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Evaluation of Barley as a Source of Protein for Chicks

I. Variety and Nitrogen Application in Relation to Protein Content and Amino-acid Composition of the Seed

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Seventy-six samples of barley embracing 14 varieties grown under various conditions of nitrogen fertilisation in different locations in the U.K. were examined for nitrogen content and 14 samples were analysed for their total content of amino acids other than tryptophan.

Top dressing with nitrogenous fertilisers during growth resulted in the production of grain with a higher nitrogen content as well as an increased overall yield of nitrogen/hectare. The growth locality had a greater effect upon the nitrogen content of the harvested seed than did the application of nitrogen to the growing crop.

Lysine content was inversely proportional to crude protein content for barleys containing between 8 and 11% crude protein and, in consequence, within this range the yield of lysine/ton of grain was of the same order.

1. Introduction

The importance of cereals as contributors of protein in practical diets for non-ruminants tends still to be underestimated. In addition to providing energy, they may contribute more than half of the total dietary protein. While considerable effort is devoted to maintaining and improving the quality of the protein in the supplementary fish meal or oilseed meal, relatively little attention is paid to the cereal protein. It is generally conceded that protein concentrates should complement the admittedly poor amino-acid composition of the cereals, but the possibility that the cereal protein itself might be improved or that individual cereals might differ in the quality of their protein constituents is rarely acknowledged.

The development of high-lysine maize has, of course, stimulated interest in improving the nutritive value of other cereals genetically and collaboration between plant breeders and nutritionists is already established in this country and in the U.S.A. with the object of developing high-lysine barley. Even if such a barley containing all of the other desirable characteristics of yield, disease resistance, etc., can be found, its development will inevitably take some years and the possibility that the conventional cereals which are already grown, might differ significantly in their nutritional qualities seems worthy of exploration.

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The relative nutritive value of the principal cereals has been studied from time to time and differences have been found to be small.[1,2,3] Although on balance the evidence available suggests that oats is superior in quality to the other cereals,[4,5,6] the sometimes conflicting results and small differences noted combine to suggest that the strain and history of individual samples of cereal might be at least as important as the difference between types. "Wheat" and "barley" may well be quite inadequate terms, each embracing samples showing a wide range of quality difference.

The work described here embodies a study of barley only and comparisons have been made between samples of barley of several varieties grown in different localities and with differing levels of nitrogen applied during growth. In part I the effects of these differences will be discussed in relation to the protein content of the seed and to its amino-acid composition.

2. Experimental

2.1. Materials

Seventy-six samples of barley were examined in all and these fell into the following four groups.

1. 18 samples (1964 harvest) supplied by the National Institute for Agricultural Botany, Cambridge, consisting of three varieties, Proctor, Rika and Zephyr each grown at three locations, Cambridge, Sutton Bonington (Leicestershire) and Wye (Kent) and with two levels of nitrogen application during growth at each location.
2. 21 samples (1966 harvest) and 24 samples (1967 harvest) were supplied by the Norfolk Agricultural Station, Sprowston. The 1966 series consisted of small samples of seven varieties—Deba Abed, Europa, Impala, Maris Concord, Mosane, Proctor and Zephyr and the 1967 series consisted of larger quantities of the same seven varieties with, in addition, Maris Druid. Each variety was treated during growth with three levels of nitrogen.
3. Seven varieties of locally-grown Aberdeenshire barleys (1967 harvest). These were Impala, Ingrid, Miln's Golden Promise, Pallas, Ruby, Ymer and Zephyr. Also one further Aberdeenshire barley of unknown variety but known amino-acid composition was added to this series.
4. Ymer grown in Aberdeenshire with five levels of nitrogen applied in the seed-bed.

2.2. Methods

2.2.1. Amino-acid analysis

The method of Moore, Spackman and Stein[7] was used. Estimations were carried out on two or more hydrolysates from each barley sample. Hydrolysis of the barley protein was by 6 N-HCl in a deep sand-bath for 18 h (sand-bath temperature = 110 to 115 °C) and separation of the amino acids was carried out employing 5.5 h runs on a Technicon Auto-Analyzer with a 75 × 0.6 cm column of Technicon "Chromo Beads". An automatic sample loading device and valve controller were used.[8]

2.2.2. Nitrogen

An A.O.A.C. method was used.[9]

TABLE 1. Proximate analysis and agronomic characteristics of 18 NIAB barley samples

Code no.	Variety	Location	Nitrogen applied (kg/hectare)	Yield (kg/hectare)	Seed protein content (N × 6.25)	Protein yield (kg/hectare)	Moisture (%)	Fat (%)	Fibre (%)	Ash (%)	m.e. (kcal/kg)
PLC	Proctor	Cambridge	44	3515	7.71	271	14.4	1.92	3.16	2.03	3013
PHC	Proctor	Cambridge	87	3992	8.74	349	13.3	2.00	3.45	1.97	2929
PLS	Proctor	Sutton Bonington	20	5109	11.6	593	12.8	0.85	3.76	2.38	3030
PHS	Proctor	Sutton Bonington	40	5222	12.5	653	13.6	2.38	3.95	2.31	3212
PLW	Proctor	Wye	41	3980	9.16	364	13.2	1.79	3.34	1.88	2928
PHW	Proctor	Wye	83	4356	10.1	439	14.5	1.39	3.78	1.90	3186
RLC	Rika	Cambridge	44	3026	8.30	251	13.4	1.95	3.56	2.03	3048
RHC	Rika	Cambridge	87	3427	9.39	321	13.5	2.35	3.37	1.89	2843
RLS	Rika	Sutton Bonington	20	5122	11.6	594	13.2	0.87	3.46	2.28	3102
RHS	Rika	Sutton Bonington	40	5147	12.1	623	13.0	1.85	4.33	2.22	2937
RLW	Rika	Wye	41	4155	9.78	407	14.4	1.18	3.65	2.06	2996
RHW	Rika	Wye	83	4093	10.3	422	13.9	1.24	3.23	1.79	2957
ZLC	Zephyr	Cambridge	44	3816	7.30	279	14.3	1.01	3.38	2.00	2910
ZHC	Zephyr	Cambridge	87	4193	8.53	358	13.3	0.76	3.42	1.92	2909
ZLS	Zephyr	Sutton Bonington	20	5787	10.4	601	12.8	1.70	3.15	2.14	2852
ZHS	Zephyr	Sutton Bonington	40	5888	11.5	677	12.4	1.83	3.43	2.35	3001
ZLW	Zephyr	Wye	41	4432	8.58	380	14.0	1.85	3.67	1.82	2964
ZHW	Zephyr	Wye	83	4833	9.98	482	12.6	0.91	3.48	2.01	2775

2.2.3. Moisture

An A.O.A.C. method was used.[10]

3. Results and discussion

3.1. The effect of nitrogen application on the yield and crude protein content of barley seed

In practically all cases increases in the level of nitrogen applied to the crop during growth have resulted in increased levels of nitrogen within the seed (Tables 1 and 2). The increases are almost always of the order of 10% based on the nitrogen content of the seed which received the lowest level of nitrogen and for each pair of samples this increase has been effected by doubling the amount of nitrogen applied: 53 to 105 kg/hectare

TABLE 2. The effect of nitrogen application on crude protein content for eight varieties of barley grown in Norfolk in 1966 to 67

Code no.	Variety	Nitrogen applied (kg/hectare)	1966 Seed protein content (N × 6.25)	1967 Seed protein content (N × 6.25)
DLN	Deba Abed	53	7.93	8.32
DMN	Deba Abed	80	8.18	8.31
DHN	Deba Abed	105	8.89	9.07
ELN	Europa	53	7.81	7.94
EMN	Europa	80	8.18	7.97
EHN	Europa	105	8.89	8.91
ILN	Impala	53	8.06	8.82
IMN	Impala	80	8.65	9.16
IHN	Impala	105	9.37	8.94
MCLN	Maris Concord	53	8.37	8.13
MCMN	Maris Concord	80	8.91	9.16
MCHN	Maris Concord	105	9.71	8.94
MLN	Mosane	53	7.79	8.18
MMN	Mosane	80	8.28	8.72
MHN	Mosane	105	8.85	8.85
PLN	Proctor	53	8.17	8.38
PMN	Proctor	80	8.47	8.82
PHN	Proctor	105	8.97	9.44
ZLN	Zephyr	53	7.43	7.66
ZMN	Zephyr	80	8.03	8.35
ZHN	Zephyr	105	8.22	8.56
MDLN	Maris Druid	53	—	8.16
MDMN	Maris Druid	80	—	9.06
MDHN	Maris Druid	105	—	9.44

(Norfolk), 44 to 87 (Cambridge), 41 to 83 (Wye) and 20 to 40 (Sutton Bonington). It is clear, however, from Table 1 that the increase in nitrogen content of the seed achieved by the use of additional fertiliser is small compared with the differences observed between the products of the same seed grown in different localities. Barleys grown at Sutton Bonington contained more nitrogen (and less lysine) than those grown in Norfolk though the latter received considerably more nitrogen during growth. Yields of grain

showed the same pattern, the highest yields being obtained at Sutton Bonington in each case, though the nitrogen applied was less than half that given at either Cambridge or Wye. It is unfortunate that no information is available regarding the soil composition at the different locations at time of sowing.

Work carried out in the United States and Canada on barley and wheat has yielded results all of which agree in confirming that nitrogen produced the largest yield increases when it was applied at sowing time or in the very early stages of growth and that later applications produced progressively smaller yield increases.[11–13] Nitrogen applied late on the other hand, to barley, wheat or oats, gives high quality grain of superior milling properties and high protein content.[13–18] However, not inconsiderable increases in protein content have been reported resulting from the application of nitrogen in the seed bed. Irish workers, for example, found an increase of 23% in seed protein from Proctor grown with 52 kg of nitrogen/hectare applied at sowing.[19] This compares with increases of the order of 40 to 60% reported from France and Canada when the nitrogen has been applied as a top-dressing during growth.[14,20,21] Smaller increases than this have been reported. Scottish-grown Ymer and Pallas barley, which received up to 101 kg/hectare, increased in protein content by 7 to 9% and none of the increase was due to the first 50 kg/hectare.[22] Though not stated in the paper referred to above, it has been ascertained that the whole of the nitrogen was applied at the time of sowing (personal communication). In Canada 54 kg of nitrogen/hectare in the seed bed increased the protein content of wheat by only 3%.[23] The five samples of Aberdeenshire-grown Ymer which were included in the present study showed no response to the provision of up to 76 kg of nitrogen/hectare in the seed bed (Table 3).

TABLE 3. Crude protein content for Aberdeenshire-grown Ymer barley with five levels of nitrogen provided in the seed bed

Nitrogen applied (kg/hectare)	Seed protein ($N \times 6.25$)
0	9.35
19	9.72
38	9.30
57	9.47
76	9.55

All barleys in the NIAB series received their supplementary nitrogen in the form of top-dressing during growth. No figures are available for corresponding samples with no added nitrogen but doubling of the applied nitrogen has increased yield by 4.3 to 16.8%. The largest increases were shown by Zephyr barley ranging from 10.6 to 16.8% and the smallest by Rika, ranging from 4.3 to 13.1%. It is clear that location is more important than variety here, because the largest increases were shown by those grown at Cambridge (Proctor 13.3%, Rika 13.1%, Zephyr 16.8%). Zephyr grown at Wye, however, increased by 16.3%. Sutton Bonington-grown samples invariably contained the

highest levels of protein, but showed the smallest difference between the two levels of nitrogen application.

A rather similar picture is shown by the grain yields. The increase in yield from the doubled nitrogen level was greatest at Cambridge (Proctor 13.5%, Rika 13.3%, Zephyr 9.9%), but Zephyr was not significantly superior to the other two varieties on the basis of yield. Again Sutton Bonington samples all showed the highest overall yields of grain coupled with low increases on doubling the nitrogen level. Rika grown at Wye was exceptional in that the yield of grain from the higher level of applied nitrogen was lower than that from the lower level.

All of the Norfolk barleys (Table 2) were grown in a single field at Sprowston. Three levels of nitrogen were applied as top-dressing during growth, but again no information is available for samples with no added nitrogen and yields are not known. Increase in the nitrogen application from 53 to 80 kg/hectare added 3.1 to 8.1% protein to the resulting seed in 1966 and 0 to 12.7% in 1967. A further 27 kg/hectare added 2.5 to 9.5% in 1966 and 0 to 11.8% in 1967. Individual varieties did not show a constant response in the two years although certain similarities were noticeable. For example, Deba Abed and Europa gave a small response to 80 kg of nitrogen/hectare in both years but responded well to the second 27 kg increment in terms of increased protein content. On the other hand Impala showed the biggest increase in protein content from 105 kg of nitrogen/hectare in 1966 (16.2%) but the smallest in 1967 (1.4%).

On the basis of these results it would be difficult to make out a case for particular varieties being more responsive to added nitrogen. Zephyr responded well, Proctor somewhat less well and Rika was poorest, in the NIAB series. Proctor and Zephyr also responded well in Norfolk in 1967 but were somewhat inferior to Europa, Impala, Maris Concord and Mosane in 1966. Maris Druid gave a high response in 1967 but was not available in 1966. Work reported to have been carried out at the Plant Breeding Institute, Cambridge, suggests that of the varieties currently available, the maximum yield of protein is likely to come from those giving the highest yield of grain, although the varieties containing the highest proportion of nitrogen are in fact the lowest yielding.[24] In particular, it was noted that Zephyr, Maris Druid, Impala and Maris Badger produced the most protein in the grain. Under the conditions of growth for the NIAB samples used in the present work, however, Zephyr was not only the highest yielder at each of the three locations but it also responded best to nitrogen treatment although it did not contain as high a level of protein as did Proctor and Rika. Despite this the yield of protein/hectare was higher than for either of the other two varieties with the single exception of Wye-grown Rika at the lower level of nitrogen application (RLW).

In general the results support the view that top-dressing of barley with nitrogen is beneficial in that it promotes a higher protein content in the grain and an overall increase in the yield of protein per hectare. Further, though varietal differences may be apparent, as has been shown in Canada for wheat,[23,25] the environmental factors may be expected to play the major part in determining the ultimate protein content of the seed.

3.2. The relationship between amino-acid composition, protein content and nutritive value

Complete analyses for all of the essential amino acids excepting tryptophan in 14 of the barley samples used, are listed in Table 4. In the present state of knowledge regarding

TABLE 4. The amino-acid composition of 14 samples of barley from Cambridge, Sutton Bonington (Leicestershire), Sprowston (Norfolk) and Aberdeenshire (g/16 g. N)

Code (variety):	PHS	RHS	RLS	PLS	ZHS	Ruby	ZLS	Unknown	PHN	EHN	PLN	ELN	PLC	Miln's Golden Promise
N application (kg/hectare):	40	40	20	20	40	Unknown	20	Unknown	105	105	53	53	44	Unknown
N × 6.25	12.5	12.1	11.6	11.6	11.5	11.2	10.4	10.3	9.44	8.91	8.38	7.94	7.71	7.06
Aspartic acid	6.7	5.2	6.6	6.8	6.1	5.7	6.1	6.8	6.4	7.2	7.6	6.7	7.7	7.0
Threonine	3.5	3.4	3.7	3.9	3.7	3.2	3.9	3.7	3.5	3.9	3.4	3.7	4.0	3.6
Serine	4.2	3.9	4.2	4.6	4.1	3.9	4.4	4.7	4.2	4.3	4.8	4.1	4.4	4.2
Glutamic acid	23.3	22.9	22.1	25.3	23.3	23.8	23.7	22.8	22.2	20.8	23.1	19.0	20.7	22.2
Proline	13.3	10.9	11.9	12.2	12.0	10.0	9.7	11.9	12.1	10.9	10.6	9.7	12.0	9.9
Glycine	4.0	3.8	3.7	4.5	4.0	4.0	4.3	4.1	4.1	4.2	4.4	4.2	4.5	4.5
Alanine	4.0	4.0	3.9	4.4	3.9	3.6	3.9	4.0	4.4	4.5	4.6	4.6	4.6	4.3
Valine	5.1	5.3	5.0	5.6	5.1	4.8	5.2	5.3	5.2	5.6	5.8	5.2	6.0	5.1
Cystine	2.5	1.8	1.4	2.0	2.0	2.1	1.7	2.4	1.9	1.9	1.9	1.7	2.6	2.0
Methionine	1.8	1.5	1.3	1.4	1.8	1.5	1.4	1.5	1.5	1.5	1.4	1.4	1.9	1.6
Isoleucine	3.6	3.6	3.6	3.9	3.8	3.4	3.5	3.3	3.7	3.7	3.9	3.4	4.1	3.7
Leucine	7.3	7.1	6.3	7.6	7.1	6.8	6.4	6.6	7.0	7.1	7.6	6.4	7.9	7.3
Tyrosine	3.0	3.2	3.3	3.4	3.2	3.7	3.1	2.7	2.9	3.3	3.0	2.9	2.8	3.4
Phenylalanine	5.2	5.1	4.9	5.7	5.4	5.0	5.6	4.9	4.9	4.9	5.3	4.7	4.7	5.7
Lysine	4.0	3.7	3.2	3.8	3.5	3.4	3.5	3.5	4.2	4.1	4.3	4.3	4.4	4.1
Histidine	1.9	2.1	2.2	2.3	2.1	2.1	2.2	2.5	2.2	2.1	2.3	2.1	2.5	2.3
Arginine	5.5	4.9	4.9	5.7	5.1	4.8	5.1	5.0	5.6	5.4	5.9	5.6	6.2	5.6

the reproducibility of results obtained using the automatic amino-acid analyser and the effects of small variations in hydrolysis conditions, it would be unwise to attach too much significance to differences in the levels of particular amino acids. However, in certain cases the differences are large enough to suggest that they may be real, and these might therefore be examined more closely to see if they are related to nutritive value. Differences between samples judged from experience to be significant, are present in the cases of threonine, glycine, valine and lysine—all amino acids which are known to be of particular importance to the chick, and also all liable to be in short supply in diets based on mixtures of cereals and protein concentrates. In particular it is of interest that the percentage of lysine in the protein decreases as the barley protein content rises.

It does not necessarily follow that increased quantity of protein in grain is accompanied by an improvement in quality. Indeed there may be deterioration as has been shown quite conclusively in the case of maize.[26] Low-lysine zein forms an increasingly large proportion of the mixture of proteins laid down in the seed and assuming that lysine is of critical importance in the diet of which the maize is to form a part, a balance must be struck between rising grain yields and falling lysine percentage. In the paper quoted above, for example, two maize samples containing 7.6 and 11.4% of crude protein, contained, respectively, 0.23 and 0.27% lysine—a far from proportionate increase. As a percentage of the protein the lysine contents were, respectively, 3.0 and 2.4. Similar observations have been made for barley[21,22,27] and for wheat and oats.[27] Jones, Cadenhead and Livingstone[22] found that an increase of 33% in protein concentration was accompanied by a 20% decrease in the proportion of lysine in the protein of barley.

Not only lysine, but other essential amino acids may be affected and it may not be sufficient merely to judge the respective qualities of high and low protein grains on the basis of their content of one amino acid only. Frey[28] studying 14 oat varieties grown in 1947 and 1949 found that the average protein content was 25% higher in 1949, but that while the lysine, leucine and methionine were, respectively, 47, 47 and 43% higher, the tryptophan was only increased by 12%. Thus he concluded that seasonal and other environmental changes could influence not only protein content but also change seriously the amino-acid spectrum of the grain proteins.

Similar effects were noted for sorghum by Waggle, Parrish and Deyoe.[29] Though high protein sorghum contained a higher percentage of 17 amino acids than did low protein sorghum, on an equal nitrogen basis the low protein grain contained a higher proportion of lysine, methionine + cystine and threonine. McElroy *et al.* noted sample differences within one barley variety affecting significantly the levels of lysine, arginine, valine and phenylalanine, the two first-named decreasing with increasing nitrogen content.[27]

All of the above studies have been carried out with grains of moderate protein content and it is of interest to note here that with grains of very high protein content it has not been found possible to demonstrate the inverse relationship between crude protein and lysine content. Using South African and Australian wheats ranging from 15.1 to 24.2% protein on a dry matter basis no changes in the amino-acid pattern were found which would create limitations from a nutritional point of view.[30]

The barleys used in the present study were not exceptional in their crude protein

content, ranging from 7.06 to 12.5 on an "as-received" basis. It will be seen from Table 4 that an inverse relationship between crude protein content and lysine content does exist and is particularly marked between 8 and 11 % crude protein content, while three of the barleys which were highest in crude protein content, all incidentally grown at Sutton Bonington, also had high lysine contents. This tends to confirm the observation of Robinson and Sageman with regard to high protein wheats.[30] Bearing in mind the reservations made above regarding the accuracy of the amino-acid analyses, the inverse proportionality of protein to lysine content is reasonably good between 8 and 11% protein and it will be seen from Table 5 that an 8% protein barley containing 4.5 g of lysine/16 g of nitrogen would yield roughly the same amount of lysine/tonne of grain as would an 11 % protein barley containing 3.5 g of lysine/16 g of nitrogen.

TABLE 5. Relationship between nitrogen and lysine content in 14 samples of barley from Cambridge, Sutton Bonington (Leicestershire), Sprowston (Norfolk) and Aberdeenshire

		Lysine content			Lysine yield
	N × 6.25	g/16 g of N	% of barley	kg/tonne of barley	per hectare (kg)
PHS	12.5	4.0	0.500	5.00	26.1
RHS	12.1	3.7	0.447	4.46	23.0
PLS	11.6	3.8	0.440	4.40	22.5
RLS	11.6	3.2	0.371	3.71	19.0
ZHS	11.5	3.5	0.402	4.02	23.7
Ruby	11.2	3.4	0.380	3.80	—
ZLS	10.4	3.5	0.364	3.64	21.0
Unknown	10.3	3.5	0.360	3.60	—
PHN	9.44	4.2	0.396	3.96	—
EHN	8.91	4.1	0.365	3.65	—
PLN	8.38	4.3	0.360	3.60	—
ELN	7.94	4.3	0.341	3.41	—
PLC	7.71	4.4	0.339	3.39	11.9
Miln's Golden Promise	7.06	4.1	0.289	2.89	—

From examination of the five Proctor samples in Table 4 it is clear that as well as lysine the other two dibasic amino acids histidine and arginine also decline with increasing protein content of the seed. Alanine and valine show a similar trend.

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Evaluation of Barley as a Source of Protein for Chicks

II. Nutritional Assessment of Barleys of Differing Variety and Composition as Complements to Protein Concentrates

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Barley samples differing in variety, nitrogen fertiliser treatment and location of growth were compared in mixtures with gelatin by the rat net protein utilisation (n.p.u.) test and in mixtures with fish, meat, soya bean or groundnut meals by the total protein efficiency (t.p.e.) chick growth procedure.

Small but significant differences were found, the order of superiority of the barleys varying according to the nature of the accompanying concentrate and in some cases there were indications that the variations in quality were related to amino-acid composition. In general the poorer the quality of the accompanying concentrate the more pronounced were the differences between the barleys.

1. Introduction

The results presented in part I have demonstrated that individual samples of barley may differ considerably in amino-acid composition and in nitrogen content and that these differences are more likely to be associated with the environmental conditions under which the barley has been grown rather than with the variety of seed. In the second part the relationship between amino-acid composition and nutritive value, as well as the effect of combining particular barley samples with various types of protein concentrate are considered.

That the nutritive value of a particular sample of barley might depend upon the nature of the other constituents of the diet, could be deduced from studies of mixed proteins which have been reported previously. Swaminathan[1] reviewed work involving the formulation of mixtures of protein concentrates with cereals, much of which was carried out in India with the object of producing a multi-purpose protein food for human consumption. These studies did not, however, extend to an investigation of the relative nutritive values of different sources and strains of a given cereal. Similarly, Bressani and Scrimshaw[2] studied mixtures of maize and legume protein, again without attempting to compare the values of different varieties of maize. Nehring and Schramm[3]

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in 1940 compared three barley varieties in pig diets and could find no difference in nutritive value. Nitrogenous fertilisers increased yield and protein content but had no effect on nutritive value. Later, Willingham, Jensen and McGinnis[4] found no difference between eight barley varieties all grown in the same locality at Pullman, Washington, and given to chicks from one day of age for 3 weeks along with soya bean and fish meal. These results contrast with those reported in 1966 by Daghir and Rottensten[5] who attempted to relate chemical composition and nutritive value for four barley varieties grown in the same locality in the Lebanon. While changes in chemical composition were found to be negligible, there were differences in nutritive value between varieties when each was supplemented similarly with the same soya bean meal and given to chicks. In addition to noting variation in lysine content for different barleys (see part I), Jones, Cadenhead and Livingstone also observed that these variations were related to carcass quality and growth rate in bacon pigs. The work of McBeath *et al.* (1960)—(see part I)—using low-, medium- and high-nitrogen barleys produced by appropriate fertiliser treatment demonstrated that rat growth and feed efficiency improved with increasing protein content of the barley and also with increased levels of lysine supplementation. Consideration of other cereals offers little help. For oats Weber *et al.* in 1957[6] and Hischke, Potter and Graham in 1968[7] found no correlation between amino-acid composition and the weight gain of rats. In contrast, it has been reported that low-protein sorghum containing improved levels of lysine, methionine and threonine gave better rat growth than did high-protein sorghums.[8] The reverse has been shown for wheat.[9] Clearly more work is needed in order to prove that barleys of different variety, or with different chemical composition produced as a result of fertiliser treatments, may differ in nutritive value.

2. Methods

2.1. Net protein utilisation (n.p.u.)

The procedure was as described by Miller and Bender,[10] nitrogen retention being measured by carcass analysis according to Bender and Miller.[11] The mineral mixture was that recommended by Hawk, Oser and Summerson.[12]

In order to accommodate the low-protein barleys, dietary modification was required and it was decided to test mixtures having a total protein content of 10% obtained by combining 5% of gelatin protein with 5% of protein from the barley under test (Table 1). In effect the gelatin was a protein diluent and the resulting n.p.u. value must be considered as a measure of the ability of the barley to complement a particularly poorly balanced protein.

