protein source supported growth as well as casein (*C*). The fermentation raised the *PER* value to that of *C*. C. V.

Interactions of water with amino acids and proteins by differential microcalorimetry. E. KARMAS (*Diss. Abstr. B*, 1968, **29** (6), 2079).—Water–protein interaction and protein denaturation were studied by differential scanning calorimetry. 30–70% water retention was shown by leucine, isoleucine, valine and methionine, indicating involvement in formation of quasi-crystalline clathrate cages. A method for determining relative water binding index (*WBI*) gave the order: egg albumin < extracted salt-sol. protein < beef muscle < isolated (vegetable) proteins. Addition of NaCl increased *WBI*. C. J. R.

Modification of vitamin D. A. W. NORMAN (*Biol. Rev.*, 1968, **43** (1), 97–137. 215 ref.).—As a working hypothesis, the concept is used that vitamin D may be acting analogously to a steroid hormone. Previously it has been considered that it was acting as a cofactor for some specific enzyme reactions. C. V.

Determination of optimum conditions for drying amino acid mixture obtained from vegetable raw materials. E. I. MEDVEDEVA, B. M. BOBOVNIKOV, S. V. KRASIL'NIKOVA *et al.* (*Pishch. Tekhnol.*, 1968, [6 (67)], 63. Russ.).— P. P. R.

Unclassified, Tobacco

Qualitative and quantitative changes of sucrose in fish canned products with tomato filling. M. I. KOMARNITSKY and V. M. KURKHANOVA (*Pishch. Tekhnol.*, 1968, [5 (66)], 107. Russ.).— P. P. R.

Thermodynamics of water fixation by pectin substances. S. L. KOVALENKO and O. D. KURILENKO (*Pishch. Tekhnol.*, 1968, [6 (67)], 14. Russ.).— P. P. R.

[Gelatin] dessert gel strength testing. II. Collaborative study. E. BORKER and K. G. SLOMAN (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 657–659. 3 ref.).—A modified Bloom gelometer method using a lower shot-flow rate was found to be more precise than the A.O.A.C. method 21·012. D. I. Rees.

Spectrophotometric determination of reducing sugars (after a short inversion) in tobacco with 2,4-dinitrophenol. B. ZIELONKA, H. CHRZAN and B. HRYCAK (*Chemia analit.*, 1969, **14** (3), 573–579. Pol.).—A sample is boiled for 15 min with water and heated with conc. HCl. The neutralised soln. is then filtered and the filtrate is heated for 6 min with the dinitrophenol reagent and the extinction of the cooled soln. is measured against a blank. The results are obtained from a calibration curve prepared using fructose as the standard. Comparison of results with those given by the method of Bertrand shows no systematic error and a smaller random error. P. Brych.

Isolation of styrenes from tobacco resins. V. V. CHENIKOV and V. G. VEIZLER (*Pishch. Tekhnol.*, 1968, [6 (67)], 69. Russ.).— P. P. R.

Determination of undegraded thiabendazole in tobacco smoke. E. KRÖLLER (*Dt. Lebensmitt Rdsch.*, 1969, **65** (3), 85–86. Ger., 3 ref.).—Thiabendazole (**I**) is used to prevent mould growth in tobacco leaf with relatively high moisture content and an analytical procedure for determination of **I** in the smoke is described. Smoke from 15 mechanically smoked cigarettes is pptd. electrostatically; the deposit, dissolved in alkaline MeOH, is steam distilled. The $CHCl_3$-extract of the distillation residue is further purified by extraction into dil. acid before t.l.c. of an aliquot on silica gel with C_6H_6–AcOH–Me_2CO–H_2O (10 : 4 : 1 : 0·4) as solvent; the **I** spot is detected under u.v. light and extracted with acid. This extract, mixed with *p*-phenylenediamine and Zn dust, is reacted with $Fe_2(SO_4)_3$ and the resultant colour extracted into butanol for measurement at 660 nm. As little as 5 μg of **I** can be estimated to ± 10%. Cigarettes made with leaf containing 600 ppm **I** yielded 120 ppm unchanged in the smoke; it is estimated that 24 μg could be ingested from each cigarette. This is not considered a health hazard. J. B. Woof.

γ-Ray irradiation of tobacco mosaic virus-infected tobacco leaves and cigarettes with special reference to the inactivation of the virus. K. TOMARU and T. SHIROYA (*Radio-Isotopes, Tokyo*, 1968, **17** (6), 247–249. Jap.). C. V.

3.—PEST AND DISEASE CONTROL, SANITATION

Plant Diseases, Pests and Weeds

Gas chromatography of organophosphorus pesticides: retention times and response data on three columns. R. R. WATTS and R. W. STORHERR (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 513–521. 7 ref.).—Retention data were obtained on 60 compounds with use of glass columns: (*i*) DC-200, (*ii*) QF-1–10% of DC-200 and (*iii*) diethylene glycol succinate, all on 80–100 mesh Gas Chrom Q. D. I. Rees.

Rubidium sulphate flame detector [for detection of organophosphorus pesticides]. Effect of design of alkali source and anode on sensitivity and stability. H. K. DELOACH and D. D. HEMPHILL (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 533–541. 9 ref.).— D. I. Rees.

Herbicides

Meiotic and morphological response of grain sorghum to atrazine, 2,4-D, oil, and their combinations. G. H. L. LIANG, K. C. FELTNER and O. G. RUSS (*Weed Sci.*, 1969, **17** (1), 8–12. 28 ref.).—Spraying sorghum with atrazine, 2,4-D (ester and alkanolamine salt) or petroleum crop oil resulted in chromosomal aberrations of pollen mother cells. Aberration frequencies were higher when combined herbicide treatments were given. A. H. Cornfield.

