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THE NUTRITIVE VALUE OF LEAF PROTEIN CONCENTRATES DETERMINED IN BIOLOGICAL TESTS WITH RATS AND BY MICROBIOLOGICAL METHODS

By KATHLEEN M. HENRY and J. E. FORD

The biological values and true digestibilities of a number of leaf protein concentrates have been determined by the balance-sheet method at an 8% level of protein intake on young growing rats. Determinations were made on preparations from the leaves of 14 different species of plants. The effects of several methods of drying the concentrates and of the maturity of the leaves at harvesting were studied. The availability of some essential amino-acids was determined microbiologically in selected samples; in all of them methionine was less available than in whole egg proteins. The microbiological results were consistent with the biological findings. In general biological values of the proteins in the leaf concentrates were of the order found for good quality plant proteins such as those of legume and cereal seeds and of yeast but true digestibilities were lower. Freeze-drying, drying by acetone extraction and drying on starch in a current of air proved satisfactory but oven-drying at 100° caused a significant decrease in biological value and a more marked loss of true digestibility. Biological values and true digestibility increased with the maturity of the leaves at harvesting because of increased availability of methionine. A possible explanation of the results is suggested.

Introduction

The value of leaf protein concentrates as supplements to cereal diets has been established in feeding experiments with chicks,[1,2] rats[2] and pigs.[3,4] Their value for man in countries where protein malnutrition is prevalent has also been demonstrated.[5]

Cowlishaw *et al.*[1] have pointed out that, although the technique used in such animal experiments has practical applications, it is not suitable for a critical examination of the digestibility or for the detection of amino-acid deficiencies in leaf proteins. They recommend that the biological value (BV) and true digestibility (TD) of such proteins should be determined and compared with amino-acid analyses of the samples.

In the experiments now reported, the BV and TD of protein concentrates prepared from leaves of several species of plants have been determined; a study is also made of the effect on these measurements of several methods of drying the concentrates and of the maturity of the leaves at harvesting. With increasing maturity, a smaller proportion of the total protein of the leaf is extractable. This change generally affects more the 'chloroplastic' than the 'cytoplasmic' fraction, so that the protein extracted from mature leaves tends to contain a larger proportion of the latter. Davies *et al.*[6] have reported higher values for the BV and TD of the 'cytoplasmic' than of the 'chloroplastic' fraction of proteins extracted from the leaves of cocksfoot. This observation has been confirmed for similar preparations from rape and lupin leaves.

Amino-acid analyses of leaf protein extracts do not, in general, correlate with present biological findings. Thus little difference has been reported between species,[7,8] between samples of barley harvested at different stages of maturity[9] or between the 'cytoplasmic' and 'chloroplastic' fractions of spinach[7] or barley.[10] On the other hand, Smith & Agiza[8] observed, in a study with four species, that the proportions of some amino-acids varied with the maturity of the plant at harvesting and that differences between species became evident if comparisons were made at similar physiological ages. Armstrong[11] also considers that there may be differences in amino-acid make up between species. All these values are for total concentrations and do not allow for the possibility that some acids may not be completely available biologically. Ford[12,13] has described a microbiological assay procedure for several of the essential amino-acids that takes into account their biological availability. Use of this method for the analysis of several leaf protein concentrates shows that the results were more closely consistent with the findings of biological tests with rats.

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Experimental

The samples of leaf protein concentrates were prepared by Mr. N. W. Pirie, F.R.S., and his colleagues at Rothamsted Experimental Station. Preparations referred to without qualification, and those called ' whole ' were made by blowing steam into fresh leaf juice so that the temperature was raised suddenly to 80°, filtering off and pressing the coagulum, resuspending it in water at pH 4 and filtering and pressing again.[14] What is loosely called ' chloroplastic ' protein was made by letting fresh juice flow through a brass tube maintained at 60° and continually scraped so that no coagulum stuck to it. As this coagulum does not filter easily, it is centrifuged down, heated to 100° to make it more compact, filtered off and pressed. The ' cytoplasmic ' protein separates when the fluid from the centrifugation is heated to 80°.