2.2. Total protein efficiency (t.p.e.)

The procedure was as described previously.[13] The method was originally designed to evaluate protein concentrates included as supplements in cereal-based diets for chickens, and it was found to be equally suitable for discriminating between cereals when the protein concentrate component of the diet was kept constant, providing that certain dietary modifications were made (Table 2). In order to accommodate barleys of widely

differing protein content it was sometimes necessary to reduce the total protein level of the experimental diet, the ratio of cereal protein to concentrate protein being held, however, at 1:2. For the first two experiments in which individually caged birds were used and in the third and seventh experiments using groups of six chicks, the total

TABLE 1. Composition of diets (%) and results for n.p.u. estimations

	PHW[a]	PLW	RHW	RLW	ZHW	ZLW	Non-protein	Egg albumin
Barley	49.6	54.6	48.7	51.1	50.1	58.0	—	—
Gelatin	5.0	5.0	5.0	5.0	5.0	5.0	—	—
Starch-maize	8.1	3.4	8.9	6.5	7.4	—	50.0	45.0
Starch-potato	—	—	—	—	—	—	10.0	10.0
Maize oil	14.3	14.0	14.4	14.4	14.5	14.0	15.0	15.0
Glucose	13.0	13.0	13.0	13.0	13.0	13.0	15.0	15.0
Vitamin premix[b]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mineral premix[c]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Egg albumin	—	—	—	—	—	—	—	5.0
n.p.u.	45.3	44.3	44.4	41.8	43.3	40.0	—	99.0

[a] For identification of samples, see part I.
[b] Bender and Miller.[11]
[c] Hawk, Oser & Summerson.[12]

TABLE 2. Composition of diets (%) for t.p.c. estimation using individually caged chicks

		%
Barley	To provide 6% crude protein	Variable
Fish meal (P968)	To provide 12% crude protein	18.0
NaCl		0.2
Steamed bone meal		1.0
Vitamin premix[a]		0.3
Lard, Maize starch	To provide 3280 kcal m.e./kg	To 100.0

[a] Woodham.[13]

protein level of the diets was 18%. In experiment 4 the total protein level was 17.4% and in the two experiments in which Aberdeenshire barleys were tested the total dietary protein was 15%. Male RIR × WL chicks were used in all eight of the experiments described below, and the final experiment was repeated using broiler chicks.

The cereal portion of the diet consisted of a single sample of barley in each case, the wheat offals and oat husk normally incorporated being omitted in order to avoid the confusion which would arise from the inclusion of a mixture of cereal products. The same batches of each protein concentrate were used throughout the series of experiments. In the first two experiments measurements were made between 3 and 10, or between 3 and 17 days of age while in experiments 3 to 7 inclusive where groups of 6 chicks were used, the experimental period was from 14 to 28 days of age.

3. Results

3.1. Net protein utilisation of barley–gelatin mixtures

Three varieties, Proctor, Rika and Zephyr, each grown at Wye, Kent, with the application of 41 or 83 kg of nitrogen/hectare were compared. Differences between the six samples were small, but it may be significant that in each pair the sample grown with the higher level of nitrogen application was superior to the corresponding sample of the same variety grown with the application of only half the quantity of nitrogen (Table 1).

3.2. Evaluations by total protein efficiency (t.p.e.)

3.2.1. Experiment 1

Each of the 18 NIAB barleys (Part I, Table 1) was combined with white fish-meal and given to two individually caged chicks in each of three separate trials. There were statistically significant differences in t.p.e. ($P < 0.01$) between the samples, and there was evidence that Rika tended on average to be the best of the three varieties, Wye the best site and low-level nitrogen fertilisation was better than high-level (Table 3). Over the 3- to 17-day test period, however, there were significant variety/site, site/level and variety/site/level interactions ($P < 0.05$ in each instance). Attention should therefore be concentrated on the 18 individual barley sample means (Table 4), rather than on overall means by variety, site or level.

TABLE 3. Total protein efficiency (t.p.e.) of 18 NIAB barley–fish meal mixtures

		t.p.e. 3 to 10 days	t.p.e. 3 to 17 days
Variety	Proctor	3.23	2.89
	Rika	3.31	3.04
	Zephyr	3.11	2.87
S.E. of differences (36 replicates):		± 0.092	± 0.080
Location	Cambridge	3.24	2.85
	Sutton Bonington	3.08	2.92
	Wye	3.32	3.03
S.E. of differences (36 replicates):		± 0.092	± 0.080
N. level	high	3.14	2.92
	low	3.29	2.95
S.E. of differences (54 replicates):		± 0.076	± 0.065

3.2.2. Experiment 2

Using individually-caged chicks again, the 6 NIAB barleys grown at one location (Cambridge) were combined with fish meal, soya bean or groundnut in order to see whether variety or nitrogen fertiliser level was important in determining which barley should be fed with particular protein concentrates. In this case differences were too small to permit useful conclusions to be drawn (Table 5), and it was decided that groups of chicks would be used for subsequent trials.

TABLE 4. Total protein efficiency (t.p.e.) of 18 NIAB barley–fish meal mixtures

Variety	Nitrogen level	Cambridge	Sutton Bonington	Wye
Proctor	High	2.73	2.79	2.84
Proctor	Low	2.97	2.99	3.00
Rika	High	3.15	2.88	3.14
Rika	Low	3.03	3.09	2.96
Zephyr	High	2.94	2.69	3.08
Zephyr	Low	2.25	3.06	3.19
S.E. of differences = ± 0.20				

(Each figure is the mean of 6 replicates measured over 3–17 days)

TABLE 5. Total protein efficiency of 6 Cambridge-grown barleys mixed with fish meal (f.m.), soya bean (s.b.) or groundnut (g.n.)

		Supplement		
		Fish meal	Soya bean meal	Groundnut meal
Variety	Proctor	3.10	2.52	2.04
	Rika	3.16	2.62	2.01
	Zephyr	3.19	2.48	2.11
S.E. of differences (10 or 11 replicates):		± 0.056	± 0.057	± 0.046
N. Level	High	3.17	2.52	2.01
	Low	3.13	2.56	2.09
S.E. of differences (10 or 11 replicates):		± 0.046	± 0.046	± 0.038

TABLE 6. Total protein efficiency (t.p.e.) for four barleys grown at Sutton Bonington when accompanied by fish meal (f.m.), soya bean meal (s.b.) or groundnut meal (g.n.)

		Supplementary protein source		
Variety	Nitrogen level	f.m.	s.b.	g.n.
Rika	High	2.50	2.32	1.86
Rika	Low	2.54	2.24	1.73
Zephyr	High	2.56	2.11	1.72
Zephyr	Low	2.50	2.15	1.90
S.E. of diff. between means = ±0.044 (27 d.f.)				

3.2.3. Experiment 3

Rika and Zephyr barleys grown at Sutton Bonington and including both high and low levels of nitrogen application were combined with fish, soya bean or groundnut meals in 18% total protein diets. No difference was apparent between the fish-meal diets, but with soya bean both Rika barleys were superior to both Zephyrs, and with groundnut, Rika (high) and Zephyr (low) were superior to Rika (low) and Zephyr (high) (Table 6).

3.2.4. Experiment 4

The same procedure was employed for testing 21 barley samples from Norfolk with supplements of soya bean and groundnut meal, but the protein level of the diet was reduced in order to accommodate the varied range of test barleys. The samples comprised 7 varieties each grown with a high, medium or low level of nitrogen application (Part 1, Table 2). In each successive pair of trials all the samples which had received the same fertiliser treatment were evaluated, the 32 available cages in each trial being divided into two blocks of 16 and accommodating each of the 7 varieties along with soya bean and groundnut.

TABLE 7. Total protein efficiency (t.p.e.) for seven Norfolk barleys when accompanied by soya bean meal (s.b.) or groundnut meal (g.n.)

Variety	Supplementary protein source	
	s.b.	g.n.
Europa	2.02	1.76
Proctor	2.08	1.71
Maris Concord	2.04	1.68
Deba Abed	2.07	1.66
Maris Druid	2.08	1.66
Mosane	2.05	1.66
Impala	2.05	1.65
S.E. of diff. between means =	± 0.027	± 0.027 (54 d.f.)

TABLE 8. Effect of level of nitrogen application on the t.p.e. of Norfolk barleys accompanied by soya bean or groundnut meal

Nitrogen level	Mean t.p.e.
Low	1.90
Medium	1.89
High	1.82
S.E. of difference between means =	±0.013 (108 d.f.)

There was clear evidence of discrimination between the groundnut diets, Europa barley being significantly better than most of the others; there was, however, no evidence of any difference between the soya bean diets. In Table 7 all nitrogen levels have been included in the comparison of varieties. There was, however, a significant effect due to the level of nitrogen fertiliser treatment ($P < 0.01$), the low and medium nitrogen levels being superior to the high level (Table 8).

3.2.5. Experiment 5

The same soya bean and groundnut meals were used in combination with seven varieties of barley from Aberdeenshire (Table 9). The range of nitrogen content exhibited by the barleys coupled with the need to exclude other cereal sources from the diets necessitated lowering the total protein content of the diets to 15%. In this case the between varieties variability was significant at the 1% level for the groundnut supplemented

diets and at the 0.1% level for the soya bean diets. With groundnut, Ruby was significantly poorer than the other varieties tested, while with soya bean Ruby and Impala were significantly poorer than Ymer, Golden Promise and Zephyr (Table 9).

3.2.6. Experiment 6

On the basis of the above results, Ruby and Golden Promise were selected as "poor" and "good" feeding barleys, respectively, and further tested in combination with fish meal, meat meal, and a 50:50 mixture of fish meal and groundnut protein. Ruby was significantly superior to Golden Promise as a supplement for either meat meal or the fish meal–groundnut mixture but with fish meal the two barleys were equal in value (Table 10).

TABLE 9. Total protein efficiency (t.p.e.) for seven Aberdeenshire barleys accompanied by groundnut (g.n.) or soya bean meal (s.b.)

Variety	N × 6.25	Supplementary protein source	
		s.b.	g.n.
Ymer	7.43	2.21	1.63
Golden Promise	7.06	2.19	1.71
Zephyr	7.56	2.16	1.62
Ingrid	6.84	2.12	1.70
Pallas	7.00	2.12	1.70
Impala	9.53	2.04	1.64
Ruby	11.21	1.99	1.47
S.E. of diff. between means =		±0.056	±0.058

TABLE 10. Total protein efficiency (t.p.e.) for two Aberdeenshire barleys accompanied by fish meal (f.m.), meat meal (m.m.) or a 50:50 mixture of fish meal and groundnut meal (g.n.) protein

Variety	Supplementary protein source		
	f.m.	m.m.	f.m./g.n.
Golden Promise	2.92	1.28	2.52
Ruby	2.87	1.51	2.69
S.E. of diff. between means = ±0.087			

3.2.7. Experiment 7

A final experiment was carried out using Aberdeenshire-grown Ymer barley which had received various levels of nitrogen incorporated in the seed-bed before planting (Part I, Table 3), with the object of ascertaining whether level of nitrogen fertilisation alone might affect the relative value of the ripe cereal as a complement to different protein concentrates. Soya bean and groundnut meals were again used because of the comparatively large differences between them in amino-acid composition. Male

RIR × WL chicks were employed as usual, but it was decided to repeat the experiment using male chicks of a broiler strain. The results are presented in Table 11, and it is clear that there is no trend with either of the concentrates or with either type of chicken.

4. Discussion

It has been noted in Part I that changes in nitrogen content of barley seed brought about by nitrogen fertilisation are accompanied by changes in the amino-acid composition. It has been now shown that these changes are reflected in the nutritive value of diets containing mixtures of such barleys with protein concentrates. The possibility that small changes in the content of sulphur amino acids may affect the nutritive value

TABLE 11. Total protein efficiency (t.p.e.) for samples of Aberdeenshire Ymer barley treated with different levels of nitrogen in the seed-bed, accompanied by groundnut (g.n.) or soya bean (s.b.) meal

		Supplementary protein source			
		s.b.		g.n.	
Variety	Nitrogen level applied (kg/hectare)	Broiler	RIR × WL	Broiler	RIR × WL
Ymer	0	2.74	2.03	2.21	1.91
Ymer	19	2.74	2.03	2.10	1.91
Ymer	38	2.78	2.12	2.21	1.84
Ymer	57	2.78	2.15	2.17	1.86
Ymer	76	2.65	2.05	2.21	1.91

S.E. of diff. between means = ±0.19

of barleys when they are combined with a protein deficient in these amino acids, is suggested by the results of the n.p.u. estimations given in Table 1. Reference to Table 4 in part I reveals that in each case where comparisons are possible between samples of the same variety grown at the same location but with differing levels of nitrogen application, the level of methionine and of methionine + cystine is higher for the sample which has received the higher level of nitrogen during growth. Gelatin is a poor source of sulphur amino acids so that its use in mixed diets with barley might be expected to reflect differences in the barley sulphur amino-acid levels, particularly in view of the well-known fact that the rat n.p.u. is known to increase with increasing sulphur amino-acid levels in the test protein.[14] Although it is tempting to speculate on the basis of the observed facts that nitrogen fertilisation of barley improves sulphur amino levels, it should be noted that the differences in both n.p.u. and sulphur amino-acid content are small and further, that the latter were not estimated on oxidised samples.

With a lysine-deficient protein source such as groundnut, differences in lysine content of the accompanying barley may affect the nutritive value. The high- and low-protein Rika barleys grown at Sutton Bonington have, respectively, 3.7 and 3.2 g of lysine/16 g of N and the t.p.e. estimation rated them accordingly (Table 6). However, the superiority of low-protein Zephyr over high-protein Zephyr cannot be explained thus as

both barleys have the same lysine content, and one might conclude that in this case the explanation could be an improvement in overall amino-acid balance due to comparatively small changes in some other of the essential amino acids, e.g. threonine, serine and glycine. A similar explanation appears to be involved in the case of the Norfolk Europa and Proctor samples (Table 8) though in both cases the low nitrogen samples are marginally higher in lysine content than the high protein samples.

No amino-acid composition data is available in the case of the six Aberdeenshire Ymer barleys but there was no evidence for any difference in nutritive value between them (Table 11) when combined with groundnut or soya bean meal. In part I, Table 3 it will be seen that the application of quite substantial amounts of nitrogen in the seed bed has not affected the protein content of the grain, so it would seem likely that amino-acid composition has also been unaffected.

For the 18 NIAB barleys examined, low-level nitrogen fertilisation yielded nutritionally better barleys than high-level fertilisation, when combined with fish meal (Table 4).

The existence of differences in nutritive value between varieties is clear from the various tables. As different groupings of samples were used in the various experiments it is not possible to arrange all the samples in a merit order for a particularly dietary combination. Nevertheless one can say that, given a choice of Proctor, Rika and Zephyr, Rika would be a suitable choice for feeding with groundnut or soya bean and probably also with fish meal (Tables 3 to 6). Of the seven Norfolk-grown barleys, Europa and Proctor would be the one of choice to feed with groundnut, but all would be equally suitable with soya bean (Table 7). With the seven Aberdeenshire barleys it would seem advisable to avoid feeding Ruby in conjunction with groundnut or soya bean, but if meat meal or fish meal is available Ruby could be usefully incorporated. Conversely Golden Promise would be indicated to pair with groundnut or soya bean, but not with meat meal (Tables 9 and 10).

In general one could conclude that the choice of barley is much wider if it is to be fed with a high-quality protein concentrate such as fish meal. The better the provision of essential amino acids by the high protein component of the diet, the less need is there to seek barleys with high lysine or high methionine contents. On the other hand, groundnut, which is a comparatively poor source of these amino acids, stands to benefit from the quite small additional amounts which could be provided by a better cereal. Soya bean as one might expect from its amino-acid composition, occupies an intermediate position between fish and groundnut meal.

The differences in nutritive value which have been found in this work, though real, are not large. The t.p.e. method is capable of fine discrimination between feeding stuffs. Reservations regarding the ion-exchange chromatographic technique for amino-acid analysis have been made in part I and it seems not unlikely that precise correlation between the chick-growth results and fluctuations in the levels of particular amino acids could be due to the inability of the chemical technique in its present form to provide sufficiently precise figures for crude feeding stuffs. Perhaps, surprisingly, it may be true that the biological test is more precise and reproducible than the laboratory procedure. More specifically, the levels of lysine, of the sulphur amino acids or of other particular essential amino acids may in fact determine the ultimate nutritive

value in every case and not merely in some. On the other hand, as has been suggested above, more complicated relationships involving a number of amino acids may operate and these could be responsible for the anomalies which have been noted.

Because the overall protein level used in these experiments is below the optimum for maximum chick growth, under practical conditions the differences in chick growth would be even smaller. Furthermore in the U.K. at least it is unlikely that a commercial

TABLE 12. Amino-acid composition of the fish meal (f.m.), soya bean (s.b.), groundnut (g.n.) and meat (m.m.) meals used in the biological tests (g/16 g of N)

	f.m. P968	s.b. PQ5	g.n. PQ11	m.m. P977
Asp	9.1	12.2	11.8	7.5
Thr	4.2	4.0	3.4	3.1
Ser	4.7	5.0	4.7	3.7
Glu	13.3	17.9	18.0	12.2
Pro	5.6	8.0	5.2	9.6
Gly	9.5	3.9	5.5	13.6
Ala	6.9	4.4	4.0	8.0
Val	4.8	5.0	4.4	4.6
Cys	1.2[a]	1.5	1.1	0.6[a]
Met	2.5[a]	1.2	0.7	0.6[a]
Iso	3.9	4.7	3.5	2.6
Leu	6.7	7.5	5.9	5.8
Tyr	2.9	3.5	3.9	1.8
Phe	3.7	5.2	5.9	3.5
Lys	7.4	6.3	3.4	4.9
His	1.9	2.6	2.2	1.8
Arg	6.9	7.7	10.6	6.9
Trp	1.4	—	—	1.2

[a] Estimated on samples oxidised with performic acid by the method of Moore.[16]

poultry ration would contain only one cereal and one protein concentrate. With increase in the number of basal cereals and accompanying protein concentrates, the more likely is it that inadequacies in amino-acid balance would be smoothed out. Only one concentrate of each type has been used in the series of experiments described and amino-acid composition of each is given in Table 12. Other samples of the same types could be somewhat different. In view of these qualifications one may well ask if a useful conclusion can be drawn from the work. Hischke *et al.* after completing their work on oats, which has already been mentioned,[7] concluded that the small varietal differences which they found might not be of prime importance from a practical standpoint. Aitken has also recently proposed that the selection of grains for improved protein quality is not of sufficient economic importance to justify the likely cost in terms of reduced yields.[15]

Though the differences noted in the present work between normal commercially available barleys are small, the possibility exists that changes of considerable nutritional

importance may result from current genetic work. In countries where protein for animal feeding is in short supply, and particularly where local economic circumstances restrict the choice of dietary constituents, the selection of an appropriate cereal to match the protein sources available might well be an important consideration. The work reported here demonstrates the possibility that matching of cereals and protein concentrates could be carried out with advantage. Whether this is of economic significance depends upon many factors including prevailing prices, and the range of feedstuffs available for use. The development by plant breeders of cereals with radically different amino-acid composition from those available at the present time could convert what is at the moment little more than an interesting academic possibility, into something of real commercial significance.

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Texture of Canned Potatoes:

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New methods are described for the objective assessment of mouthfeel (mealiness) using a Kramer Shear Press and for the amount of breakdown by sedimentation of sloughed tissue. Their application to several hundred samples of canned potatoes is reported. These objective methods correlate significantly ($P < 0.001$) with their subjective counterparts and demonstrate a clearer separation of mouthfeel and breakdown than do subjective methods. Examination of partial correlation coefficients indicates that mouthfeel is more significantly related to the specific gravity of the raw material than is breakdown. In separate experiments, late planting was shown to affect mouthfeel but not breakdown and the addition of calcium chloride to affect breakdown whilst leaving mouthfeel unaltered. Evidence is presented that mouthfeel is a rheological property controlled primarily by solids content; in contrast breakdown is interpreted as a failure of intercellular adhesion.

1. Introduction

Of the problems associated with canning potatoes, controlling texture and, in particular, breakdown has proved the most intractable. Investigations of the problem have been hampered by the ill-defined nature of the various attributes of texture which has led to a multiplicity of methods being proposed for its measurement. This paper proposes two methods which are both simple and reproducible and attempts to define what is being measured by each method.

The earliest assessments of texture were subjective and aimed at describing the feel of the potato in the mouth by ascribing to it one or more of the adjectives: mealy, floury, dry, sticky, hard, waxy, etc. According to Burton[1] these can be adequately described by a continuum which at one extreme (mealy) describes a potato which, when cooked, may retain its form but is easily broken down and if agitated with an equal volume of water will give a slurry consisting essentially of single cells. At the other extreme (waxy) the tubers exhibit no sign of being undercooked but may be cut into slices and if mashed many cell walls will be ruptured.

Zaehringer *et al.*[2] have proposed a standard method of cooking and Zaehringer and Le Tourneau[3] pointed out that "mild mashing with a fork" was the best method of judging the texture. The tensile strength of raw potatoes has also been measured and

its decrease on cooking[4] and variability in the different regions of the tuber[5] noted but this property did not correlate with subjectively assessed texture of the cooked product. Puncture tests[6] and compression tests[7] have also been tried and shown to be inadequate as a means of assessing texture.

A back extrusion device has been used successfully by a number of workers [8–10] for measuring the texture or consistency of mashed potatoes and Shannon and Bourne[11] have used a similar device with diced beetroots for assessing their texture.

Objective methods of determining the amount of breakdown on cooking have usually relied on determination of weight loss before[12] or after[13] abrasion. These procedures have the minor disadvantage of being affected by the leaching of solutes and by the uptake of water by the tissue which makes it possible to observe significant changes in weight in samples which have not broken down at all.

Most of these workers have treated breakdown as a phenomenon to be associated with excessive mealiness and many of them have observed that increasing tissue strength as exhibited by increased resistance to the passage of a shearing, puncturing or extruding device must paradoxically be associated with increasing mealiness.

This paradox, which results from the misleading assumption of a single continuum of potato texture, has led us to measure two attributes of texture—the mealiness–waxiness (referred to as "mouthfeel") on the one hand and the amount of breakdown on the other—on a large number of samples covering a wide range of these qualities and to test their interdependence.

2. Experimental

2.1. Raw materials

A large number of potato varieties including Maris Peer, Pentland Marble, Vanessa, Stormont 480, Ulster Concord, Arran Pilot, Desiree and 11 numbered experimental varieties were lifted at stages ranging from the first formation of tubers of 2 to 3 cm diameter in early June to full maturity in mid-September. This procedure ensured that samples covered a wide range of both mealiness and breakdown. Material from the 1969, 1970 and 1971 seasons was size-graded and the range 18 to 38 mm diameter selected.

Each sample was washed, its mean s.g. measured by weighing in air and in water, then it was steam peeled, filled into A2 cans and processed for 16 min at 121 °C (250 °F). Cans were stored for 3 months at room temperature before being assessed for attributes of texture.

2.2. Assessment of texture

2.2.1. Determination of the amount of sediment

The can was opened and the contents emptied onto a sieve having approximately 10-mm square apertures, the material passing through was collected in an Imhoff Cone. Any remaining fragments adhering to the potatoes were washed into the Imhoff Cone with water. The larger fragments may then be broken using a thin glass rod to ensure good sedimentation. After 20 to 30 min, to allow complete precipitation, the volume of the sediment was noted.

As the weight of the contents of the can was known with fair precision (341 ± 20 g) the amount of sediment determined in this way provides a direct measure of the extent of sloughing which has occurred. The method has a further advantage over determinations depending on measurements of weight lost on cooking in that it avoids errors due to dissolution of soluble materials. An alternative procedure is to weigh the material left on the sieve and to express the sediment in ml/kg of drained weight.

2.2.2. Use of the Kramer Shear Press

An F.T.C. Texture Test System (TP-1) equipped with a continuous chart recorder was used for all the measurements reported in this paper.

2.2.2.1. Shear–compression cell (CS-1)

Potatoes are drained from their brine and samples weighed; for routine work five 120-g samples are taken from two A2 cans. A sample is transferred to the cell as whole potatoes and the force–distance curve recorded with a speed setting of 1.0 (0.062 cm/s). The liquid and tissue passing through the cell is collected in a glass dish, mixed thoroughly with any material remaining in the cell and the whole sample returned to the cell. A second force–distance curve is recorded. Maximum peak heights are recorded and the areas under each curve determined by cutting out and weighing the chart paper. Typically two replicates from a single can would differ by less than 10% of the mean.

2.2.2.2. Grid-extrusion cell (CE-1)

Random sub-samples of tubers are taken and the cell filled and pressed down well; the force–distance curve is recorded with a piston speed of 0.062 cm/s. After an initial period of compression the tissue is extruded at an approximately constant force although this force is subject to considerable random variations throughout the period of extrusion. The average force required to extrude the sample (the extrusive force) is estimated from the force–distance curve; reported results being the mean of at least two, generally three determinations. Using this technique it is important to ensure that sufficient sample has been used to give a reasonably long record of extrusion at maximum force.

As the transducer of the shear press is subject to considerable zero error, conversions from one scale to another are unreliable. For this reason all values for use in a single experiment were recorded using the same (100% or 50%) scale.

2.2.3. Subjective assessment of texture by Q.C.6.

Canned samples were assessed for attributes of texture according to *Standards of Quality Q.C.6.*[14]

2.2.3.1. Breakdown

The numbers of tubers slightly, moderately or severely cracked, sloughed or disintegrated are counted and a number of negative marks determined for deduction from 15 by reference to a table which makes allowance for the total number of tubers in the can. The negative marks were used, higher values indicating a greater degree of breakdown.

2.2.3.2. Mouthfeel

Mouthfeel is scored on a 10 point hedonic scale, negative marks being awarded for deviations from the subjectively assessed optimum texture of canned new potatoes. Theoretically marks may be deducted for excessive waxiness but this is a rare occurrence and no samples of this type were encountered in this survey. Thus, the scores used indicated increasing mealiness with increasing values.

2.2.4. Other techniques for subjective assessment of breakdown

Although the Q.C.6 system has been used for many years for assessing the marketing quality of canned potatoes with excellent results it was felt that a more linear scale which obviated the necessity of referring to a table for the score could be useful for research purposes. The systems investigated were similar to that proposed by Rathsack.[15]

The tubers were classified and counted as follows:

a = number of tubers showing no breakdown
b = number of tubers with cracks or slight sloughing
c = number of tubers with moderate sloughing
d = number of tubers excessively disintegrated

and the following indices calculated:

$$\text{breakdown 1} = 100(b + c + d)/(a + b + c + d)$$
$$\text{breakdown 2} = 100(b + 2c + 3d)/3(a + b + c + d).$$

3. Results and discussion

3.1. Shear–compression cell

The first of the Kramer Shear Press cells which was found to give meaningful and useful results was the shear–compression cell and it was noted that peak heights correlated well with mouthfeel ($r = 0.64$, d.f. = 35)[a] but did not correlate significantly with breakdown whether this was measured by the sediment or by the Q.C.6 breakdown score.