Dissipation and phytotoxicity of dicamba. R. R. HAHN, O. C. BURNSIDE and T. L. LAVY (*Weed Sci.*, 1969, **17** (1), 3–8. 9 ref.).—Dissipation of dicamba (**I**) was more rapid in a silty clay loam than in a sandy loam, and was faster in topsoil than in subsoil. Breakdown increased with soil temp. (15–35°). Phytotoxicity of **I** and amiben (**II**) in aq. soln. increased on exposure to sunlight; **II** was more sensitive to photodecomposition. **I** soln. exposed to 60° later showed increased phytotoxicity, whilst **II** soln. were unaffected. A. H. Cornfield.

Chemical control of weeds in tobacco in northern New South Wales. A. D. DOYLE (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 12–18. 9 ref.).—Spray and furrow irrigation and disc and furrow harrowing were used on three types of area, freely draining coarse sand (granite origin), sandy clay-loam (alluvial) and sandy loam (recent alluvial). The herbicides tested were trifluralin, diphenamid, Benefin (*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine), EPTC and DCPA (chlorthal), in quantities of 0·38–14 lb of active ingredient per acre. All gave some degree of control but the amount used was critical. Benefin is suitable up to 3·6 lb/acre and trifluralin up to 1 lb/acre. Diphenamid may persist in the cured leaf. The method of incorporation could also be of great importance. M. T. Rawnsley.

Fungicides

Rapid diagnosis of viral and fungal diseases in plants by pyrolysis and gas–liquid chromatography. A. MYERS and L. WATSON (*Nature, Lond.*, 1969, **223** (5209), 964–965. 1 ref.).—This method (cf. Reiner and Ewing, *ibid.*, 1968, **217**, 191) permits rapid detection and identification of pathogens *in situ*, assessment of diseased tissue distribution in the host, and (possibly) measurement of degree of infection. Leaves were pyrolysed at 800° and the products submitted to temp.-programmed g.l.c. (40–140°) on a 1·5-m column of 'Carbowax 20*M*' plus H_3PO_4, with CH_2Cl_2 as solvent and N_2 as carrier gas. One sample per h could be analysed. Results (pyrograms) are reported for cereal leaves, both healthy and infected with rust and mildew, and for tobacco leaves infected with Potato virus Y. The typical disease patterns are not merely senescence effects, the peaks being directly attributable to fungal infection. Potentialities of this method are indicated. W. J. Baker.

Immunisation of rice plants against *Helminthosporium* infection. A. K. SINHA and N. TRIVEDI (*Nature, Lond.*, 1969, **223** (5209), 963–964. 12 ref.).—Susceptible cultivars of rice were effectively immunised against brown spot disease by pre-inoculation with a suspension of avirulent *H. oryzae* spores (10^5–10^6/ml) and less effectively by treatment with the cell-free germinating fluid or with heated spore suspension freed from dead conidia. Germ tube growth was decreased by 48–74%, owing to the development of fungitoxic substances in the host tissue after inoculation. W. J. Baker.

C_p/R_s and the disease potential of plants. J. GRAINGER (*Hort. Res.*, 1968, **8** (1), 1–40. 21 ref.).—Analysis of healthy and diseased plants affords evidence that pathogens (fungi, bacteria) deplete host plants of all essential constituents, but especially of carbohydrates. The ratio C_p/R_s, where C_p is the total plant carbohydrate (excluding cellulose but probably including hemicellulose) and R_s is the residual (carbohydrate-free) wt. of the shoot, is directly related to the disease potential. Values < 0·5 apply to healthy plants, 0·5–1·0 corresponds with 'slight disease potential', 1–10 indicates aggressive or epidemic disease and > 10 is associated with very severe damage or death of the plant. Use of the ratio as a measure of host receptivity and of other aspects of plant pathology is discussed. A. G. Pollard.

An association of infection by *Sclerotinia sclerotiorum* and potassium deficiency in Kennebec potato. P. J. FOUNTAIN and P. J. SAMPSON (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (38), 361–363. 3 ref.).—Results showed that potato crops grown on soils of low K content would be less susceptible to the fungus *S. sclerotiorum* if they were given a K supplement. M. T. Rawnsley.

Thiabendazole as a post-harvest treatment against *Sclerotinia fructicola* in dessert peaches. Y. J. FRIPP and E. B. DETTMANN (*Aust. J. expl Agric. Anim. Husb.*, 1969, **9** (36), 9–11. 9 ref.).—Trials with 37·5–2400 ppm of thiabendazole (**I**) on six varieties of peach, two in the coastal district and four in the central tablelands of N.S.W., showed that infection decreased with increasing **I** concn.; with 600 ppm of **I**, infection of 2–56% occurred, with 1200 ppm, 1–19%, and with 2400 ppm, 0–18%. The highest concn. left a white residue. M. T. Rawnsley.

Biologically active compounds derived from 3-chloromethylsulphonylaniline. A. BROJAN, K. ZIMNA, R. KOWALIK and Z. ECKSTEIN (*Bull. Acad. pol. Sci. Sér. Sci. chim.*, 1969, **17** (5), 263–268. Engl., 10 ref.).—3-Chloromethylsulphonylaniline (**I**) was obtained by reduction of the nitro deriv. of chloromethylphenylsulphone with $SnCl_2$ in conc. HCl, and was converted to halide deriv. by Sandmeyer reactions. Schiff bases were prepared by refluxing **I** with the corresponding aldehyde or by refluxing the corresponding 2-amino-4-chloromethylsulphonyldiphenyl oxide in EtOH with 5-bromo- or 3,5-dibromo-salicylaldehyde. The fungicidal activity of the azomethine deriv. formed from unsubstituted **I** was particularly high. The fungicidal activity of the Schiff bases depended mainly on the structure of the amine, and the effects of various substituents are discussed. G. W. Flinn.