Biological tests

Animals.—Rats of our hooded Norwegian strain were used. They were weaned at 21 days on to our normal stock diet.[15]

Experimental diets.—The basal ' protein-free ' diet had the percentage composition: rice starch 64, sugar 12, potato starch 10, margarine fat 10, salts[16] 4. The leaf protein concentrates replaced an equal weight of rice starch so that the diets contained about 8% protein. To each kg. of diet were added: thiamine 3·3 mg., riboflavin 5·5 mg., pyridoxine hydrochloride 3·3 mg., calcium pantothenate 11·0 mg., folic acid 0·6 mg., biotin 0·6 mg., *p*-aminobenzoic acid 11·0 mg., nicotinic acid 11·0 mg., inositol 110·0 mg., choline hydrochloride 1900 mg., menaphthone (2-methyl-1, 4-naphthaquinone) 2·2 mg., cyanocobalamin 20 μg. The diets were offered at the rate of 10 g./100 g. body weight. In addition, a weekly dose of 360 i.u. of vitamin A, 26 i.u. of vitamin D and 1·4 mg. of α-tocopheryl acetate was given separately by mouth, in arachis oil, to each rat.

Determination of BV and TD.—The BV and TD of the leaf protein concentrates were determined by the balance-sheet method of Mitchell.[17,18] Groups of twelve female rats aged 25–26 days were used in all tests. Details of the cages and experimental procedure are given elsewhere.[19–21]

Design of experiments

The samples of leaf protein concentrates were numbered 1–25 in the order in which they were received at Shinfield. Unless otherwise stated the samples were freeze-dried by the normal Rothamsted procedure[14] in which an open texture is maintained by rapid evaporative freezing or by an alternative method in which samples are pre-frozen in trays in a deep-freeze cabinet, which method gives a more gritty product.

Species.—The following species were tested in eight experiments, in each of which vitamin-low casein (Genatosan Ltd.) was included as reference protein: barley, sugar-beet, potato, wheat, mustard, tares, rye, sweet clover, rape, lupin, pea, red clover, strawberry clover and nasturtium. The leaves from the first three were dried by acetone extraction, those from the others were freeze-dried.

Effect of method of drying.—The following methods of drying were compared in three experiments: for wheat 7, acetone extraction, freeze-drying (alternative method) and oven-drying (100° in a slow current of air); for rye 8, freeze-drying (evaporative and alternative) and drying on starch in a current of warm air; for wheat 12, freeze-drying (alternative) and oven-drying (100° in a slow current of air).

Effect of maturity of the plant at harvesting.—Two experiments were done with wheat and one with tares. Wheat samples 9 (harvested 10/5/61 when 12 in. high, green and lush), 10 (harvested 19/5/61 when 12 in. high, lush) and 11 (harvested 12/6/61, ears forming) in one test; and samples 12 (harvested 3/5/62 when 10 in. high, lush), 13, (harvested 21/5/62, ears just forming and getting stalky) and 14 (harvested 18/6/62, in ear, still green but very stalky) in the second test. Two samples of tares were compared, 15 (harvested 18/7/62, buds just forming) and 16 (harvested 29/8/62, pods formed).

Effect of amino-acid supplements.—Three experiments were done in which leaf protein concentrates were supplemented with quantities of amino-acids calculated to make good the

deficiencies revealed by microbiological assay. Wheat 12 alone or supplemented with 0·3 g. of DL-methionine/16 g.N was compared with wheat 14; tares 15 alone or supplemented with 1·9 g. of DL-methionine or with 1·9 g. of DL-methionine and 0·6 g. of L-lysine/16 g.N was compared with whole egg proteins; sweet clover 17 alone or supplemented with 1·8 g. of DL-methionine or with 1·8 g. of DL-methionine and 0·8 g. of L-isoleucine/16 g. N was compared with whole egg proteins.

'Chloroplastic' and 'cytoplasmic' fractions.—These fractions were prepared from rape samples 18 and 20 at essentially the same stage of maturity and from lupin samples 19 (in flower) and 25 (coming into flower). The following four comparisons were made: (1) rape 18, its two fractions and casein; (2) the two fractions of rape 20 and casein, the whole protein not being included in this test as the amount available was insufficient; (3) lupin 19, its two fractions and casein; (4) lupin 25, its two fractions and these fractions combined in the proportions in which they were originally present in the leaves, i.e. 1 part of 'cytoplasmic' protein with 2 parts of 'chloroplastic' protein.