In investigating the reasons for this, a large number of samples of widely differing textural characteristics were tested in this cell by passing each sample through twice and noting the height of each peak and the work done (area under the peak) during each passage. In all cases the peak height (max. force) correlated extremely well with peak area (work done) ($r = 0.94$, d.f. = 159) even though the details of the shapes of the peaks varied from one sample to another. This is not unexpected if the main property of the tissue affecting the values is a flow characteristic.

Further evidence that this is the case is obtained by comparison of the forces required during the first and second passage through the cell. In general there is only a 30% reduction in each of these parameters after the first passage. The correlation coefficients between the maximum forces on the first and second passages through the shear compression cell ($r = 0.97$, d.f. = 35) and the grid extrusion cell ($r = 0.83$, d.f. = 35) are

[a] r = Linear correlation coefficient; d.f. = degrees of freedom: all r values significant at 0.1% level or better unless otherwise stated. The appearance of scatter diagrams did not justify the use of non-linear regressions.

extremely high. Assuming that the tissue structure is completely destroyed on the first passage whilst its flow characteristics remain unaltered these results indicate that more than two-thirds of the observed effect must be attributed to something other than the shearing of the tissue structure, namely flow.

This hypothesis is confirmed by comparison of the results obtained with the grid-extrusion cell—which by its design must be affected most strongly by flow properties—and those obtained with the shear–compression cell. For this experiment the samples were passed twice through the extrusion cell in exactly the same way as described for the shear–compression cell. High correlation coefficients were obtained between the forces required in each cell for the first passage ($r = 0.85$, d.f. = 35) the second passage ($r = 0.79$, d.f. = 35) and the difference in forces required for each passage ($r = 0.73$, d.f. = 35).

Szczesniak, Humbaugh and Block[16] have reported that measurements made with the Kramer shear–compression cell are strongly influenced by extrusive forces; the results reported here indicate that extrusion is the principal factor controlling peak heights and peak areas in this case and that this property could therefore be better determined with an extrusion cell.

3.2. Measurement of mouthfeel

The extrusive force measured by both the shear–compression cell ($r = 0.86$, d.f. = 93; $r = 0.73$, d.f. = 34; $r = 0.64$, d.f. = 35) and the grid-extrusion cell ($r = 0.66$, d.f. = 56; $r = 0.75$, d.f. = 34; $r = 0.73$, d.f. = 72) have always given extremely high correlations with mouthfeel (mealiness) subjectively assessed on the Q.C.6 scale and in the one experiment (d.f. = 34) in which both techniques were used the grid extrusion cell gave a very slightly higher correlation coefficient. As the latter technique requires a smaller sample and gives results independent of sample size, it is far more rapid and convenient to use. Two points must however be observed most closely in this technique; first, sufficient potatoes must be used to ensure that a reasonable length of recorder chart is obtained at the constant force to allow a good approximation of the force required at equilibrium and, second, the same scale range must be used for all samples within a population as the zero error of the transducer makes conversion from one scale to another unreliable.

For these reasons the shear–compression cell was discarded and the grid-extrusion cell used for all further measurements of extrusive force which were to be used as objective measurements of mouthfeel.

Figure 1 shows a composite scatter diagram of all experiments carried out for comparing the extrusive force measured in a grid-extrusion cell and subjectively assessed mouthfeel. The total spread in this Figure includes not only the variance of both methods of analysis but also the variance of the raw material which probably represents the largest contribution to the spread.

3.3. Measurement of the amount of breakdown

Three subjective methods and one objective method of measuring breakdown were investigated. The "breakdown Q.C.6" figure has been used as a standard procedure in the canning industry for a number of years and provides a reliable guide to the loss of marketing quality due to the disintegration of the tubers. It is, however, a complex

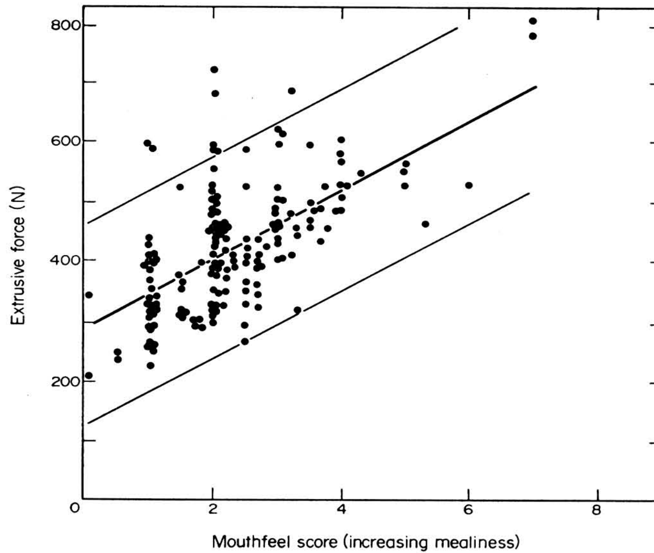

Figure 1. Scatter diagram for the relationship between mouthfeel and extrusive force showing the linear regression line ($r = 0.61$, d.f. = 166) and the 95% confidence limits.

procedure which is time-consuming and requires trained personnel for its application. Two other subjective procedures were developed which could be used by untrained staff in the hope that one of the scales would provide a linear measure of the amount of breakdown in the can. The "breakdown 2" score differs from the "breakdown 1" score in that it gives greater weight to tubers with considerable breakdown than to those with little breakdown. The latter score simply records the percentage of tubers showing any sign of disintegration. Breakdown 2 gave a higher correlation ($r = 0.73$, d.f = 56) than breakdown 1 ($r = 0.64$, d.f. = 56) with breakdown Q.C.6 as would be expected as both procedures take account of the degree of disintegration of each tuber.

The determination of the amount of sediment unequivocally measures the amount of tissue sloughed from the tubers and its high correlation with the breakdown Q.C.6 score ($r = 0.81$, d.f. = 56; $r = 0.71$, d.f. = 73) indicates that it is this absolute amount of disintegration which is the factor which detracts from the marketability of canned potatoes. This objective method of measuring the amount of disintegrated tissue correlates highly with all the subjective methods as shown in Table 1.

TABLE 1. Coefficients of correlation between different methods of measuring breakdown

	Sediment	Breakdown Q.C.6	Breakdown 1
Breakdown Q.C.6	0.81 (d.f. = 56) 0.71 (d.f. = 73)	— —	— —
Breakdown 1	0.81 (d.f. = 78) 0.61 (d.f. = 56)	0.64 (d.f. = 56)	—
Breakdown 2	0.88 (d.f. = 78) 0.70 (d.f. = 56)	0.73 (d.f. = 56)	0.97 (d.f. = 78) 0.97 (d.f. = 56)

It is clear from the high correlations over such large numbers of samples that there is little to choose between the different techniques especially as the different measures were frequently made on duplicate samples rather than on the same can and it is known that there is usually a considerable variance to be associated with the use of a single method on supposedly identical samples.

For this reason we recommend the use of sedimentation for research purposes, sediment or breakdown 2 for routine comparison of samples by untrained personnel and, of course, the retention of breakdown Q.C.6 for assessment of the marketability of

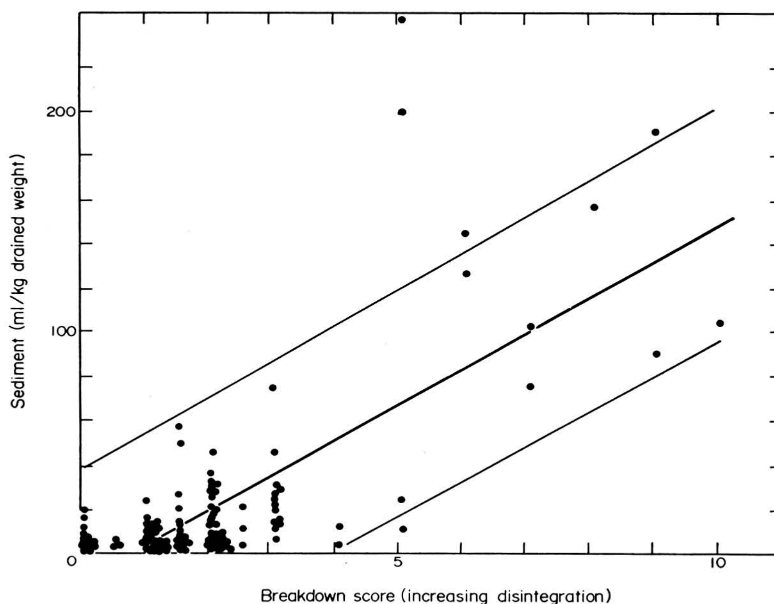

Figure 2. Scatter diagram for the relationship between breakdown score and sediment showing the linear regression line ($r = 0.68$, d.f. $= 131$) and the 95% confidence limits.

commercial samples. Figure 2 shows the composite scatter diagram for all experiments carried out for comparing the measurement of breakdown objectively by sedimentation and subjectively by the Q.C.6 method.

3.4. Relationship between mouthfeel and breakdown

The texture of a sample of potatoes may be recorded in terms of two subjective measures (breakdown Q.C.6 and mouthfeel) or alternatively in terms of two objective measures (sediment and extrusive force). In a number of experiments we have observed that the two subjective measures correlate strongly whilst the two objective measures are far less dependent on each other: the correlation coefficients are reported in Table 2. The implication of these values is that there are two independent and separate attributes of texture and that in assessing the one subjectively the tester inevitably allows the other attribute to affect the score awarded to a particular sample. Using objective techniques, however, the attributes are scored far more independently and a significant

TABLE 2. Coefficients of correlation between objectively assessed breakdown and mouthfeel and between subjectively assessed breakdown and mouthfeel

Degrees of freedom	56	73	78	93	34
Breakdown Q.C.6—mouthfeel	0.55	0.53		0.68	0.11[a]
Sediment—extrusive force	0.11[a]	0.41	0.20[b]		

[a] Not significant at 10%.
[b] Significant at 5%.

correlation between the two objective scores should be interpreted as indicating, in the population under consideration, that these two attributes are themselves correlated with a third independent variable such as maturity, growing conditions or variety. The experiment with 73 degrees of freedom involved 18 varieties grown to two maturities at seven different and widely separated sites in England and Scotland during a single season.

3.5. Relationship between specific gravity and texture

Whittenberger and Nutting[17] showed that the regions of a potato tuber where sloughing occurs have a higher specific gravity than the remainder of the tuber and that the division of a sample of potatoes into sub-samples having different specific gravities gave populations showing a direct relationship between specific gravity and breakdown. They also showed, however, that when comparisons were made between different varieties of potato or between samples treated with different amounts of calcium salts before cooking that the relationship no longer held. Whittenberger[18] also investigated samples of potatoes which had been stored for different periods and showed that the relationship between breakdown and specific gravity was not significant.

These properties were also studied by Bettelheim and Sterling[19] who confirmed, using samples of ten different varieties of potato, that the specific gravity and breakdown were not correlated significantly and similar results were obtained by Stuckey, Tucker and Sheehan[20] comparing 12 varieties over three seasons.

In contrast to this Le Tourneau, Zaehringer and Potter[21] showed that highly significant correlations ($r = 0.88$ to $r = 0.93$) existed between breakdown and specific gravity in populations of potatoes consisting of three specific gravity gradings from each of four varieties. These correlation coefficients are surprisingly high considering the variation in breakdown normally found between samples of similar specific gravity. Weckel, Scharschmidt and Reiman[13] confirmed that high specific gravity sub-samples from stored or unstored potatoes showed a higher degree of breakdown than the corresponding low specific gravity sub-sample. The percentage tissue lost on cooking showed a considerable overlap, however, the two groups ranging from 3.8 to 21.8% for the lower specific gravity group and from 7.5 to 65% in the other group.

Correlation coefficients reported or calculated from data in the above articles are summarised in Table 3.

In this work we have noted that specific gravity correlates with breakdown Q.C.6, mealiness, sediment and extrusive force as shown in Table 4. However, the breakdown Q.C.6 score is influenced by mealiness and *vice versa*, and hence, to observe the true

TABLE 3. Reported correlation coefficients between specific gravity and breakdown

Reference	No. of samples	Correlation coefficient
Whittenberger and Nutting[17]	24	+0.82
Whittenberger[18]	14	+0.39[a]
Bettelheim and Sterling[19]	10	+0.54[a]
Stuckey, Tucker and Sheehan[20]	12	+0.51[a]
Le Tourneau, Zaehringer and Potter[21]	15	+0.88
	18	+0.89
	18	+0.90
	18	+0.93

[a] Not significant at 5%.

interdependence of these factors, it is necessary to calculate the partial correlation coefficients recorded in Table 4. This Table also gives the corresponding coefficients for the objective methods of measuring texture.

TABLE 4. Partial correlation coefficients between specific gravity and the subjective and objective methods of measuring texture (d.f. = 73)

Factor 1	Factor 2	Factor 3	Correlation coeff. 1–2	Partial coeff. 1–2 with effect of 3 removed
s.g.	Breakdown Q.C.6	Mouthfeel	0.46	0.24[a]
s.g.	Mouthfeel	Breakdown Q.C.6	0.56	0.42
s.g.	Sediment	Extrusive force	0.41	0.25[a]
s.g.	Extrusive force	Sediment	0.55	0.46

[a] Significant at 5%.

Examination of the partial correlation coefficients indicates that the specific gravity has a considerable effect on mealiness and extrusive force, but little effect on breakdown or sediment; the positive value of the correlation coefficient shows that increased s.g. must be associated with larger forces being required to extrude the potato. This can be looked on as a simple dependence of the rheological properties of potatoes on the total solids content and this view sheds a new light on the anomaly pointed out by Burton[1] that mealiness is positively correlated with resistance to being cut by a wire[15, 22] and with resistance to a penetrometer.[23] This work also reports positive correlations between mealiness and the force required to extrude the samples.

In our opinion mealiness is controlled mainly by the total solids content and breakdown by another factor. Both primary factors change in a regular manner with maturity and so, in general, mealy tubers will be mature samples which will also exhibit a certain

amount of breakdown. Thus, correlations between mealiness and breakdown are to be expected in restricted populations because of the dependence of both these factors on maturity but the existence of a mathematical relationship cannot be adduced as evidence of a cause and effect relationship.

3.6. Effect of some primary factors on breakdown and mouthfeel

As an example of the application of these two objective methods of assessing texture, two further experiments were carried out. In the first, samples of potatoes of an experimental cultivar were planted at two dates differing by 4 weeks (19/4/71 and 17/5/71) and lifted at the same stage of physiological maturity as indicated by the onset of senescence of the foliage. The early planted sample took 119 days to reach this stage whilst the late planted sample required 110 days. In the second experiment mature samples of Record and Majestic were canned with and without the addition of 1.0% calcium chloride to the brine.

TABLE 5. Texture of planting date trial and calcium addition trial

	Planting date trial		Calcium addition trial	
	Early planting	Late planting	No added calcium	Added calcium
Sediment (ml/can)	1.38 (±1.16)	1.43 (±0.53)	15.1 (±11.2)	3.8 (±3.8)
Extrusive force (N)	334 (±14.1)	285 (±19.3)	349 (±82)	340 (±75)

TABLE 6. Values of Students' t test

	Planting date trial			Calcium addition trial		
Quality	d.f.	t	Probability of difference	d.f.	t	Probability of difference
Sediment	13	0.11	n.s. at 20%	19	3.04	99%
Extrusive force	14	5.78	99%	39	0.37	n.s. at 20%

[a] n.s., not significant.

This provided two sets of samples, those from the planting date trial being as similar as possible in all respects except that the later planted sample would be expected to be lower in total solids and starch whilst the two pairs of mature tubers were truly identical except for the calcium uptake during processing which would affect the pectins of the middle lamella without altering the total solids content measurably. Each set was assessed for texture using the sedimentation technique and the grid-extrusion cell to measure extrusive force. The results are given in Table 5 with the standard deviations in parentheses.

The probability of there being a difference in each of these four pairs of results was calculated using the "t" test and the results of this test are given in Table 6.

Similar results were also obtained using the subjective methods breakdown Q.C.6. and mouthfeel. The experiments show that it is possible to alter the two attributes of

texture breakdown and mouthfeel independently of each other and thus provide further substantiation for the usefulness of treating the two as independent variables. Further, the effect of calcium could indicate that the pectins play an important (although not necessarily exclusive) role in controlling the attribute of texture which we have called breakdown.

4. Conclusions

The texture of canned new potatoes can be defined in terms of two parameters—mouthfeel and breakdown—which are shown to be essentially independent of each other.

Mouthfeel can be measured objectively by measuring the force required to extrude the sample from the grid-extrusion cell of the Kramer Shear Press. Breakdown can be measured objectively by determining the volume of the sediment formed by the disintegrated tissue from a known weight of sample.

The primary factor controlling mouthfeel is total solids content whilst the amount of breakdown appears to depend on some other factor presumably associated with the pectins of the middle lamella. Correlations reported in the literature between breakdown and specific gravity and between mouthfeel and breakdown are assumed to be due to the fortuitous dependence of these three factors on a third independent variable such as maturity.

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Chemical Changes and Losses during the Ensilage of Wilted Grass Treated with Formic Acid

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Formic acid (85%) was added to wilted perennial ryegrass (36% dry matter) at the rate of 0.39%. Changes during ensilage of this material were compared with changes occurring during ensilage of untreated wilted ryegrass and freshly harvested herbage. All silages were well preserved, of low volatile N content and contained only traces of butyric acid. Formic acid restricted fermentation in the wilted grass resulting in silage of high water-soluble carbohydrate content (15.3%) compared with untreated wilted (4.7%) and fresh (1.2%) silages. Results of microbiological studies indicated that yeasts were more active in the formic acid-treated herbages. Surface waste production and fermentation plus oxidation losses were higher in the acid-treated wilted silages (21%) than in the untreated wilted materials (14%).

1. Introduction

The advantages of wilting crops to a dry matter (d.m.) content of about 30% prior to ensiling have been stressed by several workers.[1–4] Apart from reducing or eliminating effluent losses, wilting restricts or has a selective influence on microbial activity[5,6] and produces a silage more acceptable to ruminants.[4,7] One problem frequently associated with wilted herbage is the difficulty in achieving the degree of consolidation necessary to exclude air from the ensiled mass. Studies in which formic acid was applied to freshly harvested grass indicated that this additive restricted oxidation of the water-soluble carbohydrate (w.s.c.) between harvesting and ensiling.[8] It is possible that formic acid may have a beneficial effect in reducing respiration as well as in restricting microbial activity in ensiled wilted herbage. The results presented in this paper refer to an experiment in which the chemical and microbiological changes were examined in ensiled wilted grass, pretreated with formic acid, and in ensiled wilted herbage.

2. Experimental

The main silo unit in this experiment consisted of four metal silos,[9] each having a maximum capacity of about 1000 kg of fresh herbage and each suspended from a weighing apparatus sufficiently sensitive to record a change in weight in the silo and contents of 0.1 kg.

Perennial ryegrass (*Lolium perenne*) obtained from one of the school farms was cut with a mower on 8 June 1970 at 8 am and wilted for 31 h in the field before being lifted

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with a flail-type forage harvester. Untreated wilted grass was ensiled in silos A and B. The other two metal silos C and D were filled with similar wilted herbage treated with formic acid in the form of "Add-F"[a]. The Add-F was applied to the grass at the rate of 0.39% (equivalent to 0.33% pure formic acid) from a polyethylene container attached to the forage harvester as described in an earlier paper.[8]

The ensiled herbage was covered with polyethylene sheeting and after 48 h delay was consolidated with stone blocks corresponding to a surface pressure of 38 mbar (380 N/m^2). Assessment of true losses and surface waste measurements were made using a bag and marker technique already described.[10] Temperature measurements using eight thermocouples buried at different levels in each silo were recorded daily.

On 8 June 1970, two reinforced 2000 kg capacity PVC silos (E and F), similar to those described by Harris, Raymond and Wilson,[11] were filled with fresh herbage taken from the same field as that used in the wilting experiment. Only chemical changes were studied in these silos, the main object was to provide unwilted grass silage for a subsequent animal experiment. The analytical results for grass and silages are reported in this paper for comparison purposes.

Silos A, B, C, D, E and F were opened 149, 179, 156, 179, 179 and 114 days after filling, respectively.

Methods of sampling, chemical and microbiological analyses of grasses and silages were similar to those reported in earlier publications.[3,9]

In addition to the tower and plastic silos, laboratory test-tube silos (capacity 80 g) were filled with herbages similar to those used in the main experiment. An additional treatment was fresh grass plus Add-F, applied at the rate of 0.27% fresh herbage. The

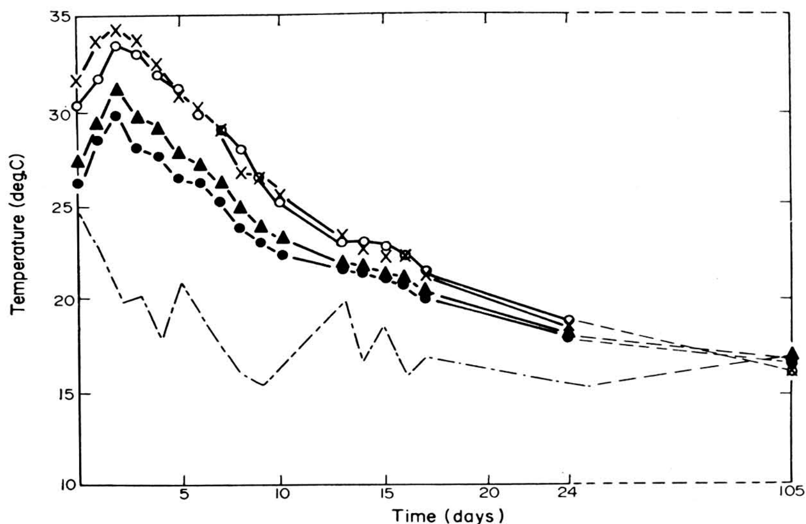

Figure 1. Temperature changes with time.
—×—×—, Silo A; —○—○—, silo B; —▲—▲—, silo C; —●—●—, silo D; - — - —, ambient temperature.

[a] A product containing 85% formic acid and manufactured by BP Chemicals International Ltd.

laboratory silos were fitted with mercury seals and duplicates were opened at intervals throughout the ensiling period. Ethanol, w.s.c. and pH values only are reported for these samples.

3. Results

3.1. Temperature changes

The temperature changes in the four silos are shown in Figure 1, each point on the graph representing the mean of eight thermocouple recordings. The temperatures followed a similar pattern, i.e. an initial rise followed by a decline after consolidation on the third day. Temperatures recorded in the formic acid-treated materials were consistently lower than those obtained in the untreated silages.

TABLE 1. Composition of grasses and silages (% of dry matter)

	Grass			Silage					
	Fresh	Wilted	Wilted and formic	A (W)[a]	B (W)	C (WF)	D (WF)	E (U)	F (U)
Dry matter (d.m.)	17.75	32.33	36.00	30.82	30.90	32.88	34.49	18.04	18.68
Organic matter	93.0	92.8	93.1	92.9	92.0	92.7	92.5	93.1	93.2
Crude protein	14.2	13.4	14.4	14.3	14.0	15.1	15.0	14.6	14.4
Ether extract	2.41	1.94	2.37	2.47	2.56	2.46	2.69	3.5	3.4
Crude fibre	26.5	26.9	25.7	28.7	29.3	28.0	29.5	31.0	29.8
Total N	2.27	2.15	2.31	2.28	2.24	2.42	2.40	2.34	2.30
Protein N	1.84	1.47	1.58	0.66	0.47	0.76	0.63	0.36	0.54
Non-protein N (n.p.n.)	0.43	0.68	0.73	1.62	1.77	1.66	1.77	1.98	1.76
Volatile N	—	—	—	0.19	0.19	0.16	0.16	0.20	0.18
Volatile N as % total N	—	—	—	8.3	8.3	6.8	6.7	8.5	7.8
Water soluble carbohydrates (w.s.c.)	17.7	16.8	18.5	4.75	4.56	15.1	15.4	1.44	0.99
Glucose	4.4	3.1	4.8	1.6	1.5	3.2	4.0	0.3	0.2
Fructose	2.9	3.4	3.9	1.4	0.9	7.5	7.4	0.3	0.3
Xylose	—	—	—	Trace	0.1	0.2	0.2	0.1	Trace
Galactose	—	—	—	0.9	0.9	1.5	1.2	0.3	0.1
Arabinose	—	—	—	Trace	0.2	0.3	0.2	Trace	Trace
Oligosaccharides (including sucrose)	4.0	5.0	5.0	0.7	0.8	2.2	2.2	0.4	0.3
Fructans	6.4	5.3	4.8	0.1	0.1	0.1	0.1	0.1	0.1
Mannitol	—	—	—	3.6	3.7	1.9	1.1	4.3	4.1
Cellulose	27.5	26.5	26.0	28.6	28.8	28.6	29.5	30.1	30.2
Lignin	4.0	4.4	4.3	4.4	4.4	4.4	4.2	4.7	4.1
Formic acid	—	—	0.92	Nil	Nil	0.5	0.6	0.1	Trace
Acetic acid	—	—	—	2.4	3.0	0.8	0.9	3.2	3.6
Propionic acid	—	—	—	0.03	0.05	0.08	0.05	0.18	0.17
Butyric acid	—	—	—	0.06	0.12	0.06	0.10	0.17	0.14
Lactic acid	—	—	—	5.9	8.2	4.3	4.8	11.1	10.2
Succinic acid	—	—	—	Nil	Nil	Nil	Nil	Trace	Trace
Ethanol	—	—	—	0.64	0.61	0.61	0.68	1.2	1.2
pH after maceration	6.08	6.21	4.93	4.18	4.29	4.39	4.45	3.94	3.94
Buffering capacity (b.c.) mequiv./100 g of d.m.	35	32	35	89	98	54	62	137	112

[a] W=Wilted; F=formic acid-treated; U=unwilted.