New antiseptic for the protection of non-metallic materials. N. M. GOLYŠHIN, V. I. MONOVA and S. D. VOLODKOVICH (*Mikrobiologiya*, 1968, **37** (6), 1109–1115. Russ., 7 ref.).—Studies were made of the antiseptic Bromtane (1,1,5-trichloro-1,2-dibromopentane), which is very active both by contact and as vapour against mould fungi, e.g., *Chaetomium globosum*, *Aspergillus niger*, *A. amstelodami*, *Penicillium cyclopium*, *P. brevicompactum* and *Stachybotrys atra*, which attack hide, fabric, pigments, paper and wood. Bromtane has d_4^{20} 1·9322, b.p. (1 mm Hg) 85° and LD_{50} for mice 1815 and for rats 1820 mg/kg. At a dose of 1 mg/ml, it is active as vapour towards all fungi except *Penicillium cyclopium*, for which it is only fungistatic. The vapour is sometimes fungicidal at doses of 0·1 mg/ml. The antiseptic is equiv. to dinitrofluorobenzene in protecting wood, hide and fabric and can be employed at concn. of 2%. It is very stable towards i.r. and u.v. radiation. L. A. Haddock.

Infra-red determination of dithianon. R. C. DOUBLE (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 659–662. 5 ref.).—Concentrates or formulated powders, containing dithianon (**I**), zineb and S, are extracted with $CHCl_3$. **I** is determined from the i.r. absorption at 1257 cm^{-1}; 20–80 mg of **I** in 10 ml of $CHCl_3$ can be determined. D. I. Rees.

Insecticides and Others

Resistance of cotton strains with high gossypol content to *Heliothis* spp. M. J. LUKEFAHR and J. E. HOUGHTALING (*J. econ. Ent.*, 1969, **62** (3), 588–591. 10 ref.).—Strains of Upland cotton with a high gossypol content (*GC*) had significantly reduced populations of *Heliothis zea* and *H. virescens*. Bioassay with lyophilised squares in the larval diet showed that *GC* was related to resistance to attack. C. M. Hardwick.

New 8-row ground sprayer with auxiliary air for *ULV* application of pesticides [insecticides] to cotton. H. M. TAFT, A. R. HOPKINS, C. E. JERNIGAN and J. C. WEBB (*J. econ. Ent.*, 1969, **62** (3), 570–574. 13 ref.).—The sprayer is fitted with external atomisers and it is at least as effective as a conventional sprayer when used with toxaphene + DDT and azodrin. C. M. Hardwick.

Termite trail-following substance; isolation and purification from *Reticulitermes virginicus* and fungus-infected wood. F. MATSUMURA, A. TAI and H. C. COPPEL (*J. econ. Ent.*, 1969, **62** (3), 599–603. 6 ref.).—Chromatographic and spectroscopic analysis of the purified termite extract and of that from wood infected by *Lenzites trabea* showed the substances to be identical. C. M. Hardwick.

Laboratory investigation on Yurimin-99 as an experimental molluscicide. K. YASURAOKA, Y. HOSAKA and Y. KOMIYA (*Jap. J. Parasit.*, 1968, **17** (5), 376–381. Engl.).—3,5-Dibromo-4-hydroxy-4′-nitroazobenzene (**I**) was a more effective molluscicide in alkaline waters (pH 7·2–8·7) when compared with the same or even greater concn. in water of pH 4·5–5·2. **I** gave better results than Na pentachlorophenate as regards photochemical action. C. V.

Toxicity of selected acaricides to susceptible and resistant strains of *Tetranychus urticae* Koch. G. F. THIELE and R. A. HARRISON (*N.Z. Jl Sci.*, 1969, **12** (2), 380–394. 31 ref.).—Et esters of both parathion and azinphos had lower LD_{50} values than the Me esters for the susceptible strain, with azinphos-ethyl being the most toxic of the four organo-P compounds tested. Susceptible strain LD_{50} levels of Mercaptodimethur, dicofol and binapacryl indicated only slight differences in contact toxicity. Quinomethionate and thioquinox showed no contact toxicity. The resistant strain showed a high level of cross-resistance to parathion-ethyl and -methyl but a lower level of cross-resistance to azinphos-ethyl and -methyl; Mercaptodimethur also showed a limited degree of cross-resistance. There was no clear evidence of cross-resistance to dicofol and to binapacryl, although there was a 2½-fold increase in the LD_{50} for the latter with the resistant strain. E. G. Brickell.

Action of thiotepa on the fertility of the confused flour beetle. J. R. PARNELL and D. F. METTRICK (*J. econ. Ent.*, 1969, **62** (3), 585–588. 18 ref.).—Females treated with 40 mg/929 cm^2 of thiotepa produced significantly fewer offspring than did those treated with 5 mg. Untreated females mated with treated males also produced a significantly lower no. of offspring (78·6% reduction by a 20 mg dose for 1 h). C. H. Hardwick.

Resistance of insect parasitoids to the defence reactions of their hosts. G. SALT (*Biol. Rev.*, 1968, **43** (2), 200–232. 86 ref.).—A discussion and review. C. V.

Reaction products of chlorourea and tertiary phosphite esters. ARMOUR AGRICULTURAL CHEMICAL CO. (Br. Pat. 1,157,316, 29.6.66. U.S., 2.7.65).—The products, especially dialkyl ureido-phosphonates and -thiophosphonates, are useful as herbicides, pesticides, and germicides. In an example, a soln. of Me_3PO_3 in MeCN is added slowly to chlorourea in MeCN at 0–20°. After 5 h at 0°, ppt. is recryst. from water, to give dimethyl ureidophosphonate, m.p. 186–187°. F. R. Basford.