Microbiological assay methods

The microbiological test procedures used were as described by Ford.[13] For the measurement of total methionine, histidine, leucine, isoleucine, valine and lysine the test proteins were hydrolysed with hydrochloric acid. Samples containing 100 mg. of nitrogen were sealed in Carius tubes, each with 40 ml. of 3 N hydrochloric acid, and heated for 18 h. in a steam autoclave at 121°. After being cooled, the tubes were opened and their contents brought to pH 7·0 with 10 N-sodium hydroxide, diluted to 100 ml. with water and filtered. Finally, 10-ml. portions were taken and diluted to 100 ml. with water. For the assays of tryptophan the samples were heated in sealed tubes for 5 h. at 121° with 40 ml. of 5 N-sodium hydroxide. The assay values were doubled to correct for the racemisation that occurs under these conditions of alkaline hydrolysis, since DL-tryptophan has only half the activity of the L-isomer for *Streptococcus zymogenes*, the test micro-organism employed.

For the assays of the available amino-acids the test samples were predigested with pepsin (cf. Ford[13]). All the test extracts were strongly coloured and growth responses in the assays were therefore assessed titrimetrically.

Results and discussion

Species of plant

Table I gives the BV and TD for protein concentrates prepared from leaves of fourteen species. Over half of the samples tested had a BV of over 70 and compared well with good

Table I

Biological value and true digestibility of several species of leaf protein determined at an 8% level of protein intake

(Mean values for groups of twelve rats)

Sample No.	Species	Biological value*	True digestibility*
1	Barley**	81·4 ± 1·41	83·9 ± 0·59
2	Sugar-beet**	67·2 ± 1·41	79·4 ± 0·59
3	Potato**	73·0 ± 1·41	81·6 ± 0·59
4	Wheat	80·3 ± 1·08	82·2 ± 0·70
5	Mustard	73·6 ± 1·08	81·8 ± 0·70
6	Tares	58·3 ± 1·08	81·1 ± 0·70
8	Rye	75·8 ± 1·01	76·9 ± 0·83
17	Sweet clover	38·1 ± 2·48	80·6 ± 0·97
18	Rape	77·9 ± 1·19	84·8 ± 0·55
19	Lupin	83·2 ± 1·61	75·9 ± 1·40
21	Pea	65·2 ± 1·82	81·0 ± 0·74
22	Red clover	53·7 ± 2·53	70·6 ± 1·08
23	Strawberry clover	62·4 ± 2·53	76·9 ± 1·08
24	Nasturtium	83·7 ± 2·53	90·5 ± 1·08

* Values with their standard errors

** Samples dried by acetone extraction: all other samples freeze-dried

quality vegetable proteins such as yeast and soya-bean, sunflower-seed and cottonseed meals;[22] values for tares and the three species of clover tested were lower. Armstrong & Thomas[23] have reported low BV's of 55 and 45 respectively for the proteins of lucerne and trefoil, while Bartlett *et al.*[24] found values of 67, 62 and 52 respectively for grass from a permanent pasture artificially dried at 300° F or 170° F or sun-dried. Davies *et al.*[6] found no differences in BV between the species tested by them. Values for TD (Table I) varied much less than those for BV, only nasturtium with a value of 90 having a TD comparable with that of good quality animal and vegetable proteins (cf. Henry & Toothill).[22] All other values were low, about 80, and were similar to the values reported by Armstrong & Thomas:[23] values found for permanent pasture[24] were much lower, about 65.