TABLE 2. Microbiological assay

Sample	Bacterial count[a] (no. of organisms/g of fresh material) YEA	TA	MA	GM	LM	pH	% Ethanol in d.m.	% w.s.c. in d.m.
Grass								
Uncut grass, 8 June, 8 am	1.2×10^3	100	<10	10^3	<10	6.20	—	19.3
Forage harvested grass, 8 June, 8.05 am	2.2×10^6	366	30	10^3	<10	—	—	—
Wilted grass, 9 June, 9 am	2.9×10^6	1.7×10^4	30	10^3	<10	—	—	—
Wilted grass, 9 June 3 pm	4.5×10^6	1.1×10^5	<10	10^3	<10	—	—	—
Forage harvested wilted grass, 9 June, 4 pm	4.6×10^6	7.2×10^5	<10	10^3	<10	6.21	—	16.8
Silage[b]								
After 1 day, silo A	6.5×10^8	1.0×10^7	373	10^4	<10	5.91	—	17.9
After 1 day, silo C	5.3×10^5	1.0×10^7	<10	10^3	<10	5.42	—	20.9
After 2 days, silo A	2.8×10^8	1.3×10^7	26	10^4	<10	5.00	—	10.3
After 2 days, silo C	4.9×10^5	1.0×10^5	103	10^4	<10	5.11	—	20.0
After 3 days, silo A	1.7×10^9	7.3×10^6	46	10^5	<10	4.85	0.5	—
After 3 days, silo C	3.1×10^6	1.4×10^6	293	10^3	<10	5.44	0.6	—
After 4 days, silo A	5.2×10^8	8.5×10^8	4.5×10^3	10^3	<10	4.80	0.5	9.8
After 4 days, silo C	1.1×10^8	1.1×10^8	1.0×10^4	10^3	<10	5.24	0.3	22.2
After 8 days, silo A	3.2×10^8	3.9×10^8	4.6×10^4	10^2	<10	4.60	0.4	9.7
After 8 days, silo C	1.2×10^8	2.1×10^8	9.8×10^4	10^2	<10	4.91	0.6	15.7
After 23 days, silo A	2.0×10^8	0.4×10^8	1.4×10^5	<10	<10	4.34	1.4	5.8
After 23 days, silo C	4.6×10^8	4.8×10^8	2.8×10^6	<10	<10	4.61	1.5	7.5
After 34 days, silo A	7.6×10^6	4.8×10^7	1.9×10^5	<10	<10	4.28	1.2	4.9
After 34 days, silo C	1.3×10^8	6.4×10^7	0.9×10^5	<10	<10	4.36	0.8	11.4
After 71 days, silo A	9.0×10^7	8.0×10^7	10^2	<10	<10	4.18	1.0	3.8
After 71 days, silo C	1.4×10^9	1.7×10^9	10^3	<10	<10	4.38	0.8	4.9
After 149 days, silo A	2.6×10^6	1.2×10^6	<10	<10	<10	4.18	0.6	4.8
After 156 days, silo C	2.5×10^5	1.6×10^7	6.4×10^3	<10	<10	4.39	0.6	15.1

[a] YEA = Yeast extract agar (total count—all organisms)
MA = Malt agar (yeasts and fungi)
LM = Lactate medium (lactate fermenters).
TA = Tween agar (lactic acid bacteria)
GM = Gelatin medium (proteolytic Clostridia)

[b] Silage samples, except final ones, were taken from the side ports using a corer.

3.2. Composition

The composition of the grasses and silages is shown in Table 1. The d.m. content of the acid-treated wilted grass (36%) was slightly higher than that of the untreated material (32.3%). The main differences in the d.m. components are seen in the carbohydrate and organic acid fractions, the soluble carbohydrate percentages being higher and the acetic and lactic acid percentages lower in the formic acid-treated silages compared with the untreated materials. These differences in acid content are also reflected in the pH values of the silages. The unwilted silages are typical of such material, being of low pH (3.94), of relatively high lactic acid content (10.7%) and containing little residual soluble carbohydrate (1.2%).

3.3. Microbiological assay

Core samples were taken from side ports in silos A and C after 1, 2, 3, 4, 8, 23, 34 and 71 days and results of microbial counts for these together with counts on original grass samples and final silages are given, using five different media, in Table 2. Although total counts of micro-organisms were consistently lower in the formic acid-treated silages there was no evidence from the Tween acetate agar counts, with the possible exception of the sample obtained after 2 days, to indicate that the activities of lactic acid bacteria were lower in the formic acid-treated material than in the untreated herbage. Microbial proteolytic and lactate fermenting activities were negligible.

3.4. Losses

The losses of d.m. are shown in Table 3. The total d.m. loss is made up of two fractions—fermentation plus oxidation and waste material. Using the bag and marker technique referred to earlier it was possible to calculate the fermentation plus oxidation losses from the well-preserved edible material separately and these are also reported in Table 3.

TABLE 3. Losses during ensilage

	Silo			
	A (W)[a]	B (W)	C (WF)	D (WF)
Herbage d.m. ensiled (kg)	280.9	280.9	281.5	281.5
Edible silage d.m. removed (kg)	244.0	238.9	218.6	231.3
Waste d.m. removed (kg)	14.7	20.6	29.4	24.1
[b] Fermentation plus oxidation loss in edible silage (%)	3.0	3.0	7.9	7.5
[c] Waste loss (%)	10.4	12.4	15.6	11.1
Total loss (%)	13.4	15.4	23.5	18.6
Loss of total N (%)	+3.0	+1.1	3.6	4.2
Loss of w.s.c. (%)	72.5	73.6	25.2	22.6

[a] W=Wilted; F=formic acid-treated.

$$^{b} = 100 - \frac{\text{kg d.m. removed as edible silage}}{\text{kg grass d.m. ensiled} - \text{kg grass d.m. to form waste}} \times 100.$$

$$^{c} = \frac{\text{kg grass d.m. to form waste}}{\text{kg grass d.m. ensiled}} \times 100.$$

3.5. Laboratory silos

The analytical results of the laboratory silages are shown in graph form in Figure 2.

The pH values of the laboratory wilted silages followed a similar pattern to those found for the cored samples taken from the large silos. The pH values of the treated and untreated fresh silages in the final stages were similar.

The w.s.c. components were consistently higher in the formic acid-treated wilted silages than in the untreated material. In the unwilted silages, formic acid had only a slight effect in preserving w.s.c.

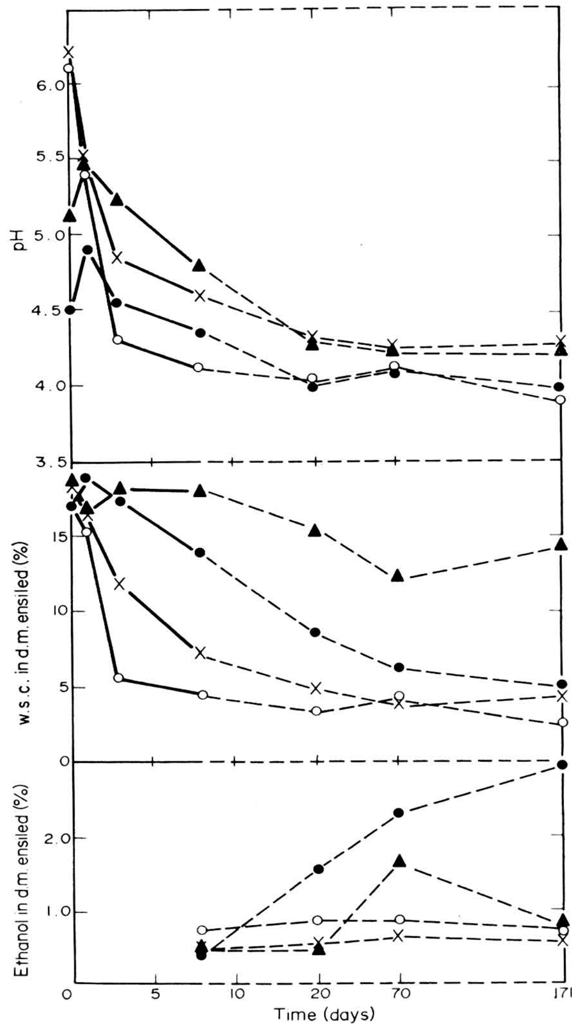

Figure 2. pH, water-soluble carbohydrates and ethanol values for laboratory tube silos. (○) Fresh; (●) fresh plus Add-F; (×) wilted; (▲) wilted plus Add-F.

The ethanol values for the acid-treated fresh herbage were about four times those obtained for the other silages in the final samples.

4. Discussion

In previous studies[8] using 14.5% d.m. fresh timothy/meadow fescue grass, Add-F applied at the commercially recommended level (0.27%) resulted in an immediate pH fall to 4.75. In the present experiment using 36% d.m. wilted ryegrass the application of 0.39% Add-F caused the pH to fall to 4.93. With the exception of d.m., w.s.c. and non-protein nitrogen (n.p.n.) the untreated and acid-treated wilted herbages were similar in composition to the fresh grass. The increase in n.p.n. during wilting confirms the results obtained in a previous experiment[3] that proteolytic enzymes become active immediately after harvesting.

Accepting that temperature rise within the silo is a direct measure of rate of respiration, formic acid apparently inhibited to some extent respiration in the early stages of ensiling; this finding agrees with the results of other workers.[12] The temperature peak in Figure 1 corresponds to the time of application of consolidation weights on day 3, confirming that adequate compression of the herbage mass in the silo is an effective method of controlling temperature in wilted material.

The silage analytical results (Table 1) indicate that formic acid effectively restricted fermentation in the wilted herbage. The lower lactate, acetate, buffering capacity (b.c.) values and higher w.s.c. components support this conclusion.

The carbohydrate components of the acid-treated silages are particularly interesting in that the residual w.s.c. were high—about 15%—and of these only 1.8% could be accounted for in terms of monomers derived from heteropolysaccharides. High residual w.s.c. values (>15%) have been reported in earlier studies[3] with heavily wilted Italian ryegrass of 47% d.m. Mannitol, a product of the action of heterofermentative lactic acid bacteria on fructose,[3] was present in all silages, although the concentration in the formic acid-treated silages was less than half that found in the non-treated wilted and fresh silages.

Formic acid had no obvious effect in preventing proteolysis during ensilage although it appeared to have a slight effect in inhibiting deamination. In previous studies using fresh grass,[8] formic acid had a beneficial effect in preventing amino-acid catabolism. The fermentation in all silages was clearly dominated by lactic acid bacteria, there being only small amounts of products derived from clostridial activity.

The microbiological data (Table 2) obtained from cored samples confirm that proteolytic clostridia and lactate fermenters were relatively inactive in the wilted silages, although surprisingly counts on the Tween acetate agar, a medium specific for lactic acid bacteria, were similar for both the untreated and acid-treated silages. In the later cored samples, taken after 8, 23, 34 and 71 days, there is some evidence from the w.s.c. values, that these were not truly representative of the silage mass and the bacteriological counts should be interpreted accordingly. In these later samples, silage had to be removed via ports which had been opened for previous sampling and although care was taken to avoid sampling from identical areas within the silo, some aerobiosis in the vicinity of the port may have affected the fermentation pattern.

The lactic acid bacteria increased on the grass during wilting and also immediately after forage harvesting. This last finding confirms the results of previous studies.[13] With the exception of the first day's samples and particularly in the final sample, yeast counts were higher in the formic acid-treated silages. These high counts can be explained by the findings of concomitant studies[14] showing that formic acid is less inhibitory to yeasts than to lactic acid bacteria. The possibility of yeasts accounting for losses in formic acid during ensilage by using it as a C-source is being investigated. The products of yeast fermentation are ethanol and carbon dioxide. There is however no apparent evidence from the ethanol contents of the silages of excessive yeast activity in the treated silages, the concentrations being similar (0.6 to 0.7 %) in the four wilted silages. Ethanol, however, is also a by-product of the fermentation of glucose by heterofermentative lactic acid bacteria[3] and it is possible that the ethanol in the untreated silages was derived via this last pathway rather than from yeast fermentation. The mannitol values lend support to this hypothesis.

The losses data in Table 3 are more difficult to interpret. Assuming restricted bacterial activity in the formic acid-treated silages it would be reasonable to expect lower d.m. losses during ensilage of the treated herbage, but this did not occur. The higher waste losses obtained in the treated silages indicate that oxidation in the surface layers was greater than in the untreated silages. This finding confirms previous results that formic acid does not have any beneficial effect in preventing the formation of surface waste.[8,12] When d.m. losses in the edible material alone are considered, the values for the untreated silage (3 %) are very low compared with the acid-treated material (8 %). A more active yeast fermentation with correspondingly high gaseous (CO_2) loss may be a partial explanation for this finding.

The analytical results using laboratory tube silos are similar to those obtained using the large silos and confirm that formic acid preserves the w.s.c. in the wilted grass. The high ethanol values obtained for the acid-treated fresh grass silages indicate that a yeast-type fermentation occurred in this material.

The significance of these differences in composition between the silages and in particular the importance of high residual w.s.c. in the acid-treated wilted silage to the ruminant animal will be reported in a later paper.[15]

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An Investigation on Laboratory and Field Methods of Determining the pH of Soil Suspensions

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A comparison was made between the pH values of soil samples, measured with a pH meter in the laboratory, and those obtained by using colorimetric indicator solutions in the field. Of the indicators tested, BDH Soil Indicator and bromocresol purple provided the closest overall comparison with laboratory results for soils with pH values from 3.8 to 7.8. Bromocresol green was useful only over a limited range (less than 5.2). Differences in procedure and analysis sometimes produced compensatory errors but the results did not often agree exactly and the magnitude of the discrepancy for any given soil was predictable only within fairly broad limits—the average standard deviation was 0.34 units and there was about a 75% chance of the result determined in the field being within 0.3 units of the result determined in the laboratory.

1. Introduction

The pH of a soil sample is usually measured with a pH meter[1,2] or by using colorimetric indicators.[3,4] With the meter the results are accurate, reproducible between different operators and have been correlated meaningfully with plant performance in the field.[5,6] Unless a portable meter is used, however, the soil has to be taken to the laboratory for analysis where the equipment required is relatively expensive and some experience is necessary before it can be operated reliably and effectively. Furthermore, it is questionable whether such a high degree of accuracy in pH measurement (often quoted to two places of decimals) is always appropriate when the variation in value between different sites in the same field may be quite considerable.[7,8]

Colorimetric indicator methods depend on a subjective assessment of the soil pH by comparing the colour of the soil suspension, after indicator and a flocculating agent have been added, with a standard pH colour chart for that indicator. This is a quick, simply operated and inexpensive method which may be used to provide an immediate assessment of soil pH in the field. In certain circumstances (e.g. diagnosis of crop failures, soils advisory work and field surveys of soil properties) these measurements might be very useful. However, there is some doubt surrounding the accuracy of the results obtained and with few exceptions[9–11] there is little published information on the subject.

The objectives of this investigation were to examine and compare the results using the two different methods of pH determination on a wide range of soil samples and to attempt to explain any discrepancies which might arise.

2. Experimental

2.1. Soils

The soils in the pH plots at Woodlands Field, North of Scotland College of Agriculture, Craibstone, were used as a starting point since they covered a wide pH range (about 4.5 to 7.5). Many different soil types including peats, podsols, brown earths and gleys, were also studied.

2.2. Methods

Three separate methods were used for assessing the pH values of soil suspensions.

2.2.1. Standard method

Moist soil samples (about ½ to 1 kg) were taken from the field to the laboratory and dried at room temperature over a period of 2 to 3 days. The sample was lightly crushed and sieved through a 2-mm sieve. A subsample (10 g) was weighed into a clean test tube and 25 ml of distilled water was added. The mixture was shaken for about 5 min and allowed to stand overnight for at least 16 h. The mixture was shaken again and the pH measured on a pH meter by immersing the electrodes in the soil suspension. Different pH meters and glass electrodes were used during the investigation; these included E.I.L. pH meter models 23A, 38B and 39A and Beckman Zeromatic with E.I.L. dual electrodes S.H.D. and S.C.D. (toughened) and Beckman electrodes. The meter was always calibrated beforehand using Buffer solutions (pH 4.0 and 7.0).

When required, conductivity measurements were made with a soil "CF" meter, (Type MC4 Electronic Switchgear Ltd) on the soil suspension after pH had been read.

2.2.2. Without preparation

With the exception that the soil was not subjected to the preparation procedure (i.e. drying, sieving and weighing), pH was determined as above.

2.2.3. Colorimetric indicators

Fine moist soil (stones greater than about 3 mm were removed) was placed in a clean, dry test tube to a depth of about 3 cm. Specially prepared (supplied by BDH Chemicals Ltd) barium sulphate powder (2 to 3 ml) was added to the soil and distilled water was introduced until the level in the tube was approximately 10 cm. The tube was stoppered and shaken lightly, about 1 ml (8 drops) of indicator solution was added and the mixture was shaken vigorously for a few seconds. The soil suspension was allowed to settle for 5 min and the pH assessed by comparing the colour of the suspension with the colour chart for that indicator. Extra barium sulphate was added if the soil suspension did not settle satisfactorily and extra drops of indicator solution were added to the suspension and stirred in lightly with a clean glass rod if the colour of the supernatant solution was not sufficiently marked or clear.

The following indicator solutions were prepared[12] and examined during the investigation: BDH Soil Indicator, bromocresol green, chlorophenol red, cresol red, Azolitmin, phenol red, methyl red, bromocresol purple, naphtholphthalein, Alizarin Red S, bromothymol blue, cochineal and litmus. In each case a calibration chart (of the colour of the solution against the corresponding pH value) was prepared by adding 1 ml of the indicator to a series of buffer standards (25 ml) made up in the required pH range.

3. Results

3.1. Variation in the pH of a soil suspension with respect to different (a) sites in the same field, (b) depths in the same profile and (c) times in the season

"Spot" samples of soil were augured to a depth of 15 to 20 cm at different sites in the same field. The pH of each sample was measured separately by the standard method. Results from five different fields are given in Table 1.

TABLE 1. pH variation at different sites in the same field

Field	Number of samples	pH Range	Mean pH	S.D. of individual observations
1	12	5.7–6.4	6.1	0.20
2	19	4.8–5.9	5.4	0.29
3	27	5.7–6.6	6.1	0.22
4	33	5.6–6.6	6.1	0.24
5	24	4.6–6.2	5.1	0.37

These figures demonstrate the considerable variation that exists in pH values of soil samples taken from different sites in the same area. All fields were less than 4 hectares. The pH values of soil samples taken from different depths in the same profile and at different times during the year exhibited a similar degree of variation. There was no consistent trend, however, between the observed pH and the depth of sample or the time of sampling.

3.2. Variability in soil pH of subsamples (10 g) taken from the same bulk sample (250 g) of soil

Samples of soil submitted to the laboratory for analysis were usually less than 1 kg (dry wt) and only 10 g were required for analysis after drying and sieving. The standard method was used to measure the pH of different subsamples taken randomly from the same well-mixed bulk sample. Some representative results are given in Table 2.

TABLE 2. Variation in pH of subsamples of soil taken from the same bulk sample

	Number of readings	Average	S.D. of individual observations
Sample 1	12	4.1	0.08
Sample 2	12	5.3	0.06
Sample 3	12	6.0	0.06
Sample 4	12	7.0	0.14

These figures show that, provided the bulk sample of soil is thoroughly mixed, the variation observed in the pH of separate subsamples is relatively small. This is further illustrated by the fact that of 450 soil samples analysed by different operators using different meters and electrodes, 99 % of duplicated values agreed within 0.3 units.

3.3. Effect of sample preparation on the observed pH of a soil suspension

In the standard laboratory measurement of soil pH the soil is dried and sieved before taking a definite ratio ($2\frac{1}{2}:1$) of water : soil and allowing an equilibration period of at least 12 h to elapse before analysis is made. Any field method for determining soil pH would differ in that the soil is neither dried, sieved or weighed. Furthermore, indicator solution and barium sulphate are added in the field and the result is assessed within minutes.

An attempt was made to assess the influence of these procedures on the measured soil pH.

The results in Figure 1 show that the preparation procedure influenced the observed pH of a soil suspension. Drying and sieving the soil sample tended to reduce the value compared to the wet, unsieved sample. The average decrease observed for 450 soil samples, analysed in duplicate, was 0.27 units. The magnitude of the effect varied from soil to soil, however, and in a few cases, an increase in pH was observed after drying the soil sample.

Increasing the ratio of soil : water from $2\frac{1}{2}:1$ to $9:1$ was accompanied, on average, by an increase of 0.2 units in the pH of the soil suspension. Addition of $BaSO_4$ powder

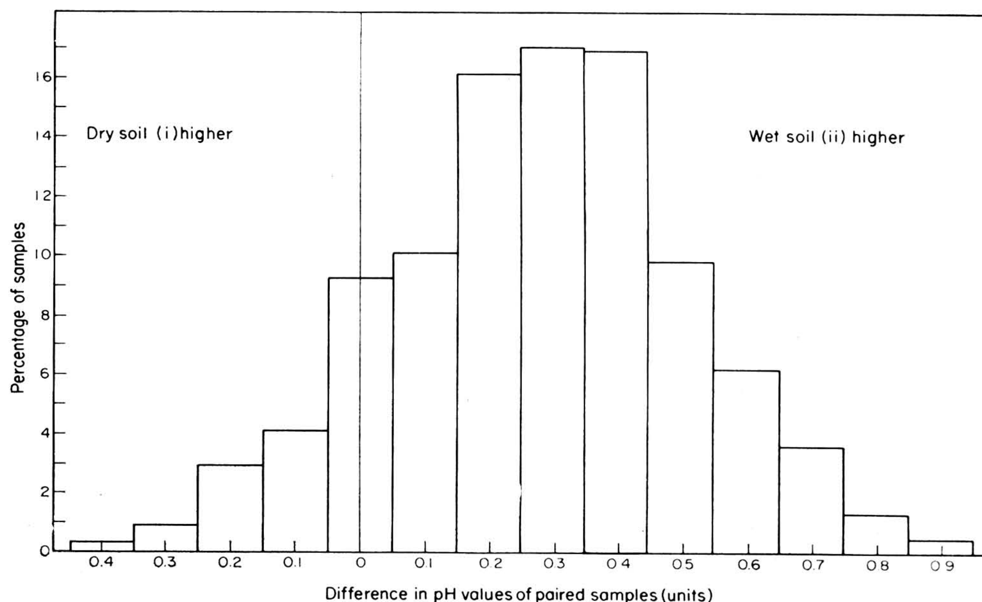

Figure 1. The effect of two different sample preparation procedures on the observed pH values of soil suspensions. (i) Sample was dried, sieved (<2 mm), fixed ratio (1 : 2.5) soil : soil solution was used. (ii) Sample was moist, unsieved and ratio soil : soil solution not fixed but in the range 1 : 2 to 1 : 6.

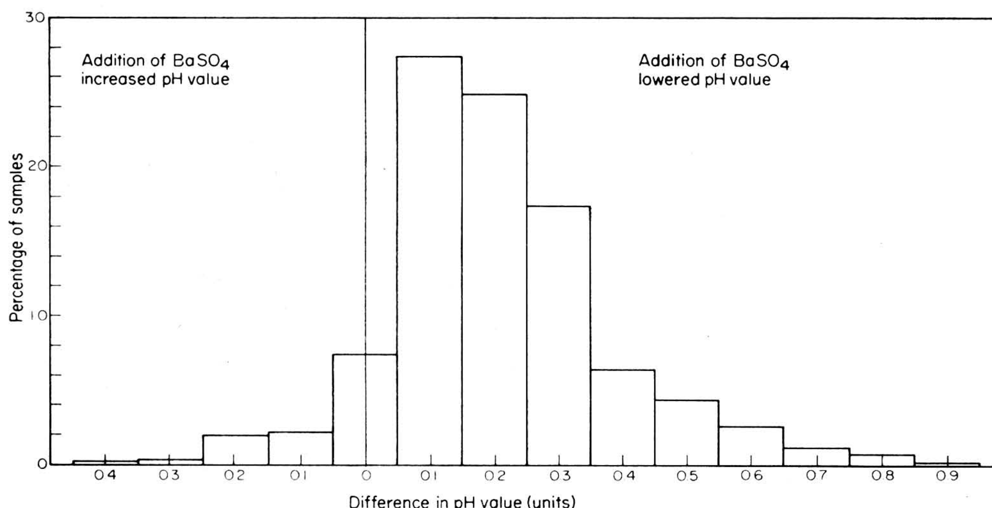

Figure 2. The effect of adding barium sulphate powder on the observed pH of a soil suspension (518 samples investigated).

usually had the opposite effect (Figure 2) and also increased the electrical conductivity of the soil suspension. Although these trends were quite clear, the precise effect varied with different soils

Finally, adding BDH Soil Indicator Solution (1 ml) had a small effect on the observed pH of the soil suspension (Figure 3). These observations were similar to those obtained with the other (BCP and BCG) solutions.

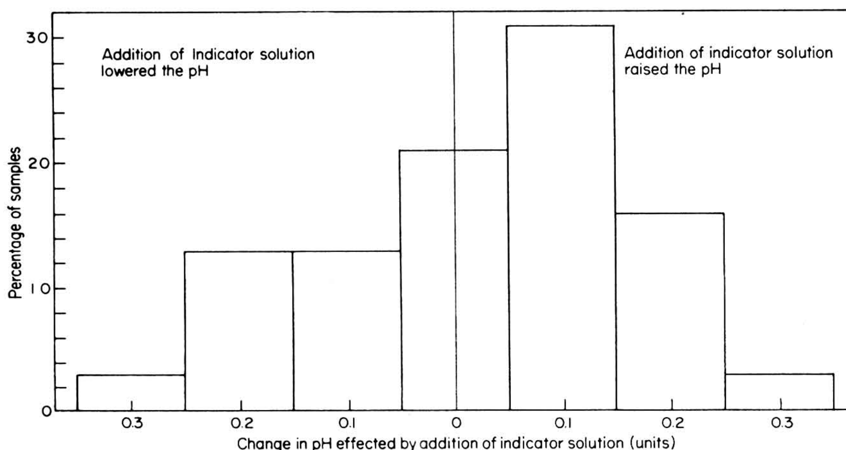

Figure 3. The effect of adding indicator solution on the observed pH of a soil suspension (100 samples investigated).

3.4. Comparison between the pH values measured by the standard method with the values assessed in the field using (a) BDH Soil Indicator solution, (b) bromocresol purple indicator solution and (c) bromocresol green indicator solution

Soils were sampled in the field and assessed under the atmospheric conditions which prevailed at the time. Several field visits were made to different areas and ten different operators were engaged, at different times, in the investigation. In all cases, the pH was assessed by the appropriate colour method in the field and then samples were immediately dispatched to the laboratory and prepared for examination by the standard method. The results for BDH Soil Indicator and bromocresol purple indicators are presented in Tables 3 and 4.

TABLE 3. Comparison between pH results of soil samples as determined by the standard method with those determined by using BDH Soil Indicator

	pH ranges						
	<5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5	Overall
Number of soils tested	320	227	261	176	138	18	1140
Mean difference between the two assessments[a]	+0.29	+0.14	+0.05	+0.10	+0.25	+0.15	+0.17
S.D. of individual differences between the two assessments	0.39	0.30	0.37	0.33	0.30	0.31	0.36
% in which meter and colour assessments agreed within 0.3 units	61	77	67	63	72	65	73
% in which colour assessment was higher than standard determination	72	60	47	52	74	55	61
% in which colour assessment was lower than standard determination	14	20	39	32	14	28	24

[a] The +ve sign indicates that on average, the colour assessment was higher than the standard determination.