Herbicidal *m*-aminophenyl *N*-substituted carbamates. FMC CORP. (Br. Pat. 1,156,046, 10.10.66. U.S., 14.10.65).—The title compd. are of formula *m*-$C_6H_4(O\cdot CONRR^1)NHCOR^2$ where RR^1N is an amine residue (including heterocyclic amines) and R^2CO is a carboxylic acid residue (not formic). In an example, *m*-(2-methylvaleramido)phenyl ethylcarbamate, m.p. 167–169° (aq. MeOH), is prep. by reacting EtNCO with 3′-hydroxy-2-methylvaleranilide (prepn. described) is presence of Et_3N. S. S. Chissick.

Herbicidal pyrazone derivatives. BADISCHE ANILIN- & SODA-FABRIK A.-G. (Br. Pat. 1,155,380, 5.10.66. Ger., 13.10.65).—The title compd. contain a tartronic ester radical and are of formula $HO\cdot C(CO_2R^3)_2\cdot NH\cdot C(R^2)\cdot CO\cdot N(R^1)\cdot N{:}CH$, where R^1 is (substituted) Ph, cyclohexyl, or cyclo-octyl, R^2 is halogen, SMe or OMe and R^3 is benzyl or (substituted) alkyl. Prepn. is by condensing a 4-aminopyridazone with a mesoxalic acid ester. E.g., 1-phenyl-4-amino-5-chloropyridaz-6-one and diethyl mesoxalate are reacted in boiling xylene for 3 h to yield diethyl *N*-[4-(1-phenyl-5-chloropyridaz-6-onyl)]-aminotartronate, m.p. 131–132° (benzene–light petroleum). S. S. Chissick.

Controlling growth of noxious plants. QUEEN'S UNIVERSITY OF KINGSTON [Canada] (Inventors: C. D. NELSON and J. I. TOOHEY) (Br. Pat. 1,157,773, 25.1.68).—A method for controlling the growth of algae and noxious weeds without apparent effect on fish, insects or mammals, consists of applying 1-phenazine carboxylic acid (**I**) or hydroxyphenazine carboxylic acid (**II**) to the growth environment. **I** is preferred where the environment is water and **II** where the environment is soil. S. S. Chissick.

Controlling pests. MOBIL OIL CORP. (Inventor: J. H. WILSON) (Br. Pat. 1,155,078, 9.5.66).—Compd. of formula $(RS)_2P(O)OMe$ where R is Pr or Bu, are useful for controlling insects, mites and symphylans, Prepn. is from the corresponding RSH which is reacted with PCl_3, then with MeOH; the intermediate product is finally oxidised. E.g., PrSH is reacted with PCl_3 at room temp. and the product added to a mixture of MeOH and Et_3N in hexane at 0–5°. The resulting *S,S*-dipropyl *O*-methyl phosphorodithioite is converted to the corresponding thioate by reaction with H_2O_2. S. S. Chissick.

Nickel-containing monoalkyl phosphites. INTERNATIONAL NICKEL LTD. (Br. Pat. 1,157,530, 9.3.67. U.S., 24.3.66).—The compd., with nematocidal activity, are of formula $Ni[OP(OR)OH]_2$, (where R is alkyl of 1–12C) or $L_2 \rightarrow Ni[OP(OR)OH]_2$, where L is a ligand containing N, O or P as electron donor. An alkali metal salt of a monoalkyl phosphite is reacted with a Ni salt in a polar anhyd. org. solvent and then, if desired, the ligand (a C_{1-12} alcohol, an org. phosphine or org. phosphite) is added. S. D. Huggins.

Diseases and Pests in Livestock; Veterinary Treatments

Control of Exogenous Pests

No abstracts

Other Treatments

Efficacy of buquinolate [ethyl 4-hydroxy-6,7-di-isobutoxy-3-quinolinecarboxylate] against artificial coccidiosis infection in broiler chickens. C. R. SADLER, E. J. DAY and J. E. FRENCH (*Poult. Sci.*, 1968, **47** (6), 1917–1921. 6 ref.).—Addition of 0·00825% buquinolate (**I**) to the diet of broiler chickens artificially infected with five species of *Eimeria* permitted normal growth. Results were superior to those obtained with other coccidiostats used at recommended levels and there were no deaths among **I**-treated birds. Caecal lesions were usually lowest among **I**-treated birds. A. H. Cornfield.

Colorimetric determination of aklomide (2-chloro-4-nitrobenzamide) in poultry feed. G. M. GEORGE, A. C. DAFTSIOS and J. L. MORRISON (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 438–441).—The coccidiostat (**I**) is extracted by heating under reflux with MeOH. It is reduced with $TiCl_3$ and the amine produced is diazotised and coupled with *N*-naphthylethylenediamine and the colour produced is measured at 545 nm. Recoveries of ~0·03% of **I** in feed were ~105%. Sulfanitran and roxarsone do not interfere. D. I. Rees.

Chick œdema disease. VII. Effects of the oral diuretic, hydrochlorothiazide. D. F. FLICK and R. G. O'DELL (*Poult. Sci.*, 1968, **47** (6), 1886–1889. 11 ref.).—Addition of hydrochlorothiazide (0·5–1·0 g per kg of feed) to the diet of chicks to 3 weeks of age prevented œdema and reduced the blood dyscrasias associated with the disease. A. H. Cornfield.

Substituted benzoic acid lactones having oestrogenic properties. COMMERCIAL SOLVENTS CORP. (Br. Pat. 1,155,066 [A] and 1,155,255 [B], 18.5.67. U.S., 29.6.66 and 3.3.67).—The lactones, useful as oestrogenic agents and growth-rate promoters in meat-producing animals, have the formula $4,6,1,2\text{-}(OR)_2C_6H_2R^IR^{II}$ wherein R^I and R^{II} together represent a $CO\cdot O\cdot CHMe\cdot[CH_2]_m\cdot B\cdot[CH_2]_n\cdot A$; A is CH:CH or $[CH_2]_2$; and [A] R is H, alkyl, CH_2Ph, etc., *m* and *n* are 3; B is CO, CH(OH), or CH_2 (in which case A is $[CH_2]_2$); and the benzene nucleus is further substituted by one or two sulphonic acid (deriv.) groups; [B] R is H, alkyl, or CH_2Ph; *m* and *n* are 2; and B is $CH(CHO)\cdot COCH_2$ or $CH_2CO\cdot CH(CHO)$. F. R. Basford.