Effect of method of drying

The results in Table II show that of the drying methods studied only oven-drying at 100° reduced the nutritive value of leaf proteins. These findings confirm those of Duckworth & Woodham;[2] they found, however, that there was no damage when oven-drying was done at lower temperatures, and that the temperature of the material in the oven was important. The results for the two samples of wheat, 7 and 12, show that oven-drying caused a significant loss of BV and a more marked loss of TD. This finding is surprising as usually losses due to heat treatment are more marked with BV than with TD.[25–29] The reduction in BV amounted to some 7% for both wheats, a figure similar to that found at Shinfield for sterilised milk.[28,29] The loss in TD was 24% for sample 7 but only 11% for sample 12. Oven-temperature and drying time were essentially the same for both samples, but unfortunately no records of load and temperature of the protein concentrate during drying are available. A difference in these conditions may well explain these findings since Duckworth & Woodham[2] found a lower gross protein value (GPV) for rye leaves when the maximum temperature of the concentrate was 94° than when it was 78°. The problem of the low TD and its sensitivity to excessive heat in leaf proteins merits further investigation.

Table II

Effect of method of drying leaf protein concentrates on their biological value and true digestibility determined at an 8% level of protein intake

(Mean values for groups of twelve rats)

Sample No.	Species	Method of drying	Biological value*	True digestibility*
7	Wheat	Freeze-dried, pre-frozen	73·7	80·1
		Acetone extraction	76·5 ± 1·41	83·9 ± 1·35
		Oven-dried	68·8	60·8
8	Rye	Freeze-dried: pre-frozen	75·8	76·5
		evaporative freezing	74·8 ± 1·01	76·9 ± 0·83
		Air-dried on starch	76·8	77·8
12	Wheat	Freeze-dried, pre-frozen	80·1 ± 0·98	83·2 ± 0·56
		Oven-dried	74·3	73·9

* Values with their standard errors

Effect of maturity of leaf at harvesting

The effect of the maturity of the leaf at harvesting on the BV and TD of its proteins is shown in Table III. The results show that the BV and, to a lesser extent, TD increased with maturity of the leaf. Davies *et al.*[6] have reported a decrease in BV with maturity, but Cowlishaw *et al.*[1] noted small increases with maturity for the GPV of lucerne and Italian rye grass. The present results for wheat indicate no change in BV during an initial period of growth (samples 9 and 10), but subsequently a marked increase (samples 10–13) followed by a smaller increase (samples 13 and 14). The increase with tares was very small and not statistically significant.

Table III

Effect of maturity of the leaf at harvesting on the biological value and true digestibility of its proteins determined at an 8% level of protein intake

(Mean values for groups of twelve rats)

Sample No.	Species	Maturity of plant at harvesting	Biological value*		True digestibility*	
9	Wheat	Harvested 10.5.61; 12 in. high, green and lush	72·0	±0·85	82·8	±0·71
10	Wheat	Harvested 19.5.61; 12 in. high, lush	70·8		82·7	
11	Wheat	Harvested 12.6.61; ears forming	79·6		84·7	
12	Wheat	Harvested 3.5.62; 10 in. high, lush	80·1	±0·98	83·2	±0·56
13	Wheat	Harvested 21.5.62; ears just forming, getting stalky	88·1		85·4	
14	Wheat	Harvested 18.6.62; in ear, still green but very stalky	91·2		86·3	
15	Tares	Harvested 18.7.62; buds just forming	57·8	±2·48	80·2	±0·97
16	Tares	Harvested 29.8.62; pods formed	60·7		82·6	

* Values with their standard errors

Amino-acid composition and effect of amino-acid supplements

Table IV gives concentrations of amino-acids, determined micro-biologically, in selected samples of leaf protein concentrates studied in the biological tests; values for whole egg proteins and casein are included for comparison.

The general interest was mainly in the ' available ' values, but for purposes of comparison a few estimations were also made of ' total ' amino-acid content, and these are included in Table IV. Most of the test samples were dark-green in colour, and it was necessary in the microbiological tests to assess the growth responses titrimetrically rather than turbidimetrically. This entails some loss in the precision of measurement, particularly in the reproducibility of the results from assay to assay. To minimise these errors all the samples were assayed simultaneously for each of the amino-acids in turn.

The leaf protein samples could not be satisfactorily assayed for available lysine by the dinitrofluorobenzene test[31] and a tentative microbiological procedure was used instead.[32] *Streptococcus faecalis* NCDO 1258 was used as the test micro-organism. Preliminary results for a limited range of protein foods have been broadly similar to those obtained with the chemical method.

Compared with whole egg proteins, the leaf proteins were low in the *S*-amino-acids and generally a little high in tryptophan and a little low in lysine, but no consistent differences were evident for the other acids determined.