TABLE 4. Comparison between pH results of soil samples as determined by the standard method with those assessed by using bromocresol purple indicator

	pH ranges					
	<5.3	5.4–6.0	6.1–6.5	6.6–7.0	>7.0	Overall
Number of soils tested	206	184	100	73	58	621
Mean difference between the two assessments	+0.06	+0.11	+0.01	−0.09	−0.28	+0.02
S.D. of individual differences between the two assessments	0.19	0.38	0.29	0.30	0.23	0.31
% in which meter and colour assessments agreed within 0.3 units	91	67	76	70	56	76
% in which colour assessment was higher than the standard determination	—	55	44	28	10	35
% in which colour assessment was lower than the standard determination	14	34[a]	39	56	84	54

[a] In 4% of these cases the colour indicated "less than 5.3" and the magnitude of the error was not exactly known.

Whereas BDH Soil Indicator produces a series of distinct colours, at intervals of 0.5 pH units, over the usual pH range of soils (4.0 to 8.0) the variation in colours with bromocresol purple was restricted to the range 5.4 to 7.0. Below 5.4 all solutions were yellow and above 7.0 a purple colouration was obtained. Frequently, the colour of the soil suspension made comparisons with the standard colours difficult. With these limitations, Tables 3 and 4 show that, for the soils tested, about 75% of all individual estimates of soil pH made in the field were within 0.3 units of the values determined in the laboratory. The average standard deviation of the difference between field and laboratory values was 0.36 and 0.31 units for BDH Soil Indicator and bromocresol purple, respectively. The level of agreement between paired results varied with the actual pH range of the soil tested. BDH Indicator was more successful when the soil pH was greater than 5.0 and bromocresol purple was better at detecting pH's less than 5.3. However, bromocresol purple could not distinguish between pH values which were less than 5.3 and, in this limited range, bromocresol green was a useful indicator.

4. Discussion

The results clearly demonstrate that the pH of a soil suspension may be expected to vary markedly at different sites within a relatively small area (the fields sampled were 2 to 4 hectares in size), at different depths within the soil profile itself and also when the samples (from an identical spot) are taken at different times of the year. Differences in pH value arising from the laboratory measurement itself were much less than those due to sampling. Greater discrepancies between measurements of the same soil may occur, however, if different sample preparation procedures are adopted.

In general, drying and sieving a soil tended to reduce the pH of the soil suspension and increasing the ratio of soil : soil solution had a similar effect. Drying, crushing and sieving a soil will probably increase the percentage of fine grained material (such as clay and humus) at the expense of the coarser material (such as stones and coarse sand). The subsequent increase in cation exchange capacity may partially account for the observed differences in acidity of soil samples, whereas diluting the sample with water should have the opposite effect. These effects were fairly well established with the soils studied but the magnitude of the changes brought about, or indeed the direction of change, was certainly not consistent or predictable. The ratio of water : soil taken in the field determination was usually greater than 2.5 : 1 and probably nearer 5 : 1. The resulting increase in pH (compared with the standard method) will often be counteracted by the addition of barium sulphate which is used to flocculate the soil and create a clear solution. Conductivity measurements indicated that the barium sulphate had dissolved to some extent. The addition of indicator had only a small effect on the pH of the soil suspension.

For these reasons alone, one cannot expect any colour assessment of soil pH in the field to compare exactly with the results measured in the laboratory on the same sample of soil. A comparison of the results between field and laboratory determinations of soil pH are presented for two different indicators (BDH Soil Indicator and bromocresol purple) used on a wide range of Scottish soils studied during this investigation. Of the indicator solutions examined, these were judged to be the most successful.

In all cases, except possibly in silty soils and other soils which will not settle in suspension, a colour will be obtained by suitable additions of water, indicator and barium sulphate. It does not follow, however, that the colour produced or the pH assessed from it is "correct". The possibility of errors in subjective judgement of colour is quite clear, and this is likely to be made more difficult by fluctuating light conditions (e.g. the same colour may appear different when viewed on a sunny day or a dull day). This consideration is more applicable to BDH Indicator where the colours are not so strong as those of bromocresol purple or bromocresol green. The strength of the colour is influenced by its concentration in solution and, in certain cases, particularly those soils high in organic matter, adsorption of the indicator by the soil particles appears to influence the colour of the resulting solution quite independently of the soil pH. Other soils may produce a fine soil suspension or dissolved soil components whose colour can interact with that of the indicator. Normally, the pH is assessed within a few minutes of shaking the sample but often small changes in colour and, therefore, in pH could be detected after somewhat greater intervals. This effect is presumably due to lack of equilibration between the soil and its suspension or to a time-dependent reaction between the components of the mixture. With experience, one may be able to make allowance for these effects but some operators may be unable to distinguish colours satisfactorily and there is always a real danger of contamination of glassware and reagents in the regular field determination of soil pH.

5. Conclusions

Although the field assessment of pH may be a useful preliminary in the investigation of field problems or conditions, the results must be treated with caution. They will rarely agree exactly with the results determined in the laboratory and the magnitude of the error in any given case may be misleading and harmful in its effect. Examination of separate samples of the same soil with two (or more) different indicators may eliminate some serious errors, and is recommended, but any field assessment of soil pH should always be backed up, or at least checked at regular intervals, with subsequent laboratory analysis of the same soil samples.

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Organic Phosphorus in Some Northern Nigerian Soils in Relation to Soil Organic Carbon and as Influenced by Parent Rock and Vegetation

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The distribution of organic phosphorus in relation to soil organic matter and the influence of parent material and vegetation of organic P level was studied in a number of soil profiles in the northern half of Nigeria. There was a close relationship between soil organic carbon and organic phosphorus contents of these soils. The ratios of these two ingredients were all less than 200:1. This fact suggests that organic P is likely to be mineralised. The effect of parent rock on organic P contents was slight, but there was an indication that soil profiles on metamorphic parent rocks contained more organic phosphorus than those on sedimentary parent material. Vegetation had a great influence on organic P as shown by the relatively higher organic phosphorus contents of profiles from southern Guinea savanna and the lower contents of the Sudan vegetation zone. The profiles from impeded drainage sites (*fadamas*) were generally richer in organic phosphorus than were those from comparable vegetation zones on well drained sites.

1. Introduction

Organic phosphorus is an appreciable ingredient of the total phosphorus supply in tropical soils. Accordingly, in tropical regions, where inorganic fertiliser use is severely restricted, organic phosphorus is a very significant source of P for plant growth. Organic P can only be used by plants after it is mineralised. The rate of mineralisation depends, in part at least, on the ratio of organic C to organic P in the soil (Acquaye).[1] The current study was designed to determine the amount of organic P and the ratio of organic C to organic P in some soils of the northern half of Nigeria as influenced by parent rock and vegetation.

Soils derived from two broad groups of rocks were compared: metamorphic and igneous on the one hand and sedimentary on the other. The metamorphic rocks were gneiss, quartzite and gneissic rock, and the igneous rock was pegmatite; the sedimentary rocks were alluvium, sandstone and iron pan. For a study of the effect of vegetation well drained soils from three zones, southern Guinea savanna, northern Guinea savanna and Sudan were compared, while also soils with impeded drainage were studied. The latter were allocated in the southern Guinea savanna, northern Guinea savanna and Sub-Sudan zones. These vegetations zones correspond with the climatic regimes described by Clayton.[4]

2. Experimental

2.1. Site selection

Soil samples from the selected sites were collected over the northern half of Nigeria. The geographic location, annual rainfall, vegetation zone and parent rock for each profile are presented in Table 1. From four to seven horizons of each profile were sampled. Soil samples were dried and sieved through a 2-mm screen. Material less than 2 mm in diameter was analysed for organic carbon, pH, texture, organic P and total P.

TABLE 1. List of soil profiles studied and their environmental data

Geographical location	Rainfall	Climate/vegetation zone	Parent material
	Effect of parent material (i) metamorphic rocks		
(a) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Gneiss
(b) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Quartzite
(c) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Gneissic rock
(d) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Pegmatite
	Effect of parent material (ii) sedimentary rocks		
(a) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Sandstone
(b) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Ironpan
(c) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Alluvium
	Effect of vegetation (i) well drained (upland) soils		
(a) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Sandstone
(b) Auna	1000 mm (39.38 in)	Northern Guinea savanna	Sandstone
(c) Bernin Kebbi	891 mm (35.12 in)	Sudan	Sandstone
	Effect of vegetation (ii) impeded drainage sites (*fadama*)		
(a) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Alluvium
(b) Bomo	1088 mm (42.85 in)	Northern Guinea savanna	Alluvium on basement complex
(c) Dugutsofo	986 mm	Sub-Sudan	Alluvium
	Comparison of an upland soil profile with a *fadama* soil profile		
(a) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Alluvium (upland)
(b) Lafiagi	1199 mm (47.25 in)	Southern Guinea savanna	Alluvium (*fadama*)

2.2. Analytical methods

Particle size distribution (texture) was determined using Bouyoucus method. Reaction was measured with a glass electrode assembly in a 1:2 soil: 0.01 M-$CaCl_2$ suspension. Organic carbon was determined by the modified Walkley–Black method. The Legg and Black[6] ignition method for organic P was found to be unsatisfactory because in sub-soil material negative results were obtained. Accordingly Mehta *et al.*[8] extraction method was followed; except that the measurement of inorganic P before and after digestion was made by Murphy and Riley's modification of the molybdenum blue method.[9]

3. Results

The texture, the pH, organic C, organic P and total P contents of the various horizons and profiles are shown in Tables 2 to 6. Texture ranged from sand to sandy clay; all the soils were acid. There were appreciable quantities of organic C in the surface horizons of each profile, and this ingredient decreased with depth. The surface horizons of profiles from the impeded drainage sites (*fadamas*) were generally higher in organic C

TABLE 2. The effect of parent material on the distribution of soil organic phosphorus

Metamorphic rocks

Parent material	Profile depth (in)[a]	Texture	pH	Organic carbon (%)	Organic P (mg/kg soil)	Total P (mg/kg soil)	Organic P as % of total P	Organic C / Organic P
Gneiss	0–4	ls	6.3	1.5	82.3	338.3	24	182
	4–14	scl	5.8	0.33	60.8	312.5	19	54
	14–25	scl	5.0	0.19	44.0	168.8	26	43
	25–44	scl	5.1	0.17	42.2	140.6	30	40
Quartzite	0–2	s	6.3	1.3	81.6	225.0	36	159
	2–10	s	4.8	0.32	56.2	120.3	47	57
	10–24	s	5.0	0.15	50.7	112.3	45	30
	24–51	ls	5.2	0.16	50.9	120.3	42	31
	51–70	ls	5.3	0.09	38.2	127.3	30	24
Gneissic rock	0–4	s	6.2	0.66	67.5	120.3	56	98
	4–12	ls	5.3	0.37	56.9	70.3	81	65
	12–24	sl	4.6	0.28	39.4	112.5	35	71
	24–39	sc	4.5	0.24	—	140.6	—	—
	39–50	scl	4.5	0.24	32.1	120.3	27	75
Pegmatite	0–5	s	6.1	0.58	71.4	140.6	51	81
	5–10	ls	5.5	0.47	58.6	140.6	42	80
	10–15	scl	5.4	0.40	59.2	148.5	40	68
	15–31	sc	5.6	0.36	54.0	148.5	36	67
	31–	sc	5.7	0.28	71.7	156.3	46	39

s, ls, sl, scl and sc indicate sand, loamy sand, sandy loam, sandy clay loam and sandy clay, respectively.
[a] Throughout this paper in = 25.4 mm.

TABLE 3. The effect of parent material on the distribution of soil organic phosphorus

Sedimentary rocks

Parent material	Profile depth (in)	Texture	pH	Organic carbon (%)	Organic P (mg/kg soil)	Total P (mg/kg soil)	% of total P	Organic C / Organic P
Sandstone	0–4	s	5.6	0.38	62.7	140.6	45	61
	4–13	s	5.8	0.24	52.1	85.9	61	46
	13–20	s	5.8	0.15	47.7	63.2	75	31
	20–28	ls	5.8	0.11	28.6	106.3	27	39
	28–43	scl	5.8	0.12	28.6	106.3	27	42
	43–65	scl	5.7	0.11	40.9	106.3	38	27
Ironpan	0–9	s	6.0	0.51	52.2	162.6	32	98
	9–16	ls	5.3	0.27	41.9	106.3	39	64
	16–23	ls	5.0	0.15	34.2	126.3	27	44
	23–31	sl	5.2	0.17	50.3	177.4	28	34
Alluvium	0–5	ls	6.1	0.70	67.4	162.6	41	104
	5–14	ls	5.7	0.18	38.8	70.3	55	46
	14–24	ls	5.7	0.06	47.8	70.3	68	13
	24–37	ls	5.8	0.06	33.6	106.3	32	18
	37–58	sl	5.7	0.04	39.1	106.3	37	10
	58–72	sl	5.6	0.08	44.4	85.9	52	18

s, ls, sl, scl and sc indicate sand, loamy sand, sandy loam, sandy clay loam and sandy clay, respectively.

TABLE 4. The effect of vegetation on the distribution of soil organic phosphorus

Well drained soils

Vegetation zone	Profile depth (in)	Texture	pH	Organic carbon (%)	Organic P (mg/kg soil)	Total P (mg/kg soil)	% of total P	Organic C / Organic P
Southern Guinea savanna	0–4	s	5.6	0.38	62.7	140.6	15	61
	4–1							
	4–13	s	5.8	0.24	52.1	85.9	61	46
	13–20	s	5.8	0.15	47.7	63.2	75	31
	20–28	ls	5.8	0.11	28.6	106.3	27	39
	28–43	scl	5.8	0.12	28.6	106.3	27	42
	43–65	scl	5.7	0.11	40.9	106.3	38	27
Northern Guinea savanna	0–3	s	4.5	0.52	48.9	120.3	41	106
	3–10	s	4.1	0.28	45.3	112.5	40	62
	10–19	s	4.1	0.16	40.9	140.6	29	39
	19–25	scl	4.1	0.21	22.7	184.4	12	93
	25–34	scl	4.2	0.23	27.8	168.8	16	83
	34–42	—	—	—	—	—	—	—
Sudan	0–2	s	5.2	0.24	13.2	168.8	8	182
	2–18	s	5.3	0.18	13.6	128.1	11	132
	18–31	s	5.1	0.13	14.4	112.5	13	90
	31–44	s	5.1	0.14	—	140.6	—	—
	44–59	s	5.2	0.11	12.6	100.0	13	87
	59–76	s	5.2	0.08	6.1	85.9	7	131
	76–92	s	5.5	0.08	19.1	56.3	34	42

s, ls, sl, scl and sc indicate sand, loamy sand, sandy loam, sandy clay loam, and sandy clay, respectively.

TABLE 5. The effect of vegetation on the distribution of soil organic phosphorus

Impeded drainage sites (*fadama*)

Vegetation zone	Profile depth (in)	Texture	pH	Organic carbon (%)	Organic P (mg/kg soil)	Total P (mg/kg soil)	% of total P	Organic C / Organic P
Southern Guinea savanna	0–4½	sl	5.9	2.1	151.9	562.5	27	138
	4½–11½	scl	5.6	0.65	100.4	295.3	34	65
	11½–35	scl	5.5	0.26	68.8	239.1	29	38
	35–46	s	5.6	0.05	47.2	184.4	26	11
	46–61	scl	5.4	0.18	67.1	267.2	25	27
	61–64	sl	5.6	0.23	54.6	253.1	22	42
	64–70	s	5.7	0.07	42.6	184.4	23	17
Northern Guinea savanna	0–3	ls	5.1	2.8	162.3	225.0	72	173
	3–12	sl	5.0	0.73	97.7	156.3	63	75
	12–20	scl	4.6	0.25	51.7	156.3	33	48
	20–30	sl	4.7	0.26	64.4	128.1	50	40
	30–37	ls	4.7	0.11	45.9	140.6	33	24
	37–58	ls	4.8	0.08	42.3	120.6	35	19
Sub-Sudan	0–3	s	5.1	1.4	96.4	226.0	34	145
	3–11	s	5.0	0.27	60.5	226.6	27	45
	11–23	s	5.0	0.12	63.5	148.5	43	19
	23–32	s	5.2	0.05	44.6	140.6	32	11
	32–50	s	5.2	0.08	40.3	128.1	31	20
	50–56	s	5.3	0.06	54.4	232.1	23	11

s, ls, sl, scl and sc indicate sand, loamy sand, sandy loam, sandy clay loam and sandy clay, respectively.

TABLE 6. Comparison of a well drained (upland) profile with a profile from an impeded drainage site (*fadama*), both within the same vegetation zone and having same parent material

Soil water regime	Profile depth (in)	Texture	pH	Organic carbon (%)	Organic P (mg/kg soil)	Total P (mg/kg soil)	% of total P	Organic C / Organic P
Upland	0–5	ls	6.1	0.70	67.4	162.6	41	104
	5–14	ls	5.7	0.18	38.8	70.3	55	46
	14–24	ls	5.7	0.06	47.8	70.3	68	13
	24–37	ls	5.8	0.06	33.6	106.3	32	18
	37–58	sl	5.7	0.04	39.1	106.3	37	10
	58–72	sl	5.6	0.08	44.4	85.9	52	18
Fadama	0–4½	sl	5.9	2.1	151.9	562.5	27	138
	4½–11½	scl	5.6	0.65	100.4	295.3	34	65
	11½–35	scl	5.5	0.26	68.8	239.1	29	38
	35–46	s	5.6	0.05	47.2	184.4	26	11
	46–61	scl	5.4	0.18	67.1	267.2	25	27
	61–64	sl	5.6	0.23	54.6	253.1	22	42
	64–70	s	5.7	0.07	42.4	184.4	23	17

s, ls, sl, scl and sc indicate sand, loamy sand, sandy loam, sandy clay loam and sandy clay, respectively.

than were those from the well drained sites within the same vegetation zone. Organic C content decreased very rapidly with depth even in the poorly drained soils (Table 5).

There were appreciable quantities of organic phosphorus in the surface layers of all the profiles and the content decreased with depth in most cases. The effect of parent material on the content of soil organic phosphorus was small but noticeable. The results of this study indicated that the profiles with metamorphic parent materials contained generally more organic phosphorus than those with sedimentary parent materials. There was also a general decrease of organic phosphorus from the southern Guinea zone to the Sudan zone in the upland soils in contrast with what happened with profiles from the impeded drainage sites. The profiles from the impeded drainage sites (*fadamas*) contained appreciably more organic phosphorus than those from the upland soils and this could be seen on comparing profiles from the same vegetation zone and having same parent material such as the profiles from Lafiagi (Table 6).

The total phosphorus contents of these profiles were relatively lower than those of temperate countries but were comparable with those reported for some Nigerian soils by Enwezor and Moore.[5] The organic carbon : organic phosphorus ratios were less than 200 in all cases. These ratios appeared to decrease with depth in some cases, but were erratic in some.

4. Discussion

The results of this study indicated that parent material had a small but noticeable effect on the organic phosphorus contents of soils within the same vegetation zone. This agrees with the previous observation by Barrow[3] who noticed only small effects of parent material on soil organic phosphorus distribution. The difference between the soil organic phosphorus contents of these two groups of parent materials could not be attributed to differences in soil texture, because a comparison of the profiles with sandstone and quartzite parent materials, which were sedimentary and metamorphic, respectively, showed that they both had sandy texture at the surface layers, but the profile with quartzite parent material contained relatively higher organic phosphorus. There was no definite relation between soil texture and soil organic phosphorus contents of most of these profiles in contrast with the previous observation by Barrow,[3] who found that organic matter of soils with the higher clay content tended to have higher phosphorus content.

Vegetation had an appreciable effect on soil organic phosphorus contents in this study. The organic phosphorus contents of the adequately drained soils with the same type of parent material generally decreased from the southern Guinea savanna to the Sudan zone. This effect of vegetation was in agreement with the previous results of some research workers in the tropics (Enwezor and Moore[5] and Nye and Bertheux[10]). They found that there was considerably more organic phosphorus in the forest than in the savanna soils. They felt that this effect could only be attributed to the type and amount of vegetation the soils carried and to the soil water regime, because they could not establish this vegetation effect for the soils from the impeded drainage sites (*fadamas*).

The vegetation effect on soil organic phosphorus of the impeded drainage sites (*fadamas*) was not well established in this study either. However, very few profiles were

studied. The northern Guinea savanna contained more organic phosphorus than the southern Guinea savanna at the surface layer and both were higher than the Sub-Sudan zone. The high contents of organic phosphorus of the profiles with impeded drainage could be attributed to the lower level of biological activity under this condition as suggested by Enwezor and Moore.[5]

It appears from the results of this investigation that more work needs to be done on the rate of mineralisation of organic phosphorus before assessing its role in phosphorus supply to plants. It has been suggested by Tisdale and Nelson[11] that organic carbon : organic phosphorus ratios less than 200 indicated the possibility of mineralisation.

The ratios of organic carbon : organic phosphorus of all the profiles studied were less than 200 indicating the possibility of mineralisation, and this may be of importance with the present systems of agriculture in most parts of Nigeria, while little inorganic fertiliser is used. Bache and Rogers[2] could not find any significant contribution of organic phosphorus to phosphorus supply to plants in their pot experiments on some soil samples from long-term manurial trials in Nigeria. In that study dung was added. This might have caused net immobilisation of the native inorganic phosphorus. However, the likelihood of this action would depend on the amount of organic phosphorus present in the dung (McLaren and Peterson).[7]

Acknowledgements

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Effect of a Varied Potassium Nutrition on the Uptake and Incorporation of Labelled Nitrate by young Tobacco Plants (*Nicotiana tabacum* L.)

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(*Accepted for publication June 1972*)

In young tobacco plants the N turnover was studied with labelled N in relation to the K nutritional status of the plants.

Plants well supplied with K showed a higher percentage of labelled N in the nitrate fraction and in the protein fraction compared with the plants of lower K status. In the latter, however, the percentage of labelled N in the soluble amino-acid fraction was higher. The experimental data support the assumption that K particularly favours the incorporation of amino acids into proteins.

1. Introduction

Acute K deficiency in higher plants results in a disturbance of N metabolism and often the accumulation of toxic amines such as putrescine and agmatine occurs.[1,2] Even when no visible K deficiency symptoms are observed, however, low K supply gives rise to higher contents of soluble amino acids and in particular glutamine and asparagine.[3,4] According to experimental results of Lubin[5] K^+ is considered to be essential for protein synthesis by enabling the binding of mRNA to the ribosomes. This finding supports the assumption that the accumulation of amino acids due to low K supply is caused by a reduced incorporation of amino acids into proteins. Recent experimental data of Mengel and Koch[6] are in full agreement with this assumption. In order to obtain further evidence that K^+ favours the incorporation of amino acids into proteins, the experiment described below considers the question of whether the K nutritional status of the plant affects the turnover of N.

2. Experimental

Tobacco plants germinated in sand, were grown in solution culture for 3 weeks. The plants were then divided into two series, one grown with and the other grown without K^+ for a further 9 days. The concentrations of the nutrient solutions in both treatments are shown in Table 1. In the treatment lacking K the K salts were replaced by Na salts. At the end of this period of 9 days' growth the nutrient solutions of both treatments were renewed and N was added in form of ^{15}N-labelled $Ca(NO_3)_2$ having a ^{15}N enrichment

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of 95%. In both treatments the plants were exposed to the labelled nutrient solution for 1, 6 and 24 h and it was thus possible to study the time course of nitrate uptake and assimilation. At the end of the appropriate exposure time the plants were taken out of the nutrient solution, their roots thoroughly washed by distilled water and then excised from the upper plant parts. Both roots and upper plant parts were cut up and extracted with 75% ethanol three times, each extraction lasting 24 h. The third extraction was carried out with hot ethanol. The extract was concentrated to dryness in a vacuum rotary evaporator at 35 °C. The residue was dissolved in water–chloroform (1 : 1, by vol.), the quantities used depending on the quantity of plant material in the samples. After thorough mixing and phase separation an aliquot was pipetted from the water phase. From this aliquot the amino N and the nitrate N were determined.

The ethanol-insoluble N still present in the residue of the ethanol extract is considered to consist mainly of protein N, and for simplicity's sake this fraction was designated as

TABLE 1. Composition of nutrient solutions applied during the last 9 days of growth

+K treatment	−K treatment	Micro nutrients (for both treatments)
4.0 mmol $Ca(NO_3)_2$	4.0 mmol $Ca(NO_3)_2$	14.0 μmol $MnSO_4$
1.0 mmol KCl	1.0 mmol NaCl	1.4 μmol $CuSO_4$
2.0 mmol KH_2PO_4	2.0 mmol NaH_2PO_4	1.4 μmol $ZnSO_4$
0.25 mmol K_2SO_4	0.25 mmol $NaSO_4$	10.0 μmol H_3BO_3
2.0 mmol $MgSO_4$	2.0 mmol $MgSO_4$	0.3 μmol $(NH_4)_6Mo_7O_{24}$
0.25 mmol Na_2SO_4		30.0 μmol Fe–EDTA

the protein fraction. The total amino N ($^{14}N + {}^{15}N$) of the protein fraction and of the soluble amino N fraction were determined by microKjeldahl distillation. The nitrate N was reduced to NH_4^+ by Devarda's reagent and also analysed by microKjeldahl distillation. The ^{15}N was determined by the emission spectroscopic technique (NOI-4 15 N-Analyzer), according to the method described by Meier,[7] Meier and Müller[8] and Faust.[9] The oxidation of the NH_4^+ to N_2 was performed with NaOBr.[10] All data presented in the Figures and Tables below are mean values of two plant samples. For the data of the ^{15}N analysis each sample was measured six times in order to obtain a representative mean value.

3. Results

The plants of both treatments grew well, but those not supplied with K^+ during the last 9 days showed a somewhat reduced growth rate resulting in lower fresh weights of about 30% compared with the plants fully supplied with K^+ throughout the experimental period. No visible K-deficiency symptoms were apparent in the plants growing in the nutrient solution without K and the K content of the aerial plant parts was 4% K in the dry matter. This may be regarded as a medium K content for young plants, the corresponding K content of the "+K plants" being 7.1% K.