Household Pests, Sanitation, Food Hygiene

General Sanitation

Mechanisms of resistance of houseflies to *p,p'*-DDT and *o*-chloro-*p,p'*-DDT. G. M. HOLMAN (*Diss. Abstr. B*, 1969, **29** (11), 4216).—Penetration and metabolism of the two compounds were compared in 2 susceptible and 3 resistant strains. Detoxication of DDT to DDE is apparently the major resistance mechanism. Reduced penetration is a secondary mechanism in the strains studied. P. P. R.

Biochemical studies on warfarin. M. A. HERMODSON (*Diss. Abstr. B*, 1969, **29** (11), 4029).—A study of warfarin (*W*) metabolism and vitamin K requirements in rats having a hereditary increased tolerance to *W* is reported. The rate and manner of metabolism appeared normal, but vitamin K_1 and K_3 requirements in the *W*-tolerant rats were slightly higher than normal. Some metabolites of *W* are discussed. P. P. R.

Determination of zinc phosphide and its stability in rodent baits. G. O. GUERRANT and J. W. MILES (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 662–666. 16 ref.).—The sample (≡ 10–60 mg of Zn_3P_2) is heated under reflux with HCl and the liberated PH_3 is absorbed in acidified $KMnO_4$ soln. After removal of excess $KMnO_4$, P is determined by pptn. of quinolinium phosphomolybdate and its titration with NaOH. Zn_3P_2 was relatively stable (> 70% activity after 99 days) when used on apple and potato baits. D. I. Rees.

Primary treatment of potato processing wastes with byproduct feed recovery. L. M. GRAMES and R. W. KUENEMAN (*J. Wat. Pollut. Control Fed.*, 1969, **41** (7), 1358–1367. 2 ref.).—Potato processing plants produce large waste streams with high org. loads. The 5-day B.O.D. is ~1750 mg/l and suspended solids concn. is ~2600 mg/l. Screening reduces the B.O.D. by 10–15% and the suspended solids by 25%. Subsequent settling in a properly designed clarifier reduces the B.O.D. by 60% and the suspended solids by 90%. Settling of the flume and wash waters, which contain 20,000 mg/l of solids (mainly inorg.), in a clarifier with a 90-min retention time, reduces the suspended solids by > 95%. If the underflow sludge from the clarifiers is properly thickened and conditioned it can be dewatered by vac. filtration. The dewatered sludge is a valuable cattle feed. J. M. Jacobs.

Water treatment in the soft drink industry. H. C. DELONGE (*J. Am. Wat. Wks Ass.*, 1969, **61** (9), 469–472).—Possible water treatment equipment for the soft drink industry is described. Coagulation equipment used for municipal water of high quality, supported by sand filtration, C purification and polishing filter is described. Processing equipment and various units such as distillation, retention tank, etc., are discussed. J. C. T. N.

Dinoflagellate bloom (*Plectodinium nucleovolvatum* Biech) causing red water in Pietà Creek (Malta). H. MICALLEF and W. H. BANNISTER (*Experientia*, 1969, **25** (6), 655).—The appearance, behaviour and occurrence of this dinoflagellate (*D*) is described; cell counts gave values of 250,000–420,000 per ml of water. The bloom coincided with the warming of the water (16·0–18·5°) together with relatively calm weather. *D* have been known to cause mass mortality amongst fishes and other marine organisms; this was not found in this area and in this case at least it is assumed that this *D* is non-toxic and a normal part of zooplankton, being probably a potential source of food for marine animals. C. V.

Food Hygiene

Naturally occurring toxicants in foods. J. M. COON (*Fd Technol., Champaign*, 1969, **23** (8), 1041–1045. 28 ref.).—Toxicants in normal foods are listed, with brief descriptions of effects. Research into their long-term effects is urged. M. T. Rawnsley.

Sporulation and survival of *Clostridium perfringens*. F. R. STEELE, JUN. (*Diss. Abstr. B*, 1969, **29** (11), 4220).—A medium for growing food poisoning strains of *C. perfringens* is described. P. P. R.

Efficacy of methyl, propyl, butyl and heptyl esters of *p*-hydroxybenzoic acid as inhibitors of *Clostridium botulinum* Types A, B and E. A. A. GONSALVES (*Diss. Abstr. B*, 1969, **29** (11), 4014–4015).—In pork infusion agar, these compounds were inhibitory to *C. botulinum* in concn. which are feasible for use in foods. Antimicrobial effectiveness increased with length of alkyl ester side chain. Much greater concn. were required to prevent growth in fish homogenate and the compounds exhibited a reversed order of effectiveness,

i.e., the heptyl ester was least effective. Presence of NaCl slightly reduced the concn. required to prevent growth. P. P. R.

Bacteriological survey of chicken eviscerating plants. B. F. SURKIEWICZ, R. W. JOHNSTON, A. B. MORAN and G. W. KRUMM (*Fd Technol., Champaign*, 1969, **23** (8), 1066–1069. 20 ref.).—Salmonellae were isolated from 20·5% of 244 eviscerated birds, and also from swabs and chill-tank waters. The incidence of this infection was, unlike bacterial counts, not greatly altered by continuous chilling. It is suggested that chlorine or similar compounds should be added to chill-tanks and/or pre- or post-chill dips. M. T. Rawnsley.

Extraction and analysis of aflatoxins from cured and aged meats. L. B. BULLERMAN, P. A. HARTMAN and J. C. AYRES (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 638–641. 15 ref.).—The homogenised sample is extracted with $CHCl_3$, interfering substances are removed on silica gel and aflatoxins (**I**) are determined after t.l.c. by comparison of the fluorescent spots with standards. Recoveries of added **I** are high, and 0·0025 ppm of **I**-B_1 in meat can be detected. D. I. Rees.