Table IV

Amino-acid concentrations in whole egg proteins, casein and selected samples of leaf protein concentrates determined microbiologically

Sample No.	Description of sample	Amino-acid, g./16 g. N*															
		Methionine		Cystine†		Histidine		Tryptophan		Leucine		Isoleucine		Valine		Lysine	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
	Egg	3·5	3·6	—	2·4	2·0	2·4	1·5	1·6	8·5	8·0	4·9	5·7	6·7	6·7	7·2	7·8
	Casein	3·1	—	—	—	2·8	—	1·2	—	9·3	—	5·2	—	6·1	—	—	—
12	Wheat harvested 3.5.62	1·9	2·3	—	1·2	1·8	2·3	2·0	2·2	7·4	8·0	4·5	5·3	6·4	6·5	5·1	6·8
14	Wheat harvested 18.6.62	2·2	2·2	—	1·4	2·0	2·3	2·3	2·2	7·7	7·9	4·7	5·3	6·9	6·8	5·9	7·1
15	Tares	1·6	1·8	—	1·2	2·0	2·6	2·0	2·0	8·3	8·6	4·5	4·4	6·6	6·6	5·6	7·2
17	Sweet clover	1·7	1·8	—	1·0	1·8	2·6	2·0	2·1	8·0	9·0	4·1	5·3	6·9	6·8	5·4	7·3
21	Pea	1·8	—	—	1·1	1·2	—	1·4	—	10·7	—	4·3	—	6·0	—	5·3	—
18	Rape: whole	1·8	1·9	—	1·6	1·2	2·3	1·2	—	8·1	—	3·4	—	5·1	—	4·6	—
	' cytoplasmic '	2·2	1·8	—	1·7	2·4	2·8	1·9	—	10·7	—	4·0	—	6·0	—	—	—
	' chloroplastic '	1·8	1·5	—	1·4	1·0	2·0	1·0	—	7·6	—	3·2	—	4·0	—	—	—
20	Rape: ' cytoplasmic '	2·2	2·0	—	1·8	2·4	3·2	1·9	—	9·7	—	4·0	—	6·2	—	—	—
	' chloroplastic '	2·1	2·0	—	1·5	1·2	2·6	1·3	—	8·3	—	3·7	—	5·0	—	—	—
19	Lupin: whole	1·9	1·9	—	1·2	1·6	2·3	1·7	—	9·3	10·2	4·6	—	6·2	—	6·4	—
	' cytoplasmic '	2·0	1·9	—	1·2	2·4	2·4	1·9	—	9·5	10·1	4·9	—	6·4	—	—	—
	' chloroplastic '	1·6	1·8	—	0·8	1·2	2·3	1·2	—	6·9	10·7	4·0	—	4·7	—	—	—
25	Lupin: whole	1·7	1·7	—	—	1·7	2·3	1·8	—	10·8	10·7	4·6	5·8	6·4	7·6	6·5	—
	' cytoplasmic '	1·9	1·9	—	—	2·5	2·6	2·3	—	11·3	10·9	4·5	5·5	6·9	7·7	—	—
	' chloroplastic '	1·6	1·5	—	—	1·4	2·0	1·2	—	8·5	10·5	4·2	5·9	5·6	7·5	—	—

* (a) = available, (b) = total † Determined chemically by the method of Moore[39]

There were indications in the results of microbiological assay that the content of available methionine was marginally higher in the proteins of the mature (14) than in those of the less mature (12) wheat leaf. The difference was small but was found consistently in several assays, and it seemed possible that it might explain the relatively higher BV of sample 14 (Table III). Table V shows that when the apparent deficiency was made good by the addition of 0·3 g. of DL-methionine/16 g.N to sample 12 its BV was increased to that of sample 14.