From Table 2 it can be seen that the nitrate content of roots and upper plant parts did not differ much between treatments. The contents of labelled nitrate, however,

TABLE 2. Nitrate content of roots and upper plant parts (mg of N/100 g of FM)

	Roots		Upper plant parts	
	+K	−K	+K	−K
1 h	19.2	22.2	47.7	54.1
6 h	18.3	21.5	48.5	48.6
24 h	28.4	28.9	53.0	47.1

were markedly different for the two treatments. As is shown in Figure 1 the percentage of labelled nitrate in the nitrate fraction was higher in the plants well supplied with K^+. This indicates that the rate of uptake and the rate of nitrate reduction were greater in the plants with the higher K concentration. The total amount of labelled nitrate taken up by the +K plants was considerably higher compared with the −K plants. This is particularly true for the upper plant parts (Figure 2). This effect is probably caused by the different growth rates in the treatments. The data presented in Figure 3 support this assumption. The rate of incorporation of labelled nitrate into the protein fraction may be regarded as a measure of growth rate, because in young plants protein synthesis occurs predominantly in the meristematic tissues. The difference in the content of labelled nitrate in the protein fraction between the treatments is particularly obvious in the upper plant parts (Figure 3). The plants which were well supplied with K^+ showed a dramatic increase in their content of labelled N in the protein fraction, whereas

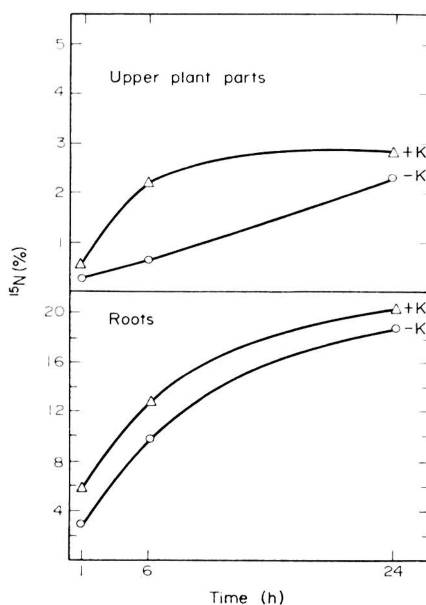

Figure 1. Percentage of ^{15}N in the nitrate fraction.

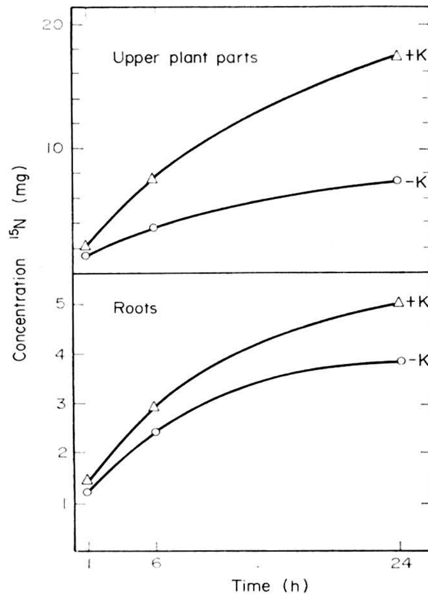

Figure 2. Quantities of ^{15}N in the upper plant parts and the roots in relation to the K concentration of the plants.

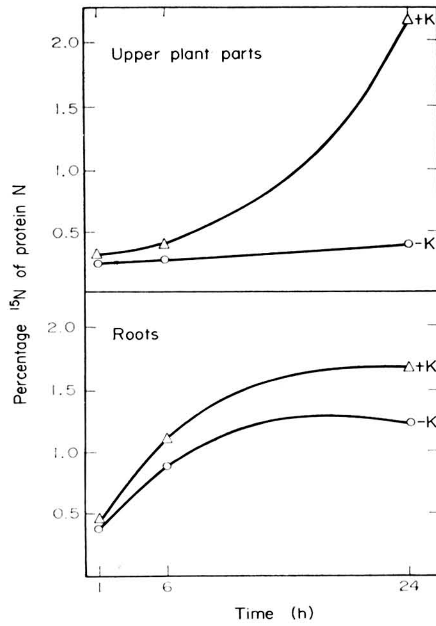

Figure 3. Percentage of ^{15}N from the protein N in relation to the K concentration of the plants.

with the −K plants only a very slight increase of the content of labelled N in the protein fraction occurred during the 24 h experimental period. Plants well supplied with K^+ not only had a lower content of soluble amino acids, but also the percentage of labelled N in this fraction was lower compared with the −K plants (Table 3).

TABLE 3. Percentage of ^{15}N in the soluble amino fraction

Application time (h)	Roots +K (%)	Roots −K (%)	Upper plant parts +K (%)	Upper plant parts −K (%)
1	3.2	4.6	0.4	0.5
6	4.6	13.9	1.3	2.6
24	8.4	14.1	1.9	3.0

4. Discussion

The uptake of labelled N by the plants well supplied with K^+ was significantly higher compared with the plants of lower K concentration. As the nitrate content of the plants of both treatments was rather similar, it must be concluded that the +K plants had a higher N turnover. This conclusion is supported by the finding that the percentage of ^{15}N of the nitrate fraction was higher in the +K treatment plants (Figure 1). This agrees well with the higher ^{15}N percentage of the protein fraction in plants with a high K status (Figure 3). In the soluble amino fraction, however, the reverse was the case. Here plants with low K status showed the higher percentage of ^{15}N. The soluble amino fraction is mainly affected by two processes, nitrate reduction, which supplies N to the soluble amino fraction (amino-acid pool), and the consumption of amino acids for protein synthesis, which drains off amino acids from this pool. Both transfer steps are favoured by K^+, as is evident from the higher ^{15}N label in the nitrate and protein fraction of plants of higher K status. But the latter step, the incorporation of amino acids into proteins, appears to be more affected by K^+. This means that for the plants low in K^+ the supply of N into the amino-acid pool is relatively higher than the rate of amino-acid consumption for the protein synthesis. This need not necessarily result in a higher labelling of the soluble amino acids of the −K plants, as the rate of turnover is considerably different for individual amino acids. It can be assumed that amino acids needed in larger quantities such as glutamic acid and aspartic acid are synthesised rapidly and used for protein synthesis. This should be particularly true for the plants with high K concentration. Nevertheless, in the −K plants some accumulation of labelled N occurred, resulting in a higher percentage of ^{15}N in the soluble amino fraction of these plants.

The data presented here support the assumption that the accumulation of amino acids in plants with a low K concentration is caused by a reduced incorporation of amino acids into proteins. This does not mean that the higher turnover of N, due to an increased K status, is conclusive proof that K is directly involved in the protein synthesis, although this assumption is probable.

A higher N turnover may also simply be caused by the higher growth rate of the +K plants. Whether this growth rate is primarily dependent on protein synthesis in meristematic tissues, or whether it is caused by other processes favoured by K^+, i.e. by a better supply of carbohydrates[11] or a better supply of ATP[12] still remains an open question.

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Influence of Nitrogen Source on the Synthesis of Fat from Sucrose by *Aspergillus terreus*, *Aspergillus ochraceus*, *Cladosporium fulvum*, *Cladosporium herbarum* and *Penicillium gladioli*

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(*Accepted for publication June 1972*)

The influence of ammonium nitrate, ammonium sulphate, ammonium chloride, sodium nitrate, potassium nitrate and urea as nitrogen sources on the synthesis of fat by *Aspergillus terreus*, *Aspergillus ochraceus*, *Cladosporium fulvum*, *Cladosporium herbarum* and *Penicillium gladioli* has been studied. Sodium nitrate has proved to be the best source of nitrogen for production of fat by *A. terreus*, *C. herbarum* and *C. fulvum* where the highest yields obtained were of fat contents in the felt of 51.0, 29.2 and 23.5%, respectively. In the case of *A. ochraceus* the highest yield of fat was obtained with urea as nitrogen source giving a fat content in the mycelium of 47.5%. The highest yield of fat by *P. gladioli* was obtained using ammonium nitrate, giving a fat content of 31.0%.

1. Introduction

The production of fat by micro-organisms mainly depends on the strain specificity of the organism and on the cultural conditions. This subject has been recently reviewed by Woodbine.[1] In a preliminary survey of the fat-forming capacities of 24 strains of five species of moulds Singh and Sood[2] reported that five moulds, namely *A. terreus* Thom 309, *A. ochraceus* Wilhelm 312, *C. fulvum* Cke 793, *C. herbarum* Link 705 and *P. gladioli* Machacek 723 gave promising results in media containing salts and glucose and the yield of fat was quite comparable with that of fat-rich vegetable seeds. The production of fat on an industrial scale by the utilisation of moulds, however, depends upon the use of less expensive carbon sources such as cellulose waste and molasses. Since sucrose is the major component of molasses the behaviour of these five moulds with regard to sucrose has also been investigated.[3] It was thought of interest to study the effect of different sources of nitrogen on the synthesis of fat by these five moulds.

2. Experimental

2.1. Strains

The strains of the moulds used were subcultures of the moulds which were obtained from the Culture Collection of Indian Agricultural Research Institute, New Delhi.

2.2. Maintenance

The strains were kept on slants prepared from a medium with the following composition:

agar, 20 g; glucose, 20 g; malt extract, 20 g;
peptone, 1 g; distilled water, 1000 ml.

All the strains were subcultured at intervals of 2 months.

2.3. Medium

The basal medium used contained in g/100 ml: sucrose, 15; $ZnSO_4 \cdot 7H_2O$, 0.005; $FeCl_3 \cdot 6H_2O$, 0.016; K_2SO_4, 0.044; $MgSO_4 \cdot 7H_2O$, 0.5; $NaH_2PO_4 \cdot 2H_2O$, 0.73; made up to volume with distilled water. The amount of ammonium nitrate added was 0.225 g and when ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrate and urea were used as alternative nitrogen sources, the quantities taken were such that would supply the same quantity of nitrogen as was contained in 0.225 g of ammonium nitrate. The initial pH of the medium was 3.5. Aliquots (50 ml) of this solution were placed in 250-ml Erlenmeyer conical flasks and sterilised by autoclaving for 15 min at a steam pressure of 10 lb (4.5 kg).

2.4. Inoculation and harvesting

The inoculum was prepared from 3 to 4 days old cultures grown on malt agar slopes. The spores were shaken with 10 ml of sterile distilled water per slope. 2 ml of such a suspension was used to inoculate each flask which was then incubated at 25 °C. The period of incubation was 10 days for *A. terreus* and *A. ochraceus* and 12 days for *C. fulvum*, *C. herbarum* and *P. gladioli*. A set of 5 flasks was removed in every case and sterilised by autoclaving for 15 min at 10 lb steam pressure. The contents of the flasks were allowed to cool to room temperature and filtered. The mycelium was washed thoroughly with distilled water to remove the adhering sucrose solution and the filtrate was collected for sucrose estimation.

2.5. Estimation of fat

The felts were dried in air for 2 to 3 days at room temperature and then in an oven at 60 °C to a constant weight. The dried felts from 5 flasks in each case were thoroughly powdered, added to Whatman extraction thimbles and extracted in a Soxhlet extraction unit on a water bath for 10 h using petroleum ether (b.p. 60 to 80 °C). Petroleum ether was recovered by distillation on a steam bath and the fat remaining was weighed after drying at 85 °C.

2.6. Estimation of sucrose

To the filtrate and washings from a batch of 5 flasks 5 ml of hydrochloric acid were added and the solution heated for 30 min in a water bath to hydrolyse unchanged sucrose. The solution was made up to a known volume and analysed by the method of Lane and Eynon.[4]

3. Results

The results of experiments on felt and fat formation by *A. terreus*, *A. ochraceus*, *C. fulvum*, *C. herbarum* and *P. gladioli* when grown on sucrose using different compounds as sources of nitrogen are given in Tables 1, 2, 3, 4 and 5, respectively.

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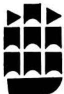

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This is the Proceedings of the first in a series of annual symposia of the American Association of Pathologists and Bacteriologists.

TABLE 1. Influence of source of nitrogen on felt and fat formation from sucrose by *A. terreus*

Source of nitrogen	Weight of felt (g)	Fat content of dry felt (g/100 g)	Weight of sucrose consumed (g)	Fat coefficient[a]	Economic coefficient[b]
NH_4NO_3	1.240 (±0.036)	38.3	4.9	9.7	25.3
$(NH_4)_2SO_4$	0.470 (±0.040)	47.2	3.4	6.5	13.8
NH_4Cl	0.740 (±0.045)	56.7	4.3	9.7	17.2
$NaNO_3$	1.304 (±0.024)	51.0	5.0	13.3	26.0
KNO_3	1.350 (±0.050)	48.5	5.0	13.0	27.0
Urea	1.466 (±0.026)	40.0	4.8	11.7	30.5

Incubation period = 10 days at 25 °C.
Vol. of culture medium, 50 ml, containing 7.5 g of sucrose and 0.0393 g of nitrogen.
The standard deviations are given immediately underneath the mean felt weight figures.

[a] The amount of fat expressed as percentage of sucrose consumed.
[b] Felt weight produced/100 g of sucrose.

TABLE 2. Influence of source of nitrogen on felt and fat formation from sucrose by *A. ochraceus*

Source of nitrogen	Weight of felt (g)	Fat content of dry felt (g/100 g)	Weight of sucrose consumed (g)	Fat coefficient[a]	Economic coefficient[b]
NH_4NO_3	1.290 (±0.020)	38.2	5.1	9.6	25.3
$(NH_4)_2SO_4$	0.654 (±0.046)	33.6	5.6	3.9	11.7
NH_4Cl	0.580 (±0.070)	41.0	5.7	4.1	10.2
$NaNO_3$	1.344 (±0.035)	44.8	5.2	11.5	25.8
KNO_3	1.380 (±0.043)	46.5	5.3	12.1	26.0
Urea	1.504 (±0.054)	47.5	5.4	13.2	27.8

Incubation period = 10 days at 25 °C.
Vol. of culture medium, 50 ml, containing 7.5 g of sucrose and 0.0393 g of nitrogen.
The standard deviations are given immediately underneath the mean felt weight figures.

[a] The amount of fat expressed as percentage of sucrose consumed.
[b] Felt weight produced/100 g of sucrose.

TABLE 3. Influence of source of nitrogen on felt and fat formation from sucrose by *C. fulvum*

Source of nitrogen	Weight of felt (g)	Fat content of dry felt (g/100 g)	Weight of sucrose consumed (g)	Fat coefficient[a]	Economic coefficient[b]
NH_4NO_3	2.000 (±0.042)	23.0	6.2	7.4	32.2
$(NH_4)_2SO_4$	0.566 (±0.014)	6.3	2.5	1.4	22.6
NH_4Cl	0.583 (±0.033)	5.6	2.7	1.2	21.6
$NaNO_3$	2.114 (±0.092)	23.5	6.8	7.3	31.0
KNO_3	2.110 (±0.090)	22.1	7.1	6.6	29.7
Urea	2.024 (±0.043)	21.7	6.2	7.1	32.6

Incubation period = 12 days at 25 °C.
Vol. of culture medium, 50 ml, containing 7.5 g of sucrose and 0.0393 g of nitrogen.
The standard deviations are given immediately underneath the mean felt weight figures.

[a] The amount of fat expressed as percentage of sucrose consumed.
[b] Felt weight produced/100 g of sucrose.

TABLE 4. Influence of source of nitrogen on felt and fat formation from sucrose by *C. herbarum*

Source of nitrogen	Weight of felt (g)	Fat content of dry felt (g/100 g)	Weight of sucrose consumed (g)	Fat coefficient[a]	Economic coefficient[b]
NH_4NO_3	1.830 (±0.049)	22.4	6.1	6.7	30.0
$(NH_4)_2SO_4$	0.625 (±0.063)	5.1	2.7	1.2	23.1
NH_4Cl	0.500 (±0.022)	4.5	2.7	0.8	18.5
$NaNO_3$	2.500 (±0.055)	29.2	6.9	10.6	36.2
KNO_3	2.500 (±0.069)	27.6	7.1	9.7	35.2
Urea	2.177 (±0.060)	20.4	6.3	7.0	34.5

Incubation period = 12 days at 25 °C.
Vol. of culture medium, 50 ml, containing 7.5 g of sucrose and 0.0393 g of nitrogen.
The standard deviations are given immediately underneath the mean felt weight figures.

[a] The amount of fat expressed as percentage of sucrose consumed.
[b] Felt weight produced/100 g of sucrose.

TABLE 5. Influence of source of nitrogen on felt and fat formation from sucrose by *P. gladioli*

Source of nitrogen	Weight of felt (g)	Fat content of dry felt (g/100 g)	Weight of sucrose consumed (g)	Fat coefficient[a]	Economic coefficient[b]
NH_4NO_3	1.166 (±0.031)	31.9	6.5	5.7	18.0
$(NH_4)_2SO_4$	0.373 (±0.038)	14.7	3.7	1.5	10.0
NH_4Cl	0.383 (±0.034)	24.0	4.8	1.9	8.0
$NaNO_3$	0.516 (±0.034)	13.5	3.5	2.0	14.7
KNO_3	0.635 (±0.015)	14.9	2.1	4.5	30.2
Urea	0.786 (±0.046)	18.5	4.4	3.3	17.9

Incubation period = 12 days at 25 °C.
Vol. of culture medium, 50 ml, containing 7.5 g of sucrose and 0.0393 g of nitrogen.
The standard deviations are given immediately underneath the mean felt weight figures.
[a] The amount of fat expressed as percentage of sucrose consumed.
[b] Felt weight produced/100 g of sucrose.

4. Discussion

For the production of fat by *A. terreus*, *C. fulvum* and *C. herbarum* potassium or sodium nitrates were the best nitrogen sources. Urea was the most suitable source for *A. ochraceus* and ammonium nitrate for *P. gladioli*. Ammonium sulphate and ammonium chloride were not suitable sources of nitrogen for any of the mould species examined. Garrido and Walker[5] also found that ammonium chloride and ammonium sulphate were not suitable as sources of nitrogen for the production of fat by *A. nidulans*. For *P. javanicum* ammonium sulphate was reported by them to be the most unsuitable nitrogen source. They believe that the utilisation of ammonium ions by the moulds results in the development of acidity in the medium which is detrimental to fat synthesis. They have further stated that acidity developed by the utilisation of ammonium ions from ammonium sulphate is more detrimental to mycelial fat production than the acidity developed by the utilisation of ammonium ions from ammonium chloride. Lockwood *et al.* also observed similar behaviour in the case of *P. javanicum*. Whereas the results obtained from *A. terreus*, *A. ochraceus* and *P. gladioli*, using ammonium sulphate and ammonium chloride as nitrogen sources, are in agreement with the observations made by Garrido and Walker, the results given by *C. fulvum* and *C. herbarum* do not agree. In these two cases ammonium sulphate has not been found to be more harmful than ammonium chloride for fat synthesis.

The effect of pH and the development of acidity in the media during the process of fat synthesis by moulds need a detailed study and will constitute a subject for further investigations.

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Changes in the Ethanol-insoluble Material of Tea Leaves (*Camellia sinensis* L.) during Maturation

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(Accepted for publication March 1972)

The ethanol-insoluble material (e.i.m.) of immature and mature tea leaves was fractionated into hot-water-soluble polysaccharides and proteins, ammonium oxalate-soluble pectic acid, hemicelluloses A and B and α-cellulose, by successive extraction with hot water, ammonium oxalate, sodium hypochlorite and cold alkali. The final residue was termed α-cellulose. The hot-water extract and the hot-water-insoluble residue were found to contain appreciable quantities of protein nitrogen. Each fraction was hydrolysed and the mixture of sugars was separated on paper chromatograms and estimated. It appeared that each stage of the extraction procedure removed from the e.i.m. a complex mixture of polysaccharides. The sugars produced on hydrolysis of the arbitrary fractions from immature and mature leaves were qualitatively similar, although there were quantitative differences and were glucose, galactose, xylose, arabinose, rhamnose, galacturonic acid and an unidentified uronic acid. Maturation was mainly accompanied by an increase in the content of lignin, hemicelluloses and α-cellulose.

1. Introduction

Physiological and biochemical changes occur in the tea leaf as it matures. Leaf cell walls in particular undergo great changes during maturation and a knowledge of the changes is of interest to the tea industry. For the manufacture of good quality black tea, only the shoot tips of tea plant (*Camellia sinensis* L.) comprising the buds, the first two leaves and the included stems, collectively called the "flush" are used. The flush could be suitably conditioned for the subsequent manufacturing processes. Slightly mature leaves are not only difficult to process, but the tea manufactured from them is of an inferior quality and contains a lot of fibre stalks, which have to be carefully removed before the product is marketed. Increased lignification and other changes accompanying maturation of the tissues also markedly influence the "appearance" and texture of the final product.[1] Despite the fact that these characteristics play an important part in determining the market value of the made tea, little work has been done on the cell wall material of tea leaves and their changes during maturation. The present study was undertaken to elucidate the function of some of the structural constituents of tea leaves and provide information about the maturation process itself.

Leaves from free-growing tea shoots of Clone TRI 2024 were used in this investigation. Free-growing shoots provide excellent material in which to study leaf formation, development and maturation as the various stages are arranged in approximately

linear order. The analysis of consecutive leaves yields information on changes accompanying cell development. Detailed studies carried out on the structural constituents present in the first and eighth leaves are reported in this paper.

2. Materials and methods

2.1. Source of plant material

All material (leaves from free-growing shoots of Clone TRI 2024) used in this investigation was collected from 6-year-old plants grown in a field near the laboratory (1500 m elevation). Processing of the material was started within 1 h of harvesting.

2.2. Preparation of the ethanol-insoluble material

The leaves were plunged into boiling 80% (v/v) aqueous ethanol for 3 min, cooled to room temperature and then macerated for 5 min in a blender. The macerate was filtered through a sintered glass funnel and the residue washed with 80% alcohol until the filtrate gave a negative test for carbohydrates. It was then washed with absolute alcohol and ether. The product was dried in a dehumidified room at 28 °C. The e.i.m. thus prepared would contain cell wall and cytoplasmic material.

2.3. Fractionation and analysis of the ethanol-insoluble material

The e.i.m. was extracted according to a procedure similar to, but not identical with, that of Jermyn and Isherwood.[2] It differs from the latter principally in that the residue after hot-water extraction, was further extracted with ammonium oxalate to solubilise the calcium salts of pectic acid before delignification with acid sodium hypochlorite.[3] The protein content of the e.i.m. was calculated from nitrogen determination and the starch was determined by the method of Hassid and Neufeld.[4]

The polysaccharide fractions were hydrolysed with 2 N-sulphuric acid and analysed as described earlier.[5]

Histological studies were carried out with transverse sections of the leaves of increasing maturity.

3. Results

The dry matter content of tea leaf increased during maturation ranging from 26 to 35.5%. From Figure 1 it is clear that there is a marked increase in the structural constituents from the second to the fourth leaf. The protein detected in the e.i.m. may mainly be cytoplasmic in origin and it did not show much variation with maturation. The starch content of the leaves increased with maturation, but its contribution to the e.i.m. was slight.

These studies were supported by histological observations using transverse sections of the leaves at different stages of maturity. The most obvious changes were seen in the mid-rib region and in the vascular bundles of the lamina. In the mid-rib region, the parenchyma adjacent to the upper and lower epidermis showed gradual differentiation to collenchyma. Bundle sheath consisting of sclerenchyma developed. Gradual development of the xylem was also noted. Again notable differences were seen between the second and fourth leaves.

3.1. Qualitative and quantitative composition of the ethanol-insoluble material

A study of the composition of each of the arbitrary fractions in the first (immature) and eighth (mature) leaves showed that there were no consistent ratios between the sugar residues present, such as would have been expected if the fractions represented definite

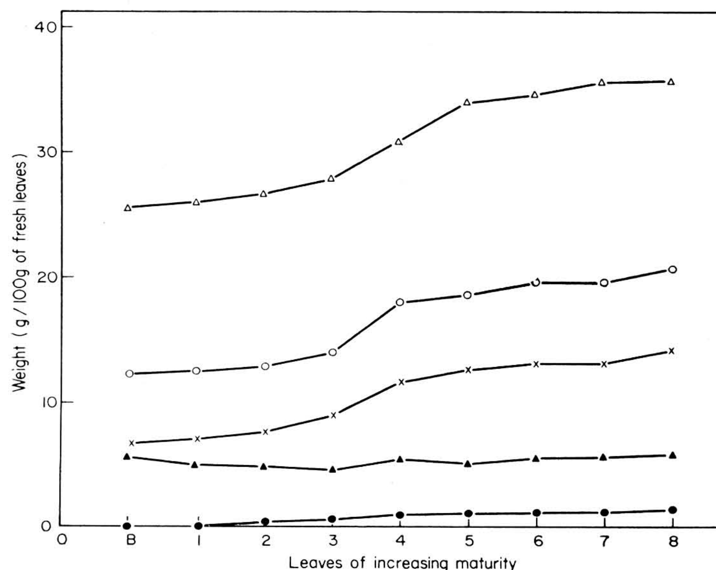

Figure 1. Changes in the dry wt and constituents present in the ethanol-insoluble material of tea leaves during maturation. –△–△–, Dry wt; –○–○–, ethanol-insoluble material (e.i.m.); –×–×–, structural constituents (e.i.m.—proteins + starch); –▲–▲–, proteins present in the e.i.m.; –●–●–, starch. The condition of the leaves is indicated by appropriate description in the X-axis (B, buds; and 1 to 8 represents the leaf number).

TABLE 1. Percentage composition of the ethanol-insoluble material of immature and mature tea leaves (dry wt basis)

Constituent	Immature (1st leaf)	Mature (8th leaf)
E.i.m.	47.5	57.2
Hot-water-soluble polysaccharides and proteins	11.0	6.8
(Hot-water-soluble proteins—N × 6.25)	(3.7)	(1.8)
Ammonium oxalate-soluble pectic acid	3.3	6.0
(Hot-water-insoluble proteins)	(14.5)	(14.8)
NaClO-soluble compounds (lignin and hot-water-insoluble proteins)	15.3	17.7
Hemicellulose A	3.4	5.0
Hemicellulose B	7.6	12.6
α-Cellulose	6.2	9.9

The values within brackets were computed from nitrogen determinations and the rest except for NaClO-soluble compounds (which was obtained from calculate difference) by direct weighing.

components of the cell wall. It appears that each stage of the extraction procedure removes from the e.i.m. a complex mixture of polysaccharides, the composition of which is governed by the age of the sample. The results of analysis are given in Tables 1

TABLE 2. Relative proportions of different polysaccharides in the arbitrary fractions extracted from the ethanol-insoluble material of immature and mature tea leaves

Polysaccharides present	Hot-water-soluble		Ammonium oxalate-soluble		Hemicellulose A		Hemicellulose B		α-Cellulose	
	Immature (1st leaf)	Mature (8th leaf)	1st leaf	8th leaf	1st leaf	8th leaf	1st leaf	8th leaf	1st leaf	8th leaf
Glucosan	0.05	0.28	—	—	0.36	0.19	1.0	0.19	1.0	0.87
Galactan	0.53	0.38	0.53	0.05	0.09	0.10	0.39	0.17	0.12	0.006
Xylan	0.04	0.02	0.05	0.006	0.68	1.0	0.51	0.35	0.02	0.02
Araban	0.37	0.17	0.31	0.09	0.27	0.08	0.30	0.14	—	—
Ribose	0.03	—	—	—	—	—	—	—	—	—
Polygalacturonic acid	1.0	0.36	0.96	1.0	—	—	—	—	—	—
Uronic acid	—	—	—	—	0.08	0.17	0.05	0.18	0.003	0.02
Rhamnan	0.02	0.03	0.03	0.17	—	0.002	—	0.01	—	0.009

and 2. From Table 1 it is clear that the lignin, hemicelluloses and α-cellulose components increased considerably during maturation. In Table 2 each sugar has been designated as a hexosan or pentosan; this is not meant to imply that they existed as distinct polysaccharides. Further, the results are expressed in the form of ratios, the polysaccharide present in largest amounts in each fraction for both first and eighth leaves being equated to unity. This method of presentation was used to bring out more clearly the variation in the composition of the arbitrary fractions. From this Table it is clear that the hydrolysates of the various polysaccharide fractions from first and eighth leaves were qualitatively similar although there were quantitative differences.