Modification of method for [determining] aflatoxins in milk. M. S. MASRI, J. R. PAGE and V. C. GARCIA (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 641–643. 2 ref.).—The modification (for aflatoxin M) consists of direct extraction of the sample with MeOH–H_2O (1 : 1). Aflatoxin M is then separated by t.l.c. D. I. Rees.

Method for extraction of light filth from flours by defatting and a mineral oil flotation. J. J. THRASHER (*J. Ass. off analyt. Chem.*, 1969, **52** (3), 467–469. 2 ref.).—A method involving acid hydrolysis of the defatted flour was found to be applicable to flours of high bran content. D. I. Rees.

An acid autoclave method for mineral oil extraction of light filth from spaghetti and macaroni. J. J. THRASHER (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 463–465. 2 ref.).—The new method is faster and gives improved recoveries compared with the A.O.A.C. method 36·025. D. I. Rees.

Comparison of white gasoline and n-heptane for recovery of insect fragments and rodent hairs from food products. J. J. THRASHER and P. M. BRICKEY, JUN. (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 465–467. 2 ref.).—n-Heptane used as a flotation oil [A.O.A.C. method 36·002(o)] gave higher recoveries than did gasoline. D. I. Rees.

Method for extraction of extraneous materials from unground cassia [cinnamon]. A. L. ROAF and P. M. BRICKEY, JUN. (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 469–470).—Adhering extraneous material is removed from the bark with HCl, double sieving separates larger pieces of bark from the filth elements, and a further extraction follows. Av. recoveries of whole insects and rodent hairs were 95 and 73%, respectively. D. I. Rees.

Evaluation of some antifungal agents for use in the dairy industry. S. G. KULKARNI, R. A. SRINIVASAN and A. T. DUDANI (*Indian J. Dairy Sci.*, 1968, **21** (4), 221–227. 9 ref.).—None of several commercial antifungal agents were found to have a fungicidal effect on *Penicillium*, *Aspergillus* or *Mucor* spp. at concn. of 10,000 ppm and an exposure time of 10 min. The addition of 0·15% of K sorbate to processed cheese considerably improved keeping quality at 4° without affecting flavour. M. O'Leary.

New technique and equipment for evaluating the cleanliness of washed milk bottles. S. D. SHARMA, R. K. MURALIA and D. S. SARASWAT (*Indian J. Dairy Sci.*, 1968, **21** (4), 255–257).—A description is given of a light measuring device, based on nephelometric principles, for evaluating the cleanliness of washed milk bottles. M. O'Leary.

Contamination by Pesticides

Soil, Air, Water

Distribution of pesticides in surface waters. P. H. KING, H. H. YEH, P. S. WARREN and C. W. RANDALL (*J. Am. Wat. Wks Ass.*, 1969, **61** (9), 483–486. 7 ref.).—Batch-type sorption studies were designed to indicate pesticide distribution in selected environments and to enable development of an isothermal relationship describing the results. The sorbents included soil, algae, coal and activated C. Pesticides used were lindane and parathion. The Freundlich equation was used. In adsorbing parathion, the algal systems were one order of magnitude greater than soil, coal was 2–2½ orders greater and activated C about 4 orders. In contrast to the soils, the sorption by algal systems did not decrease proportionally with increasing pesticide concn. Thus, pesticides may be introduced into the food chain and may eventually be concentrated in higher animals, e.g., fish. J. C. T. N.

Crops, Livestock, Food

Alkaline pre-column for use in gas chromatographic pesticide residue analysis. G. A. MILLER and C. E. WELLS (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 548–553. 4 ref.).—The pre-column consists of a series of layers of KOH and of NaOH (25% of each) on Gas Chrom Q. This pre-column destroys some pesticides (organo-phosphate type), changes the retention of others (e.g., DDT) and leaves some (e.g., aldrin) unaltered. Interfering non-pesticide peaks from crops are often eliminated. D. I. Rees.

Bratton–Marshall reagent for detection of substituted urea herbicides and metabolites on t.l.c. plates. J. H. ONLEY and G. YIP (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 545–547. 5 ref.).—Analysis of 8 urea herbicides in crop samples by t.l.c. is described. Recoveries of 0·025–2·0 ppm of the herbicides added to crops were > 70%. D. I. Rees.

Analysis of a single crop extract for substituted urea herbicides and metabolites, chlorinated insecticides and amitrole. J. H. ONLEY and G. YIP (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 526–532. 10 ref.).—The MeCN extract is extracted with light petroleum and analysed for chlorinated pesticides (*ibid.*, 1963, **46**, 186). The substituted ureas and metabolites are then extracted from the residual MeCN with CH_2Cl_2 and CCl_4, and analysed, after clean-up, by t.l.c. on alumina GF. Amitrole remaining in the MeCN layer is isolated by ion-exchange chromatography and determined colorimetrically. D. I. Rees.

Charcoal column clean-up method for many organophosphorus pesticide residues in crop extracts. R. R. WATTS, R. W. STORHERR, J. R. PARDUE and T. OSGOOD (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 522–526. 12 ref.).—The EtOAc extract is placed on a column of charcoal-hydrated Sea Sorb 43–Celite 545 (1 : 2 : 4); the pesticides are eluted with EtOH–C_6H_6 (1 : 4) and analysed by g.l.c. D. I. Rees.

Gas chromatographic determination of residues of dimethoate and its oxygen analogue in field-sprayed kale. R. W. STORHERR and R. R. WATTS (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 511–513. 9 ref.).—Significant residues of the O-analogue of dimethoate were found when 0·5 or 1 lb/acre of dimethoate was sprayed on kale. The ratio of dimethoate to its O-analogue was 1 : 4 after 14 days. D. I. Rees.