Table V

Effect of amino-acid supplements on the biological value and true digestibility of leaf protein concentrates

(Mean values for groups of twelve rats)

Sample No.	Protein source	Amino-acid supplement, g./16 g. N	Biological value*	True digestibility*
12	Wheat	None	75·0	85·2
		0·3 g. DL-methionine	81·5 ± 0·72	85·4 ± 0·62
14	Wheat	None	81·9	86·2
15	Tares	None	53·7	79·1
		1·9 g. DL-methionine	94·5 ± 2·16	83·1 ± 1·18
		1·9 g. DL-methionine + 0·6 g. L-lysine	92·5	82·2
	Whole egg	None	93·8	98·0
17	Sweet clover	None	28·9	77·3
		1·8 g. DL-methionine	78·3 ± 4·38	84·4 ± 0·78
		1·8 g. DL-methionine + 0·8 g. L-isoleucine	78·0	85·2
	Whole egg	None	93·0	98·3

* Values with their standard errors

Of the early samples studied tares 6 had the low BV of 58·3; subsequently a much lower value, 38·1, was found for sweet clover 17. Amino-acid analysis (Table IV) revealed no obvious difference between these two species in the availability of the amino-acids measured. Compared with whole egg proteins both were markedly, and equally, deficient in methionine and cystine. Table V shows that when a fresh sample of tares (15) was supplemented with sufficient methionine to bring the concentration of this acid to that in whole egg proteins the BV of tares was increased to that of egg. When this test was done, only total lysine had been determined microbiologically, and this assay suggested that lysine might be the second limiting amino-acid in tares; the biological test, however, showed that this was not so. The microbiological assays (Table IV) showed that the content of total lysine was essentially the same in tares and sweet clover. Subsequently it was found that available lysine, although lower than total lysine, was the same in the two samples. However, available isoleucine was some 9 and 16% lower in sweet clover than in tares and egg, respectively, and it seemed possible that a slight deficiency of this acid might contribute to the very low BV of sweet clover. Table V shows that when the methionine deficiency in sweet clover was made good, its BV was markedly increased but was still inferior to that of egg; a further supplement of isoleucine was ineffective. It is clear that a low concentration of some acid other than methionine contributed to the low BV of sweet clover and that it is probably threonine or phenylalanine and tyrosine for which satisfactory microbiological methods are not yet available.

It may be noticed in Table V that the BV's of the two wheats (12 and 14), tares (15) and sweet clover (17) were lower than the initial values for these samples given in Tables I and III. This suggests that there may have been some loss in nutritive value of these proteins between tests; during this period (9–23 months) the samples had been stored in an air-tight tin at 0–4°.

Comparison of ' chloroplastic ' and ' cytoplasmic ' fractions

Table VI gives the results of the biological experiments with ' cytoplasmic ' and ' chloroplastic ' fractions of rape and lupin leaf proteins. In agreement with the findings of Davis *et al.*[6] the BV and TD of the ' cytoplasmic ' fractions were higher than those of the ' chloroplastic ' fractions for both species. The BV of the ' cytoplasmic ' fractions of both rapes

Table VI

Biological value and true digestibility of rape and lupin leaf proteins: values for total proteins and for their 'cytoplasmic' and 'chloroplastic' fractions

(Mean values for groups of twelve rats)

Sample No.		Protein source	Biological value*	True digestibility*
18	Rape	whole	77·9 ± 1·19	84·8 ± 0·55
		'cytoplasmic' fraction	87·0	95·9
		'chloroplastic' fraction	71·3	66·7
	Casein		85·5	99·4
20	Rape	'cytoplasmic' fraction	91·0 ± 1·82	93·9 ± 0·75
		'chloroplastic' fraction	79·8	74·9
	Casein		89·0	97·9
19	Lupin	whole	83·2 ± 1·61	75·9 ± 1·40
		'cytoplasmic' fraction	64·0	86·0
		'chloroplastic' fraction	53·0	61·4
	Casein		85·2	99·0
25	Lupin	whole	78·5 ± 2·00	82·2 ± 1·58
		'cytoplasmic' fraction	86·8	77·4
		'chloroplastic' fraction	72·6	53·7
		1 pt. 'cytoplasmic' + 2 pts. 'chloroplastic'	82·5	61·4

*Values with their standard errors

did not differ significantly from that of casein, and their TD was only slightly inferior to that of casein. The BV of the whole protein of rape 18 was lower than that of the 'cytoplasmic' fraction and was approximately the value that could be expected from the relative proportions of the two fractions in it. The results of the experiment with lupin 19 did not confirm those with rape 18 in that the BV of the whole protein did not differ significantly from that of casein and the BV's of both fractions were significantly lower. This experiment was repeated with lupin 25. In addition to testing the whole protein and its fractions, the latter were recombined in approximately the same proportions in which they occurred originally, as it seemed possible that lupin proteins might be susceptible to some damage during preparation of the fractions. The results of this experiment were similar to those with rape 18 and the BV of the combined fractions did not differ significantly from that of the whole protein. The results for the TD of the two lupin samples were inconclusive.