4. Discussion

The major structural constituents of immature and mature leaves differ in their relative proportions rather than in their presence or complete absence. The increase in the structural constituents, namely, lignin, hemicelluloses and α-cellulose, with maturation is consistent with the view that they are being gradually laid down on the cell wall.[6] The increase in the ammonium oxalate-soluble pectic acid with maturation suggests the probable participation of Ca and Mg in the natural cross-linking of the cell wall.[7] These studies were confirmed by histological observations with transverse sections of leaves of increasing maturity. Maturation of the leaves is accompanied by a multitude of morphological and chemical changes, many of which may directly or indirectly influence the texture of the leaf. Perhaps an important one is the increase in size and shape of intercellular spaces. Alterations in the thickness and firmness of the cell wall and of the cementing substance in the middle lamella have a pronounced effect on the appearance of the leaf. What essentially appears to be happening during maturation is that the gaps in the cell wall associated with radial enlargement are being filled with cellulose, pectic substances and hemicelluloses and some of the cell walls become encrusted with lignin. The polysaccharide matrix and lignins interpenetrate and in many instances the latter becomes the dominant interstitial polymer. The juxtaposition of lignins and cellulose microfibrils introduces a structural element of comparatively constant volume into the reticulum thereby providing the cell wall with a dimensional stability to a degree otherwise impossible. This property may influence the transpiration rate and permeability characteristics of the cell walls with maturation and hence their "tea manufacturing" properties.

Acknowledgement

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Variation in Lipid Composition through the Skin and Subcutaneous Adipose Tissue of Pigs

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(Accepted for publication July 1972)

Serial samples were taken at regular intervals through the skin and subcutaneous adipose tissue of pigs and the lipid and fatty acid composition of each was determined. The triglycerides from the region immediately beneath the skin contained a much higher proportion of oleic acid and correspondingly less of the other fatty acids than did the triglycerides from the remainder of the tissue; there was a similar but less marked increase in the proportion of oleic acid in the triglycerides from the innermost sections of adipose tissue. There was a distinct discontinuity in the fatty acid composition at the line of connective tissue between the inner and outer layers of adipose tissue. The chemical changes were correlated with the morphological appearance of the tissue by histological examination.

1. Introduction

Pig back fat consists of two layers of adipose tissue separated by a thin layer of connective tissue; the outer layer is immediately below the skin. These layers perform the dual function of serving as an energy store and as insulation for the animal. The composition of the outer layer, in particular, is readily altered by changes in the ambient temperature during growth[1–3] and by various dietary means;[4–6] saturated fats are laid down at higher environmental temperatures and unsaturated fats at lower. Thus, the triglycerides from the outer layer of back fat have higher contents of oleic and linoleic acids and a lower melting point than do the triglycerides from the inner layer.[5] In most of these studies, each layer was considered as a homogeneous entity. However, the temperature of a pig back fat is known to exhibit a continuous gradient through the layer,[7] so it seemed possible that the fatty acid composition of the fat would vary continuously also. Therefore, serial samples have been analysed and also examined histologically to determine whether any such changes occur and can be correlated with morphological changes in the tissue.

2. Experimental

2.1. Samples

Samples approximately 1 cm wide and 10 cm long were obtained from the loin region of Large White pigs slaughtered at 90 kg body weight. Pig diets and husbandry were as

described earlier.[5] The samples were kindly supplied by Dr K. G. Mitchell of the Pig Husbandry Department, National Institution for Research in Dairying, Shinfield, Reading. These samples were 2 to 3 cm deep and consisted of skin, outer and inner layers of back fat and a little adhering muscle tissue. In four preliminary experiments with four different animals, slices 1 cm^2 and 1 to 2 mm thick were cut sequentially from the tissue with a scalpel. With three other carcasses, slices 15 μm thick were accurately cut parallel to the skin surface with a cryostat (Pearse-Slee Ltd, London) and 20 or 40 successive slices were combined and extracted. Such pooled samples represented layers 0.3 or 0.6 mm thick, respectively, and were extracted more readily than were single thick slices. The average weight of these pooled samples was obtained by dividing the total weight of the adipose tissue sample by the number of pooled samples taken.

2.2. Lipid analysis

Whenever possible samples were handled under nitrogen and 2,6-di-*tert*-butyl-*p*-cresol was added to solvents and reagents to minimise autoxidation. Lipids were extracted from the adipose tissue slices with chloroform–methanol (2 : 1 v/v). Each sample was subjected to thin layer chromatography on 20 × 20 cm glass plates coated with layers of Kieselgel G (E. Merck, A. G., Darmstadt, Germany) 0.5 mm thick; the solvent system was hexane–diethyl ether–formic acid (80 : 20 : 2 by vol.). Bands were visualised under u.v. light after spraying with 2,7-dichlorofluorescein in methanol (0.1 % w/v), scraped into test tubes and a standard solution of methyl pentadecanoate in methanol was added to each before the lipids were transesterified for gas–liquid chromatography (g.l.c.) analysis.[8,9] The methyl esters of the constituent fatty acids were analysed in a Pye 104 chromatograph (Pye-Unicam Ltd) on 7 ft × $\frac{1}{4}$ in glass columns packed with 15% EGSS-Y on Chromosorb W (100 to 120 mesh, acid washed and silanised; Applied Sciences Inc., U.S.A.) at 185 °C. Fatty acids were identified by their retention times relative to authentic standards. The amount of each component was measured by multiplying the height by the retention time of each; replicate analyses of the same sample indicated that results for major components were reproducible within a limit of 0.4% absolute. The amount of each lipid class and the total amount of lipid present were calculated by relating the total amount of fatty acids found by g.l.c. in each lipid class to the amount of the internal standard.[8,9]

2.3. Histology

From each of three pigs, four back fat samples were obtained at random and these were cut without prior fixation at 25 to 30 μm perpendicular to the skin surface. The histological sections were in general mounted in water mounting medium and examined unstained although a few were stained by haematoxylin and Sudan IV prior to mounting. For each sample, 100 measurements were made of the thickness of the skin and the subcutaneous layers; this total was achieved by making five measurements on five random sections from each of the four back fat samples. Back fat specimens from all three animals were also fixed in 12% formal-saline, dehydrated in alcohol cleared in xylene and embedded in paraffin wax. Sections 10 μm thick were stained with haematoxylin and eosin and examined.

3. Results

Two lipid classes only were detected in significant amounts—triglycerides and phospholipids. The former was always the major component and the latter only assumed significance in the lipids of the skin itself where the total amount of lipid was very small.

Only six fatty acids were present in significant amounts, i.e. myristic (14 :0), palmitic (16 :0), palmitoleic (16 : 1), stearic (18 :0), oleic (18 : 1), and linoleic (18 :2) acids. In Figure 1, the fatty acid compositions of the triglycerides from each of the slices are plotted against the depth from which they were taken through the tissue. 14 :0 was a

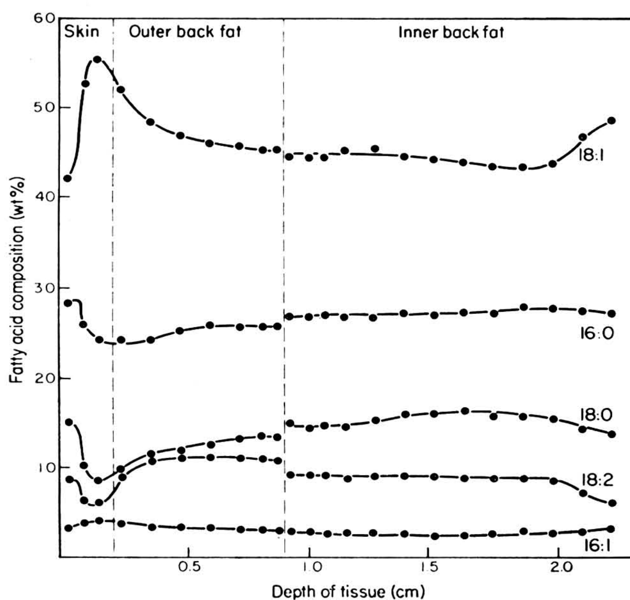

Figure 1. The variation in fatty acid composition with depth in pig back fat (for the sake of clarity alternate points have been omitted).

minor component and did not vary significantly through the tissue so is omitted from the figure.

The concentration of 18 : 1 increased sharply in the first 2 mm from the outer surface of the skin and then decreased to a fairly constant level by the time the connective tissue between the two layers was reached. At the junction of the two layers of adipose tissue there was a definite discontinuity in the manner in which the fatty acids were distributed. Thus, on the interior side of the connective tissue the concentrations of 18:0 and 16 :0 were distinctly higher and the concentration of 18 :2 was distinctly lower than the corresponding values observed on the outer side of the connective tissue. The composition in the inner layer of adipose tissue did not change appreciably, although there was a suggestion of an upward trend in the concentrations of saturated components and a downward trend in the concentrations of unsaturated constituents until the last 2 to 3 mm before the muscle tissue was reached. At this point, the concentration of 18 : 1

began to rise sharply together with a slight rise in the concentration of 16 : 1 while the concentrations of the saturated fatty acids and 18 : 2 tended to decrease. The discontinuity in fatty acid composition at the line of connective tissue between the inner and outer layers was observed in both of the other samples examined in detail and in one of these was even more marked than that illustrated.

The outer 1 mm of the skin, which represents the epidermis, the stratum papillare and the outer reticulare, was found to be clear of adipose tissue cells. The small amounts of triglyceride isolated from this region may originate from the sebaceous glands. In the next 1 mm of the skin which represents the inner layers of the stratum reticulare, adipose tissue cells were found around the hair follicles and sweat glands. In this region, the fatty

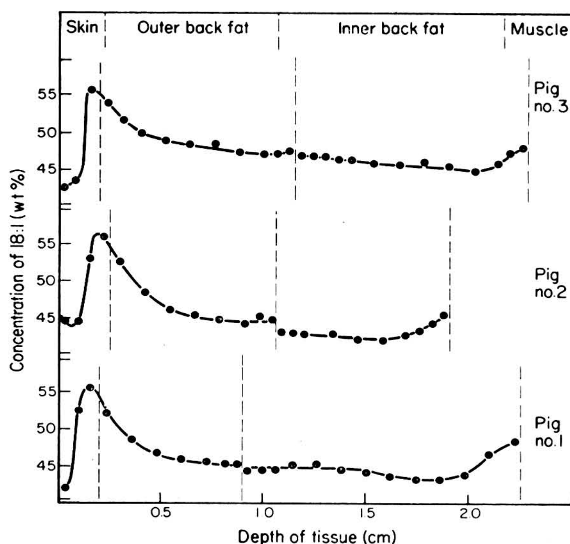

Figure 2. The variation in 18 : 1 concentration with depth in pig back fat for the three animals used in the experiment (for the sake of clarity, alternate points have been omitted).

acid composition of the triglycerides began to change towards values that were more characteristic of the deeper layers of adipose tissue. Nevertheless, the major changes in fatty acid composition occurred in the adipose tissue immediately below the skin. The fat cells immediately below the skin were smaller than those deeper in the tissue, but there were no obvious morphological changes detectable by light microscopy between the adipose tissue cells on either side of the connective tissue between layers.

Qualitatively similar results were obtained with two other animals although the thickness of the skin and adipose tissue layers varied considerably. To illustrate this, the concentration of 18 : 1 in each of the animals is plotted against the depth of the tissue in Figure 2.

4. Discussion

It is well established that subcutaneous adipose tissue in pigs is responsive to changes in environmental temperature and that the fat deposited in the outer layers is considerably

softer and contains a higher proportion of unsaturated fatty acids than that deposited further into the tissue.[1–3] As a result, it has been suggested that changes in fatty acid composition of this nature occur such that the physical properties of the fat in the cooler outer layers remain constant and similar to that in warmer regions of the animal. This study illustrates that there are continuous gradients in the concentrations of individual fatty acids throughout the outer layer of adipose tissue and these are more marked than is the gradient of total unsaturated fatty acids as the main components, 18 : 1 and 18 :2, alter in different directions. Pig back fat differs from the adipose tissue of other species in that a remarkably high proportion of its fatty acid constituents are synthesized *de novo* in the tissue.[10] It seems possible that the softer fat found in the layer immediately below the skin surface is formed principally by increased desaturation of saturated fatty acids by the appropriate enzyme system in this region rather than by selective deposition of unsaturated fatty acids formed elsewhere in the body or of those that may be of dietary origin.

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Effect of Calcium on Production of Volatiles by Apples

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The addition of calcium to Jonathan apples caused a large increase in the production of ethanol by the fruit for the first three weeks after injection. Later weeks showed a reduced level of ethanol in the fruit. Calcium addition also reduced the level of acetic acid in the fruit. The reduction in acetic acid could be the method by which calcium retards the development of low-temperature breakdown.

1. Introduction

Apples with low levels of calcium have been found to have a higher incidence of the physiological disorders, low-temperature breakdown[1] and bitter pit.[2] The incidence of both disorders can be reduced by adding calcium either pre-harvest[2–4] or post-harvest.[5,6]

Calcium deficiency has also been found to be a cause of internal browning of brussels sprouts,[7] internal tip-burn of cabbage,[8] blossom end rot of tomatoes and black heart of celery[9] during their growth.

No satisfactory explanation has been put forward to explain the effect of calcium in reducing breakdown or bitter pit. This paper examines whether the addition of calcium affects the levels of volatile compounds produced by Jonathan apples as Wills and his co-workers[10–13] have shown that acetic acid and a number of acetate esters may be involved in the development of breakdown.

2. Experimental

Jonathan apples were obtained from commercial orchards at Bilpin, New South Wales and stored at −1 °C. The fruit selected were considered to be susceptible to breakdown but not susceptible to bitter pit. Eight weeks after harvest, 180 fruit were injected into the core with calcium nitrate (80 μmol/fruit in 0.2 ml of water) and 180 were injected with water only. Two groups of 20 fruit from each treatment were placed in glass jars and the volatiles given off by the fruit were determined at various times from 0 to 4 weeks after injection by gas chromatography.[10] The volatiles in the fruit were determined at various times from 1 to 12 weeks after injection by vacuum sublimation[12] of a 100-g sample obtained from five fruit. Two samples per treatment were extracted at each time.

Fruit from a different orchard were distributed into 10 units, each of 20 fruit, after storage at −1 °C for one week. Four units were injected with 80 μmol of calcium nitrate

as above, but using ethanol as the solvent, four units were injected with 40 μmol of gibberellic acid (GA_3) in ethanol and two units with ethanol only. The volatiles given off were collected and analysed 1, 3 and 5 weeks after injection. The effect of GA_3 was examined since Wills and Patterson[14] have shown that it also reduces the incidence of breakdown in apples.

3. Results

Figure 1 shows that the addition of calcium markedly increased the rate of loss of ethanol from the fruit soon after injection but the effect had disappeared after four weeks. During this period, the level of ethanol in the calcium-treated fruit also increased but later it fell below that of control fruit. The addition of calcium also caused a reduction in the level of acetic acid in the fruit. This effect was present from the second week after injection (Figure 2). Calcium addition tended to reduce the level of other volatiles in the fruit but this effect was not statistically significant (Table 1). There was no effect of calcium on the rate of loss of acetic acid or volatiles other than ethanol (see also Table 1).

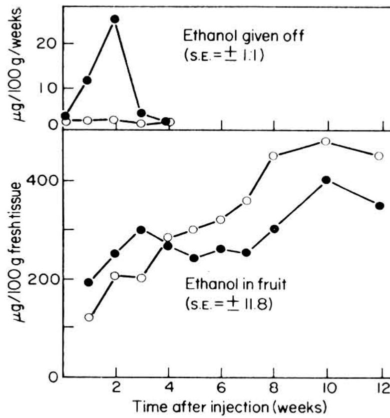

Figure 1. Effect of calcium on ethanol production by apples. —●—●—, Ca; —○—○—, water only.

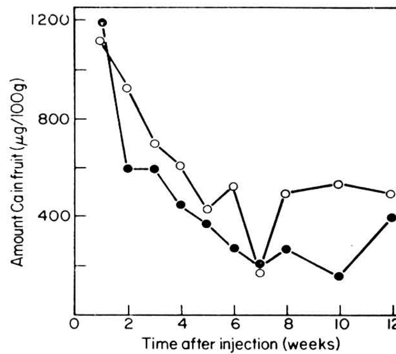

Figure 2. Effect of calcium on acetic acid levels in apples. —●—●—, Ca^{2+}; —○—○—, water only. S.E. = ±25.2.

TABLE 1. Effect of calcium on volatiles produced by apples

Compound	Amount in fruit[a] (μg/100 g) Ca^{2+}	Water	S.E.	Amount given off[b] (μg/100 g) Ca^{2+}	Water	S.E.
Ethanol	271	330	±11.8	45.2	6.0	±4.6
Acetic acid	504	606	±25.2	19.3	20.5	±11.6
Butyl acetate	372	394	±10.2	10.2	10.0	±1.1
Butanol	680	705	±18.4	10.5	10.2	±1.3
Isopentyl acetate	42	48	±2.1	5.4	5.5	±0.6
Isopentanol	<30	<30	—	5.1	6.4	+0.5
Hexyl acetate	49	57	±3.0	19.4	18.9	±1.5
Hexanol	79	82	±4.1	39.2	43.2	±2.9
Acetaldehyde	<10	<10	—	<1	<1	—

[a] Values are means of 20 readings (two samples, ten times).
[b] Total amount given off in four weeks was calculated from weekly readings.

When a large amount of ethanol (about 115 mg/100 g of apple tissue) was added to the fruit together with calcium, calcium still increased the rate of loss of ethanol (Figure 3). The effect was still present after five weeks. GA_3 had no effect on the loss of ethanol. There was also no effect of either treatment on the loss of other volatiles.

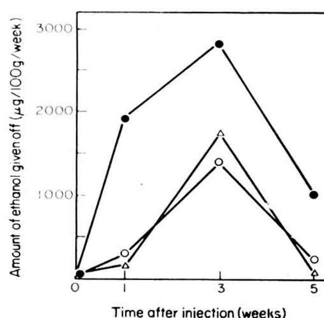

Figure 3. Effect of calcium and GA_3 on loss of ethanol from apples which have been injected with ethanol (115 mg/100 g of tissue). —●—●—, Ca^{2+}; —△—△—, GA_3; —○—○—, ethanol only. S.E. = ±434.

4. Discussion

The initial effect of calcium is to cause a decrease in the level of acetic acid and an increase in the level of ethanol. As both compounds are derived from pyruvate, this would suggest that calcium could be inhibiting the conversion of pyruvate to acetate with an increase in the rate of production of ethanol preventing an accumulation of pyruvate. The later effect of calcium, which results in a decrease in the level of ethanol while sustaining the reduced level of acetate, could be due to calcium inhibiting the production of pyruvate. The main source of pyruvate in apples is from phosphoenol-pyruvate (PEP) which is produced by glycolysis[15] and from malate.[16] Calcium has been

shown to inhibit both pyruvate kinase (which converts PEP to pyruvate) and pyruvate carboxylase (which converts oxalacetate to pyruvate) in animal tissues.[17]

The action of calcium in reducing the level of acetic acid in the fruit could be the method by which calcium decreases the incidence of breakdown as other methods for reducing the disorder such as increasing the rate of water loss from the fruit and storage at higher temperatures, have been shown to act in this way.[12,13]

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Effect of Water Loss from Apples during Cool Storage on the Water Content of the Fruit

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Apples which were stored in air of different relative humidities had different rates of water loss from the fruit but the water content of the fruit remained the same. It is suggested that water lost by evaporation is partly replaced by the water liberated from increased esterification of acids and alcohols in the fruit.

1. Introduction

Apples which have been harvested continue to lose water by evaporation, with the rate of loss depending on the temperature of the fruit and the relative humidity of the surrounding air. Scott and Roberts[1] found that the incidence of the physiological disorder, low temperature breakdown, was reduced if the rate of loss of water from the fruit during cool storage was increased. The reduction in breakdown was quantitatively related to the increase in water loss. Increasing the water loss by 1 % of the weight of the fruit resulted in a substantial reduction in breakdown. To help understand how this increase in water loss affects the fruit, we examined the effect of a similar increase in water loss on the residual water content of the fruit.

2. Experimental

Jonathan apples were individually weighed and distributed into two groups, each of 90 fruit. One group was stored at −1 °C in air of 70 % relative humidity (r.h.) and the other group at 95 % r.h. After 6 months, the fruit were reweighed and the weight loss calculated for each fruit. The water loss was considered to be the weight loss (expressed as % of original weight) less 0.4 %, which allows for the amount of respiratory carbon given off from the fruit in 6 months. This correction factor was calculated from the data on Jonathan apples of McGlasson and Hall (unpublished).

A cylinder of tissue (2 cm diameter) was taken from each fruit with a cork borer which was inserted at the equator of the fruit and pushed through the centre of the fruit. The core area was removed and the remaining tissue was weighed, freeze-dried, re-weighed and the water content was calculated as a percentage of the wet weight.

The mean values for water loss and water content were calculated for each group and compared by statistical analysis. The 90 values for each group were arranged in

decreasing order of magnitude of water content and grouped into nine sets each of ten fruit. Mean values of the water content in each set and the associated water losses were calculated. Regression analysis was carried out on the derived data.

3. Results and discussion

For each storage condition (high and low humidity), there was a slight but significant ($P<0.01$) inverse relation between water content and water loss (Figure 1). However, although the fruit stored at low humidity had an increased water loss ($P<0.001$) over

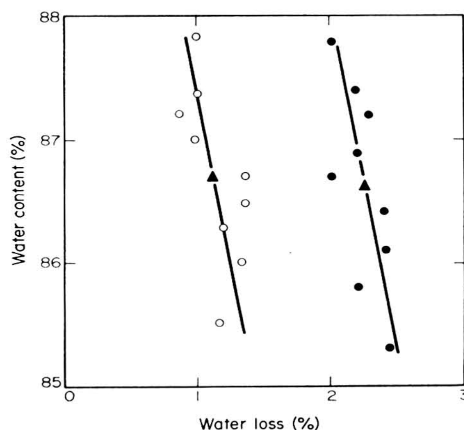

Figure 1. Relation between water loss during cool storage and water content of apples. (○) Stored in air of 95% r.h.; (●) stored in air of 70% r.h.; (▲) mean value of treatment.

that at high humidity, there was no significant effect on the water content. Thus the fruit must have a mechanism for maintaining an approximate water content even when appreciable amounts of water are lost.

The extra water lost from the fruit at the lower humidity could be partly replaced by chemical reaction within the fruit. A possible source of water is from an increased rate of esterification of the acids and alcohols in the fruit. In studies on the causes of the storage disorder, low temperature breakdown, Wills[2] and Wills and McGlasson[3] found increased esterification of the major volatile alcohols and acetic acid in fruit stored at low humidity. Thus the ability of the fruit to replace lost water could be the mechanism that results in a reduction of breakdown.

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A Note on the Effect of Elevated Plate Temperature on Freeze-dried Porcine Myofibrillar Proteins

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Electrophoretic differences between the myofibrillar proteins of fresh porcine *longissimus dorsi* and *psoas* muscles, still detectable after freeze-drying at a plate temperature of 60 °C, largely disappear when the temperature is 80 °C.

1. Introduction

The reduced hydration and dry texture which tend to impair the quality of meat on reconstitution after subjection to the Accelerated Freeze-Drying (a.f.d.) process[1] have been attributed to the effect of plate temperature on the muscle proteins during the sublimation phase. The degree of change which a.f.d. causes appears to differ according to the part of the carcass involved. Thus after subjection to the a.f.d. process, the myofibrils of *l. dorsi* in both ox and pig are less able to reabsorb water, on reconstitution, than those of *psoas*.[2,3] Moreover, when the isolated myosins from these muscles of the pig are freeze-dried (at a plate temperature of 50 °C) more change was detected in the electrophoretic pattern for myosin from *l. dorsi* than in that from *psoas*.[4]

According to Aitken, *et al.*[5] there is a distinct change in the appearance of reconstituted muscle and a degradation in the quality of meat, when plate temperatures above 80 °C are employed. Furthermore, Tilgner[6] reported that free H_2S begins to form in meat heated to temperatures above 80 °C. The work reported here was carried out to establish to what extent elevated plate temperatures affect the electrophoretic pattern of the myofibrillar proteins freeze-dried without prior separation from the meat; and, in particular, whether the differences in pattern between porcine *l. dorsi* and *psoas* still persist under these conditions.

2. Materials and methods

2.1. Muscles

Samples of porcine *l. dorsi* and *psoas*, from the region between the 4th and 6th lumbar vertebrae, were obtained at 24 h *post mortem*, trimmed of excess fat and connective tissue and cut into 3.5-cm cubes. The latter were blast-frozen at –22 °C in a Frigidaire Tunnel Freezer; and subsequently freeze-dried in a Vickers AFD pilot plant using plate temperatures of 60 and 80 °C. The ultimate pH of both muscles was 5.5 to 5.6. Any effect of the latter on the electrophoretic pattern[7] was thus obviated.