Degradation of Supracide in hay, silage and the rumen. C. E. POLAN, R. A. SANDY and J. T. HUBER (*J. Dairy Sci.*, 1969, **52** (8), 1296–1299. 6 ref.).—Supracide, an organo-P insecticide, was shown to have a logarithmic rate of degradation in silage and hay for 200 and 120 days, resp. Incubation of ^{14}C-labelled Supracide with bovine rumen contents resulted in a linear increase in formation of water-sol. products. Disappearance of the insecticide from the intact rumen was logarithmic, and after 2 h < 20% of the initial concn. remained. M. O'Leary.

Responses from cows fed silages containing Dursban residues. J. C. JOHNSON, JUN., M. C. BOWMAN and D. B. LEUCK (*J. Dairy Sci.*, 1969, **52** (8), 1253–1257. 20 ref.).—Spraying of maize with Dursban [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate] at rates of 0·28, 0·56 and 1·12 kg/ha resulted in levels of 2·71, 5·87, and 11·59 ppm of Dursban (dry basis), resp., in maize silage 92 days after ensiling. Trials with cows showed that these residues did not cause a depression in silage intake or milk production. The milk and urine of cows ingesting residues at averages of 0·04–0·17 mg/kg body wt. for 49 days were free of Dursban (< 0·002 ppm) and its oxygen analogue (< 0·005 ppm). Except for a slight depression of blood cholinesterase activity, ingestion of the Dursban residues had no ill effect on the cows. M. O'Leary.

Pesticide residues. III. Ronnel [fenchlorphos] residues in the perirenal and omental fat of cattle following dermal applications. J. E. MCLAUGHLIN (*Queensland J. agric. Anim. Sci.*, 1968, **25** (1), 1–5. 10 ref.).—Residues were determined in these fats 1, 4, 7, 10, 13 or 16 days after one application (2 gal, 0·1%). Max. level (2·63 ppm) was noted in the omental fat after 4 days and min. (0·09 ppm) in the perirenal fat 16 days after treatment. When deposition was

complete the residues were consistently higher in omental than in perirenal fat. C. V.

Application of malathion to the laying hen. W. W. MARION, J. M. J. NING and SHU MIN NING (*Poult. Sci.*, 1968, **47** (6), 1956–1961. 14 ref.).—Addition of 250–500 ppm of malathion (**I**) to the feed or supplying an equiv. amount orally did not result in any egg or tissue residues of **I**. Spraying the hens and nest with **I** (1·5 gal of 1% soln. per 1000 ft²) produced significant residues in nest litter and on the skin and feathers of the birds for at least 9 weeks. Only trace amounts of **I** were detected in egg contents. Trace amounts were found in the blood and kidney, but not in the liver.
A. H. Cornfield.

DDT induces a decrease in eggshell calcium. J. BITMAN, H. C. CECIL, S. J. HARRIS and G. F. FRIES (*Nature, Lond.*, 1969, **224** (5214), 44–46. 13 ref.).—Japanese quail (39-days-old) fed *o,p'*- and *p,p'*-DDT (100 ppm of each to a low Ca diet) produced eggs with thinner shells (6·2–6·8 mil) and lower Ca content (~1·95%) than the control birds. Although seed-eating birds are hardly likely to encounter such high amt. of these pesticides, there is ample evidence that carnivorous birds have been exposed to very high concn. Possible mechanisms by which chlorinated hydrocarbons affect eggshell thickness are indicated. W. J. Baker.

[Pesticide] residues in food and feed. (*Pestic. Monitoring J.*, 1969, **3** (2), 70–101).—Seven papers describing a cooperative study. **Residues of endrin and DDT in turnips grown in soil containing these compounds.** W. B. WHEELER, H. A. MOYE, C. H. VAN MIDDELEM, N. P. THOMPSON and W. B. TAPPAN (72–76. 7 ref.). **Residues of endrin and DDT in soyabeans grown on soil treated with these compounds.** B. F. BARRENTINE and J. D. CAIN (77–79. 2 ref.). **Residues of DDT and dieldrin in peanuts [groundnuts] and tobacco grown on contaminated soil.** T. J. SHEETS, M. D. JACKSON, W. J. MISTRIC and W. V. CAMPBELL (80–86. 10 ref.). **DDT residues in tobacco and soyabeans grown in soil treated with DDT.** J. K. REED and L. E. PRIESTER (87–89. 2 ref.). **Residues of DDT and endrin in peanuts [groundnuts] and soyabeans grown in soil containing these pesticides.** H. W. DOROUGH and N. M. RANDOLPH (90–93. 4 ref.). **Residues of dieldrin and DDT in peanuts [groundnuts] and turnip greens grown in soil containing these compounds.** R. W. YOUNG (94–99. 3 ref.). J. C. T. N.

Gas chromatographic method for ronnel in feeds. A. J. GEHRT (*J. Ass. off. analyt. Chem.*, 1969, **52** (3), 435–438).—The cattle feed containing < 0·1% of ronnel is shaken with acetone and analysed by g.l.c. Recoveries were 81–98%. D. I. Rees.

Possible toxicity of fish flour [due to dichloroethylene residues]. ANON. (*Nutr. Rev.*, 1968, **26** (2), 58–61).— C. V.

4.—MISCELLANEOUS

Loss of cerium, cobalt, manganese, protactinium, ruthenium and zinc during dry ashing of biological material. P. STROHAL, S. LULIĆ and O. JELISAVČIĆ (*Analyst, Lond.*, 1969, **94** (1121), 678–680. 10 ref.).—Results obtained with *Mytillus galloprovincialis* Lam. after uptake (1–7 days) of radioactive Ce, Co, Mn, Pa, Ru and Zn from sea-water showed that during heating of the soft tissues up to 800° in air, all these elements were partly volatilised, even at low ashing temp. of 100–110°. Wet ashing with H_2O_2 at room temp. is therefore recommended for detn. of radioactive contaminants in such biological samples. W. J. Baker.