Amino-acid analyses (Table IV) show that the availability of those acids measured was higher in the 'cytoplasmic' fractions than in the 'chloroplastic' fractions and, to a lesser extent, in the whole protein, the differences being particularly marked for histidine and tryptophan. The somewhat anomalous results for the BV's of lupin 19 and its fractions are not explained by the amino-acid results from which one would have expected values of the same order as for rape 18 and lupin 25. The BV's of the 'cytoplasmic' fractions of both samples of rape and one sample of lupin (25) were similar to those found for casein although their available methionine content was some 30% lower. It can be calculated that the ratio between methionine and histidine was the same in these fractions as in casein, and this favourable balance may well explain the biological results.

General conclusions

Although these experiments are incomplete in many respects, it is clear that drying methods that are satisfactory for other protein concentrates can be applied to those obtained from leaves. The observation that the nutritive value of leaf proteins increases with maturity is of some practical significance. There is an optimum harvest time since, as the yield per cut and the BV of the extracted proteins increase, the quantity of protein that can be extracted decreases. Consequently this time is probably a little later than that when optimum yields, per year and acre, are obtained because of the better quality of the more mature protein. In confirmation of *in vitro*[33] and microbiological[13] findings, the digestibility of these protein concentrates was

almost always lower than is normal for preparations of animal or of legume and cereal seed proteins. There were some marked differences between species for BV, but on the whole these values were of the order observed for good quality vegetable proteins. Some of the smaller differences between species may arise because all species were not tested at a comparable stage of maturity; such information was not available for samples used in some of the earlier tests as the significance of this factor had not then been recognised.

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STUDIES ON THE NUTRITIONAL VALUE OF FOODS TREATED WITH γ-RADIATION

II.*—Effects on the Protein in some Animal Feeds, Egg and Wheat

By T. S. KENNEDY

The effect of γ-radiation on the protein nutritive value of certain animal feeds (protein concentrates), frozen whole egg, whole wheat and wheat gluten was measured by use of a microbiological method. There was little change in nutritive value of the animal feeds with doses of 0·5 and 1·0 Mrad and no change with frozen egg at 0·5 and 5·0 Mrad. Whole wheat showed no loss at 0·2 Mrad; a 6% loss at 1·0 Mrad was not increased further at 5·0 Mrad. Gluten prepared from wheat and then irradiated was unchanged at 0·02 Mrad but losses of 5, 7 and 26% were shown with doses of 0·2, 1·0 and 5·0 Mrad respectively.

Following amino-acid supplementation studies and microbiological assays for methionine, loss of availability of this amino-acid was demonstrated to be principally responsible for the lowering of the nutritive value of the 5·0-Mrad-irradiated wheat gluten.

Introduction

Radiation processes have been proposed for the disinfestation of grain[1] and for the elimination of Salmonellae from frozen whole egg and certain animal feeds[2, 3] used as protein concentrates. The effect of radiation on the protein quality of these products is described in this paper; a similar investigation on some B-complex vitamins in egg and wheat has previously been reported in Part I.[4] The doses chosen are those recommended in each specific application and higher doses are included to exaggerate any possible effect. A detailed study on wheat gluten is also reported in relation to some observed changes in its amino acid composition following radiation treatment.