2.2. Preparation of myofibrils

Myofibrils were prepared by the method of Perry and Grey[8] after homogenising the freeze-dried muscle samples in ice-cold 30 mM-sodium β-glycerophosphate (adjusted to

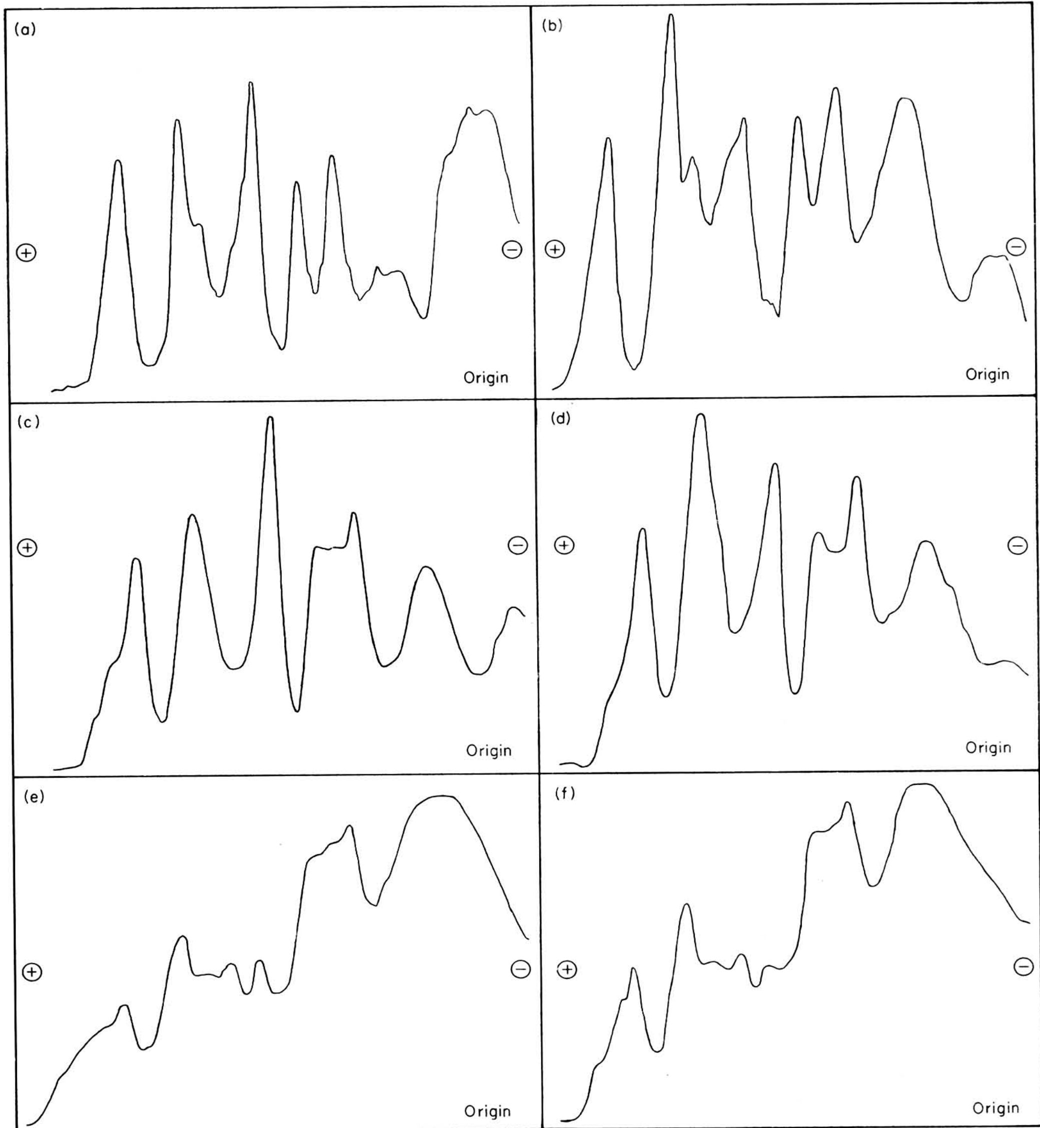

Figure 1. Densitometer tracings of starch gel electrophoretograms of porcine myofibrillar proteins extracted at 24 h *post mortem*. (a) *l. dorsi* fresh. (b) *psoas* fresh. (c) and (d) *l. dorsi* and *psoas*, respectively, after freeze-drying at plate temperature of 60 °C. (e) and (f) *l. dorsi* and *psoas*, respectively, after freeze-drying at plate temperature of 80 °C.

pH 6.5 with N-HCl). Preparations were washed twice by alternately adding buffer and centrifuging at 2000 **g** for 15 min.

2.3. Extraction and electrophoresis of myofibrillar proteins

Aliquots (2 ml) of the final suspensions of myofibrils from above were pipetted into 6 ml of 8 M-urea and left for 24 h at 0 °C with occasional shaking. The supernatants, after centrifuging at 20 000 **g**, were subjected to electrophoresis and the stained electrophoretograms scanned by laser beam, following the procedures already described.[4]

3. Results and discussion

Typical scans obtained for the myofibrillar proteins extracted from unheated *l. dorsi* and *psoas* are shown in Figure 1 (a) and (b), from those freeze-dried at a plate temperature of 60 °C in Figure 1 (c) and (d) and from those freeze-dried at a plate temperature of 80 °C in Figure 1 (e) and 1 (f), respectively. It is clear that freeze-drying affects the patterns in both muscles; and that the higher temperature causes greater change. Moreover, whilst differences between the proteins in the two muscles are quite evident after freeze-drying at a plate temperature of 60 °C, the patterns after the treatment at 80 °C are largely indistinguishable. This is in agreement with expectations from the work of Aitken *et al.*[5] The particular myofibrillar proteins involved in the above reactions have not so far been identified.

Acknowledgements

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Ascorbimetry: Determination of Peroxides in Certain Edible Oils

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The iodometric determination of peroxides in certain edible oils by the use of standard L-ascorbic acid solution is reported. Calculation of Lea number by the proposed method and the previously recognised sodium thiosulphate method is also given.

1. Introduction

Previously reported methods to detect the degree of rancidity in edible oils and fats almost all depend upon the determination of peroxides in the oil or fat.[1,2,3] Lea number is the amount of 0.002 N-$Na_2S_2O_3$ needed to react with the iodine liberated from the peroxides in one gram of oil or fat.[4]

The photometric method based on absorbance of free iodine solution for the determination of peroxides gives an error of 5.9%, while the earlier iodometric method[2] gives an error as high as 11.2%.

The present work describes an iodometric method for the determination of peroxides in certain rancid edible oils involving the use of standard L-ascorbic acid solution from which Lea number can be calculated.

2. Experimental

An accurately weighed amount of the oil, e.g. 10 g, was placed in a glass-stoppered Erlenmeyer flask. Then 5 ml of 10% potassium iodide solution, 2 ml of 5% sodium bicarbonate solution and 2 ml of 10% dilute acetic acid (v/v) were added at room temperature (30 to 35 °C). The flask was stoppered and the contents were shaken vigorously at intervals for 10 min away from direct sunlight to allow the reaction to go to completion. The iodine liberated was titrated against standard L-ascorbic acid solution after adding 1 ml of soluble starch indicator solution and 10 ml of distilled water. The standard L-ascorbic acid solution (0.002 N) was added dropwise with continuous shaking until no blue colour was observed even after shaking for 5 min. The volume of the titre was noted.

Lea number can be calculated from the expression:

$$\text{Lea number} = \frac{\text{titre of 0.002 N-L-ascorbic acid (ml)}}{\text{weight of edible oil taken (g)}}$$

3. Results

Samples of six different edible oils, e.g. arachis oil, available on the market were collected at random. All samples gave a positive Kreis Test for rancidity. Peroxides in these rancid oils were simultaneously determined by the proposed method and the official method.[5]

Lea number was then calculated for each sample of oil. The results are shown in Table 1.

TABLE 1. Peroxide value of certain edible oils

Edible oil	0.002 N-L-ascorbic acid/g of oil (ml)	0.002 N-Sodium thiosulphate /g of oil (ml)
Arachis oil	1.28	1.30
Cottonseed oil	1.92	1.90
Linseed oil	2.86	2.90
Maize oil	1.26	1.26
Olive oil	3.87	3.82
Sesame oil	2.48	2.46

4. Discussion

Earlier methods for the determination of peroxides in edible oils and fats are very sensitive to atmospheric oxygen and show poor reproducibility.[6]

The addition of the mineral acid before potassium iodide leads to disintegration of peroxides.[7] In the proposed method, potassium iodide was added before acetic acid to minimise any destruction of peroxides by the acid.

The effect of atmospheric oxygen is avoided by adding sodium bicarbonate and acetic acid so that the evolved carbon dioxide will replace oxygen in the flask.[8]

The iodometric and benzidine peroxide value gives a reproducible measure of rancidity.[1]

The proposed method is simple, relatively rapid and can detect minute amounts of peroxides without the need for elaborate equipment. The results of the proposed method at local room temperature (30 to 35 °C) show great analogy to those obtained by the official method (Table 1) which involves the use of equal amounts of boiling chloroform and glacial acetic acid to dissolve the oil.

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Zinc, Iron and Copper Contamination in Home-produced Alcoholic Drinks

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The contamination of home-brewed alcoholic drinks with zinc, iron and copper is fairly widespread. It is largely unrelated to the material fermented but usually depends on the type of vessels used during production.

A change in equipment used in home brewing in many African countries has resulted in production of drinks which are contaminated by heavy metals and may be responsible for an increased incidence of certain forms of cancer.[1,2] Oil drums and other metal containers have replaced traditional clay pots and gourds. Analyses of more than 150 samples showed how widespread such contamination is (Table 1). The contamination is independent largely of the materials fermented and is related to the type of vessel used (Table 2). Distillation does not necessarily remove the contaminating metals, as results for spirits show. The zinc, iron and some of the copper come principally from the containers through the action of acids produced during fermentation. African beers are made with a mixed microbial flora containing various *Lactobacilli* and are highly acid. Copper, at least in Zambia, has been traced in some cases to contamination by copper-rich soil. Lead contamination is rare and the highest concentration recorded

TABLE 1. Metal contamination in alcoholic drinks from different countries

Country of origin	No. of samples	Copper	Zinc	Iron
			Concentration range (mg/l)	
Zambia	120	0.05–58(73)	0.025–65(93)	1.0–245(15)[a]
S. Africa	10	— (0)	0.025–3.6(4)	0.5–7.3(2)[a]
Kenya	5	— (0)	0.025–6.2(5)	0.5–22.7(2)[a]
India	15	1.2–8.9(5)	0.4–68.0(11)	No results[a]
Ireland	3	7.0–26.2(3)	0.4–12.3(3)	No results[a]
Switzerland	4	0.1–3.7(4)	1.3–17.7(4)	0.2–2.3(3)
Canada	4	0.05–2.4(3)	5.7–32.0(4)	0.5–3.3(2)

All of the samples were home-produced. Metals were analysed by direct aspiration of filtered samples in a Techtron Model 1000 Atomic Absorption Spectrophotometer. The numbers of contaminated samples are given in parentheses.

[a] Only 20 Zambian, 4 South African and 2 Kenyan and no Irish or Indian samples were analysed for iron.

TABLE 2. Metal contamination in different types of beer and spirit

Sample	Type	Fermentation vessel	Metal content (in mg/l) Copper	Zinc	Iron
1	Millet beer	clay pot	0	0	0.5
2	Honey beer	clay pot	0	0.03	1.3
3	Maize beer	galvanised metal drum	0.1	5.5	140
4	Maize beer	enamel bowl	0	8.7	17.7
5	Maize spirit	galvanised metal drum	0	19.4	2.5
6	Maize spirit	steel drum	0.05	1.8	2.8
7	Cassava spirit	galvanised metal drum	8.3	1.6	3.3

was 0.5 parts/million. Some commercial alcoholic drinks show similar contamination. A Spanish sherry had 0.3 parts/million of copper, 7.5 of zinc and 2.5 of iron. Italian Cinzano, a French brandy and a Scotch whisky had between 0.1 and 0.4 parts/million of copper[3]. Metal contamination at a high level is reported in illicit spirits in the U.S.[4] and in wines.[5] The health hazards of heavy metal contamination in African drinks have been discussed by Burrell,[6] Lowenthal *et al.*,[7] Reilly and McGlashan[8] and others.

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I wish to thank Dr N. D. McGlashan for supplying most of the African and all of the Indian samples. A generous grant towards purchasing a Techtron Atomic Absorption Spectrophotometer was made by the Roan Selection Trust and the Nchanga Consolidated Copper Companies.

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J. Sci. Fd Agric. 1972, **23**, 1145–1150

Agriculture Group and Fertiliser Society Symposium March 1972

Soil Sulphur and Plant Growth

The following are summaries of papers presented at a joint meeting of the Agriculture Group and the Fertiliser Society on 14th March 1972. The reports so published are entirely the responsibility of the authors and in no way reflect the views of the Editorial Board of the Journal of the Science of Food and Agriculture

Methods for the Determination of Total Sulphur in Soils and Plant Materials

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Research on the role of sulphur as a plant nutrient requires the determination of the sulphur contents of soils and plants. For soils, comparison of published data shows that values obtained by the chemical methods of Arkley,[1] Bloomfield,[2] Jenkinson,[3] Steinbergs *et al.*[4] and Tabatabai and Bremner[5] are in close agreement. Of these the method of Tabatabai and Bremner[5] is preferred because of ease of manipulation and safety. X-ray fluorescence spectrometry[6] gives results that agree well with those obtained by chemical methods for mineral soils but corrections are necessary for soils containing much organic matter.

For plants most chemical methods involve two steps, oxidation of sulphur to sulphate and determination of the sulphate produced. A comparative study has been made using the same samples. Two oxidation procedures were tested; that using fuming nitric acid alone and the method of Butters and Chenery[7] using fuming nitric acid followed by ignition of the dry residue at 400 °C with magnesium nitrate. The latter procedure is more efficient and leads to larger values for sulphur content. Determination of sulphate by turbidimetry using the Technicon Auto-analyzer,[8] by Cunningham's[9] indirect flame photometry and by a modification of the Johnson and Nishita[10] method, using titration with mercuric acetate solution with dithizone as indicator[11] to determine H_2S produced, have been compared. The turbidimetric method was found to be unreliable whereas the modified Johnson–Nishita procedure gave the most consistent results. X-ray fluorescence spectrometry using standards prepared by addition of methionine to cellulose and making an allowance for the difference in X-ray absorption between cellulose and average plant material gives reasonably accurate results for most samples. For the most accurate and reliable results, corrections for the matrix effects such as have been evaluated by Norrish and Hutton[12] are required. These corrections require knowledge of the major element composition of the plant material.

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Sulphur in Rainwater and Air at some Relatively Clean Sites in the British Isles from 1959 to 1970

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Monthly measurements of the concentrations of sulphur (and other substances) in air and rainwater at 17 sites throughout the United Kingdom and Eire for the period 1959 to 64 were reported by Stevenson.[1]

Sampling has continued at three sites in the United Kingdom to monitor any long term trends. Lerwick is situated in a clean maritime environment, Eskdalemuir in an isolated, largely uncultivated rural area and Bracknell in an area of rapid urban growth about 30 miles south-west of London.

The 10 percentile, median and 90 percentile concentrations of sulphur in rain water for the periods 1959 to 64 and 1965 to 69 are shown in Table 1.

TABLE 1. Concentrations of sulphur in rainwater in mg l^{-1}

	Eskdalemuir		Lerwick		Rothamsted	Bracknell
	1959 to 64	1965 to 69	1959 to 64	1965 to 69	1959 to 64	1965 to 69
10 percentile	0.7	0.6	1.7	0.8	1.4	0.9
Median	1.2	1.4	3.3	2.7	2.1	1.8
90 percentile	2.0	2.6	6.7	5.0	4.2	3.2

The concentrations at Bracknell, where sampling was started in 1965 are similar to those at Rothamsted for the period 1959 to 64. At Lerwick most of the sulphur appears to originate from sea-salt particles. However, some "excess" sulphur is present, the amount increasing in the summer months. Additionally, some sea-salt chloride seems to be replaced by sulphate in the summer months. At Eskdalemuir and Bracknell most of the sulphur in rain water is not associated with sea salt.

Median monthly deposition rates in rainfall range from a minimum of about 1 kg/hectare/ month at Bracknell to a maximum around 3 kg/hectare/month during the winter months at Lerwick.

Mean monthly air concentrations of sulphur at Lerwick throughout the year and at Eskdalemuir in the winter months have been very similar for the period 1959 to 64 and 1965 to 69 but concentrations of sulphur in the summer months at Eskdalemuir have almost doubled, resulting in an almost constant sulphur concentration throughout the year for the period 1965 to 69.

Some measurements taken from July 1969 to August 1971 indicate that, on average, more than 70% of the total airborne sulphur at Lerwick and Eskdalemuir is in particulate form. Similar measurements have not been made at Bracknell which is the only site to show a significant annual variation of air sulphur concentration in the period 1965 to 69.

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Inorganic Sulphur Reactions in Anaerobic and Aerobic Soils

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In anaerobic soils sulphate is reduced microbiologically to sulphide, which reacts with iron to form ferrous monosulphide. In this form the sulphide seems to be harmless to plants, but free H_2S is toxic to rice. Free H_2S can be formed in soils containing a considerable excess of ferric oxide and the proportion of H_2S decreases asymptotically with increasing amounts of soil—Fe_2O_3.

The disulphide, FeS_2, often forms in sulphidic soils; although the concentration of monosulphide seems seldom to exceed a few 100 parts/million, percentage amounts of pyrite are common, and the oxidation of pyrite is the essential cause of acidification when such soils become aerobic. Moist pyrite oxidises slowly at neutral or mildly alkaline reactions, in the first instance giving Fe^{2+} and SO_4^{2-} and the rate of oxidation increases with increasing acidity. The process is catalysed by Fe^{3+} and once the pH decreases to 3.5 to 4.0, the intervention of *Thiobacillus ferro-oxidans* causes a considerable acceleration. In view of this mechanism, it is to be expected that liming would inhibit the oxidation of pyrite and experiments with undisturbed soil cores confirm this expectation.

As well as causing acidification, in pyritic soils *T. ferro-oxidans* is also responsible for the blocking of field drains by a form of ochre that is quite distinct from the well-known form produced by filamentous iron bacteria.

Sulphate Adsorption and Leaching in a Latosol

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Uncropped lysimeters were used to study the leaching of ammonium sulphate, rock phosphate and potassium chloride through a Malayan latosol (ferralsol) at pH 4.8 and pH 5.2 (water). 27 mm of water was added weekly to the surface for up to 26 weeks.

The added sulphate leached much more slowly than nitrate or chloride especially at the lower pH. Soil analyses showed zones of adsorbed sulphate (soluble in N-ammonium acetate) in the lysimeter profiles. These zones moved slowly down the profile with further leaching. The adsorbed sulphate in these zones lowered the soil pH when measured in both water and N-potassium chloride suspensions.

A Langmuir isotherm relating adsorbed sulphate to sulphate concentrations in solution was derived from analyses of the final leachates and of soil from the bottom of the lysimeters.

The isotherm was then used to calculate the rate sulphate should move down nine successive "plates" of a model soil profile, assuming each unit addition of water to the surface displaced the soil solution from the "plate" to the next. A computer program enabled patterns of sulphate adsorption in the soil and of sulphate in the leachate to be predicted after adding up to 200 units of water. Calculated rates of leaching and adsorption patterns were similar to those observed in the experiments.

Sulphate adsorption by these soils probably explains why S-deficiencies have not been observed in crops grown on them (mainly rubber and oil-palm). These might be expected with 2000 mm annual rainfall and only small amounts of mineralisable organic matter in the soils and sulphur dioxide in the atmosphere. The isotherm shows that this soil adsorbs about fifty times more sulphate than would be present in the soil solution at pH 5.2.

Sulphur in the Nutrition and Flavour Development of Vegetables

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The conclusions to be described were based on growth of three *Allium* spp. (onion,[1] garlic and *A. vineale*[2]) and five Cruciferae (cabbage, radish, *Sinapis alba*,[3] *Brassica juncea*[4] and watercress[5]) in sand culture in a glasshouse. The nutrients, other than sulphur were at the optimal levels. Sulphate was supplied at six concentrations ranging from 0 to 3 mequiv./l with four intermediate values. Indicators of sulphur deficiency included: visual sumptoms; weight responses; total, inorganic and organic sulphur concentrations in the plants; and N:S ratios.

Flavour strength was determined by sensory, gas chromatographic and chemical methods, all of which were in agreement with the conclusion that flavour in these vegetables was closely related to sulphur nutrition up to the maximum genetically controlled limits. In the case of the *Allium* spp. virtually all the important flavour components contained sulphur and there was a correlation between the sensory observations and total peak areas in headspace gas chromatograms. For radish, *Sinapis alba* and watercress, however, there was no such correlation but a definite relationship between sensory observations, sulphur nutrition and flavour components, present in solvent extracts of the vegetables, resolved by gas chromatography at relatively high temperature. It was concluded that the flavours of cruciferous plants consist of at least two types of components: (a) compounds which impart a bland, generalised "vegetable" flavour to the food and (b) a specific component or components which are perceived as a burning sensation on the tongue sometimes accompanied by a characteristic odour. The former are independent of sulphur nutrition whilst the concentrations of the latter are highly correlated with sulphur nutrition.

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Supply of Sulphur to Grass from some Soils of England and Wales

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The sulphur status of 16 soils was investigated by chemical and pot-culture procedures. One group of soils was from the wetter, western region (annual rainfall above 900 mm) and the other from the drier, central and eastern region. The soils from the two regions had previously received similar inputs of sulphur through fertilisers and rainfall, but differed in a number of properties as shown in the Table.

TABLE 1. Differing properties of the soil samples investigated

Soils		pH	"Free" Fe_2O_3	Organic C	Organic S	KH_2PO_4-extractable S
Region	Number	(H_2O)	(%)	(%)	(parts/million)	(parts/million)
Wet	7	6.0	2.2	3.90	550	25.3
Dry	8	7.5	1.2	2.04	355	13.8
Dry	1 (peat)	6.7	2.7	19.38	7040	367.8

Perennial ryegrass was grown on the soils in a growth cabinet, using sulphur-free water and air filtered to remove gaseous sulphur compounds. Sulphur, as K_2SO_4, was added to half the pots of each soil and all received N (at 2 levels), P, K, Ca and Mg. With one exception (a peat) the soils failed to supply sufficient sulphur for maximum growth.

Plant uptake of sulphur was measured in the ryegrass tops at 4 successive harvests and in the roots at the final harvest. It was generally greater from soils of the wet than of the dry region: means, 22.3 and 13.6 mg S/kg of soil, respectively, excluding the peat (76.4 mg S/kg of soil). For the 15 soils on which there were responses to sulphur, plant uptake was highly correlated with the original levels of KH_2PO_4-extractable S (soluble plus adsorbed sulphate), the regression equation being:

$$\text{uptake} = 3.37 + 0.75x$$

where x is extractable S (mg/kg of soil). Decreases in extractable S under plants accounted for 80 to 94% of uptake and release through mineralisation, as measured in unsown soil, contributed the remainder. The improved relationship between uptake and soil sulphur is given by the equation:

$$\text{uptake} = 1.42 + 0.78x,$$

where x is the decrease in extractable S plus mineralised S (mg/kg of soil).

The higher levels of available sulphur in the soils from the wet region were due mainly to their higher levels of adsorbed sulphate (means: wet region, S, 13.6 parts/million; dry region excluding peat, S, 2.1 parts/million), which are, in turn, significantly correlated with soil pH ($r = -0.68$**) and with "free" Fe_2O_3 ($r = 0.80$***).

Soil Sulphur Levels in Relation to Sulphur Deficiency and Crop Responses under Irish Conditions

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The main objective is to identify the combination of soil, crop, fertiliser and meteorological conditions conducive to sulphur deficiency in the Republic of Ireland. Earlier work has already been summarised.[1]

Since 1969 the following developments have occurred.

1. A series of 27 field experiments to measure responses to sulphur in grassland throughout the country were laid down in 1970. Responses to date have been negligible.
2. Pot culture experiments with 24 of the major soils have shown an average increase of 26% in clover dry matter yields with sulphur applications.
3. Sulphur application, as gypsum, increased the nitrogen content of clover significantly in pot culture.
4. Soil tests for available sulphur and total soil sulphur contents have given significant correlations with dry matter yields in pot culture.
5. A soil test and a crop diagnostic service for sulphur were introduced during 1971 for the national agricultural and horticultural advisory services.
6. An intensive soil and plant sampling programme on a stratified random basis was completed, in the South-East.
7. An automated analytical technique for soils and plants was introduced during 1971.

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Sulphur Requirement of Crops in the United Kingdom

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In 1963, in pot culture at Jealott's Hill, sulphur deficiency was established in the third year in grass receiving only N.P.K. fertilisers and deionised water. This was corrected by the use of ammonium sulphate or by using rainwater in place of the deionised water.

As a result of these findings and with the knowledge of the decline in use of the main sulphur-containing fertilisers, sulphate of ammonia and superphosphate, the level of sulphur in a series of kale leaf samples was examined in the winters 1966 to 67 and 1970 to 71 to see if there was any relationship between the sulphur level in the kale and sulphur contamination in the atmosphere. Kale was chosen as the test crop as it has a high sulphur demand and it has been reported that for optimum yield the sulphur content of the kale leaves should not fall below 0.7% S.

In the 1966 to 67 survey, although there were some differences in the sulphur contents of the samples from areas of low and heavy contamination, the average sulphur content was 1.07% S. In 1970 to 71 the difference between areas was maintained, but there was a general fall in the kale sulphur contents and the average levels for industrial and rural areas were 0.92 and 0.78%, respectively.

In long-term field and lysimeter studies to measure nutrient losses from grassland treated with 2 differential rates of nitrogen, preliminary findings have indicated, in the 10 month period April 1971 to January 1972, that although the rainfall supplies 20.1 kg/ha (18 lb/acre) sulphur per annum, there was a negative sulphur balance of about 44.8 kg/ha (40 lb/acre) on the lysimeter where the grass crop was cut and removed. The concentration of sulphur in the drainage water was similar from both lysimeters and field trial and varied between 30 and 200 mg of S/l dependent on the flow rate and in the period under test and averaged about 70 mg of S/l.

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SAFETY IN MICROBIOLOGY

edited by D. A. Shapton and **R. G. Board**

Microbiological Laboratory
H. J. Heinz Co. Ltd., Hayes, Middlesex England

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With safety as the central theme of the Autumn 1970 Demonstration Meeting of the Society for Applied Bacteriology microbiologists reviewed many of the methods which have evolved for the safe handling of pathogenic and saprophytic microorganisms as well as radioisotopes, etc. Covering many branches of microbiology, the contributors have provided a comprehensive and definitive text together with references, which will be of undoubted value for use in laboratories, whether medical, veterinary, pharmaceutical, food or fermentation.

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