Separation of bacteria by adsorption on to ion-exchange resins. S. L. DANIELS (*Diss. Abstr. B*, 1968, **29** (4), 1336).—Particles of either anion- or cation-exchange resins were contacted with agitated suspensions containing bacterial cells. The variables of cell concn., resin concn. and particle size, pH, salt concn., agitation, time of contact, and temp. were evaluated. Mixed suspensions of 2 bacterial species were resolved with > 90% efficiency by either selective adsorption of one species, or non-selective adsorption of both species followed by desorption. Potential applications of the phenomena in fermentation technology, waste and water treatment, and clinical microbiology are mentioned. P. R. G.

Radiation safeguards for microwave ovens. C. E. TIBBS (*Non-ioniz. Rad.*, 1969, **1** (2), 73–76).— P. C. W.

5.—RECENT BOOKS AND JOURNALS

Soil organic matter: its nature, its rôle in soil formation and in soil fertility. M. M. KOMONOVA, transl. T. Z. NOWALOWSKI and A. C. D. NEWMAN. 1966, 2nd Engl. edn., 544 pp., 66 pp. bibliog. (Oxford, etc.: Pergamon Press). S. C. H.

Tea growers handbook. 1969. TEA RESEARCH INSTITUTE OF EAST AFRICA. 1969, 151 pp. (Nairobi: Tea Boards of Kenya, Tanganyika & Uganda). S. C. H.

Animal nutrition. P. MCDONALD *et al.* Reprinted 1969, 407 pp., 2 gns. (Edinburgh: Oliver & Boyd). S. C. H.

Selenium in biomedicine. Ed. O. H. MUTH. 1967, 445 pp. (Westport, Conn: Avi Publishing Co. Inc.).—Of the 26 contributions, the following are specially noted: **Relation of geochemistry of selenium to its occurrence in soils.** H. W. LAKIN and D. F. DAVIDSON (29 ref.). **Distribution of selenium in plants.** C. M. JOHNSON, C. J. ASKEW and T. C. BROGER (33 ref.). **Levels of selenium in animal tissues and methods of selenium administration.** W. J. HARTLEY. **Analytical methods for selenium in biological material.** J. H. WATKINSON (73 ref.). **Selenium in human nutrition.** L. L. HOPKINS, JUN. and A. S. MAJAJ (17 ref.). **Selenium deficiency: clinical aspects and physiological responses for farm animals.** H. E. OKSANEN (57 ref.). **Selenium deficiency in chicks and poults.** M. L. SCOTT (12 ref.). C. V.

Canned foods: an introduction to their microbiology. A. C. HERSOM and E. D. HULLAND. 1969, 6th edn., 319 pp. (London: J. & A. Churchill Ltd.). S. C. H.

Human nutrition and dietetics. S. DAVIDSON and R. PASSMORE. 1969, 4th edn., 899 pp., 5 gns. (Edinburgh & London: E. & S. Livingstone Ltd.). S. C. H.

Pests of rice. D. H. GRIST and R. J. A. W. LEVER. 1969, 520 pp., 150/-. (London & Harlow: Longmans Green & Co. Ltd.). S. C. H.

World crop protection. Vol. 1. Pests and diseases. J. H. STAPLEY and F. C. H. GAYNER. **Vol. 2. Pesticides.** K. A. HASSALL. 1969, vol. 1, 270 pp.; vol. 2, 249 pp., 170/- net. (London: Iliffe Books Ltd.). S. C. H.

Degradation of herbicides. Ed. P. C. KEARNEY and D. D. KAUFMAN. 1969, 394 pp. (New York: Marcel Dekker Inc.). S. C. H.

Fungicides. Ed. D. C. TORGESON. **Vol. 1. Agricultural and industrial applications, environmental interactions.** 1967, 697 pp. **Vol. 2. Chemistry and physiology.** 1969, 742 pp. (New York & London: Academic Press). S. C. H.

Tropical nematology. Ed. G. C. SMART, JUN. and V. G. PERRY. 1968, 153 pp., $8.50. (Gainesville, Fla.: University of Florida Press).—Papers presented at the 5th Ann. Meeting of the Soc. of Nematologists, Aug. 1966, including: ***Radopholus similis* and other nematode species on banana.** E. J. WEHUNT and D. I. EDWARDS (1–19. 83 ref.); **Burrowing nematode decline of citrus: A review.** E. P. DU CHARME (20–32. 93 ref.); and **Plant parasitic nematodes and soil management practices.** J. M. GOOD (113–138. 81 ref.). S. C. H.

Micro-organisms in foods: their significance and methods of enumeration. F. S. THATCHER and D. S. CLARK. 1968, 234 pp., $12.50. (Toronto: University of Toronto Press). S. C. H.

Economics of water utilisation in the beet sugar industry. G. O. G. LÖF and A. V. KNEESE. 1968, 125 pp., $4.00. (Washington, D.C.: Resources for the Future Inc.). S. C. H.

Methods in microbiology. Ed. J. R. NORRIS and D. W. RIBBONS. 1969, vol. 1, 712 pp. (London & New York: Academic Press). S. C. H.

Comprehensive biochemistry. Vol. 17. Carbohydrate metabolism. Ed. M. FLORKIN and E. H. STOTZ. 1969, 308 pp. (Amsterdam, etc.: Elsevier Publishing Co.). S. C. H.

Non-ionising Radiation. 1969, **1** (1), quarterly. (London: Iliffe Science & Technology Publications Ltd.).—Effects, uses and safety of electromagnetic radiation, from r.f. through microwaves and i.r. to the optical region. P. C. W.

Journal of the American Association for Contamination Control. 1968, **1** (1), semi-annual. (Boston: American Association for Contamination Control). P. C. W.

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