A microbiological method is used to measure protein relative nutritive value (R.N.V.); results are expressed relative to casein which is rated at 100. This method of estimating the nutritive value of protein has been shown to correlate well with other methods based on rat feeding studies.[5]

Experimental

Animal feeds

Two fish, one meat and one meat-and-bone meal were obtained commercially. The fish meals were of different nutritive value, one being Viobin, a vacuum-dried, low-temperature, fat-extracted meal, and the other Provimi 66, a standard white fish meal air-dried at 100°. Samples were milled to 200-mesh size to facilitate pipetting of the suspensions at a later stage. After thorough mixing the moisture contents were estimated by a Karl Fischer method;[6] water was extracted from the samples by refluxing in methanol at 90° for 15 min.[7] Meals were irradiated at 0·5 and at 1·0 Mrad in a cobalt-60 ' Hotspot ' source at a dose-rate of approximately 0·2 Mrad/h. and temperature of 18–20°. Further samples of meals were prepared at a moisture content of approximately 14%—the highest that might be expected in such meals—and irradiated as before.

Frozen whole egg

Thoroughly homogenised fresh egg was canned and then frozen to −15° prior to γ-irradiation at doses of 0·5 and 5·0 Mrad using the Spent Fuel Rod Assembly at A.E.R.E., Harwell; the dose-rate was approximately 1·0 Mrad/h. The egg was maintained at −15° during transport, irradiation and subsequent storage prior to analysis. The egg used as control was unirradiated but otherwise treated identically.

* Part I: *J. Sci. Fd Agric.*, 1965, **15**, 81.

Wheat

It was originally intended to estimate the R.N.V. of wheat protein by direct measurement on wholemeal flour prepared from irradiated wheat. However, preliminary results showed a steep rise in R.N.V. after irradiation. This was found to be due to an increase in turbidity in the assay tubes caused by radiation-induced breakdown of the starch component. This could be observed in the absence of the assay organism and could be corrected by enzymic digestion of the starch with takadiastase. The fragmentation of the starch molecule by irradiation has previously been observed[8] and this might well lead to a lowering of gelatinisation viscosity causing particles to remain in suspension for long periods in the assay tubes. A method of estimating growth based on the reduction of 2,3,5-triphenyltetrazolium chloride[9] was tried in order to overcome this difficulty, but was found to be unsatisfactory owing to high blanks, probably caused by the presence of reducing sugars formed during a necessary autoclaving procedure. Removal of the starch from untreated wheat before assay did not alter the R.N.V. of the gluten and it was decided, therefore, to measure (*a*) the R.N.V. of a starch-free preparation from irradiated wheat and (*b*), for comparative purposes, to measure the effect of irradiation on crude gluten itself.

(*a*) Whole wheat (Manitoba No. 2) was irradiated at room temperature at a moisture content of 11% in a 10-kilocurie cobalt-60 source at dose levels of 0·2, 1·0 and 5·0 Mrad with a dose-rate of 0·03 Mrad/h. A starch-free preparation was made in a similar manner to that described by Kent-Jones[10] for the preparation of crude gluten, but, during the washing process the dough was enclosed in a silk bag to contain the husk and bran. It was observed that the time taken to obtain clear washings increased with the radiation dose from 15 min. for untreated wheat to 37 min. for the 5·0-Mrad treatment. The preparation was dried under vacuum at 46° for 16 h. and milled to pass through a 100-mesh sieve. It was stored in airtight containers at 2° until required.

(*b*) Crude gluten prepared from wheat flour was irradiated in a similar manner at levels of 0·02, 0·2, 1·0 and 5·0 Mrad. The method for the gluten preparation was similar to that described above, the dough also being enclosed in a silk bag since the presence of husk and bran interfered with the formation of large particles of gluten, making recovery difficult. Husk and bran were removed through a coarse sieve under a stream of water prior to drying, milling and storing as for the starch-free preparation.

Measurement of protein R.N.V.

This was carried out by the method of Ford[5] using the proteolytic organism *Streptococcus zymogenes* NCDO 592. Samples were assayed in a multiple assay in which the reference standard and test extracts were set up in one rack per replicate, thus giving a direct comparison between treatments. From each sample, preparations were made in triplicate and each preparation was assayed at three levels, each level being set up in duplicate. Two or more multiple assays were prepared on different days for each product. Basal medium, water, egg and gluten preparations were dispensed by means of an automatic filler, suspensions being agitated with a magnetic stirrer. The automatic filler was not suitable for the animal feeds and these were dispensed with volumetric pipettes. The setting-up of the tubes in racks and the measurements of growth were made in random fashion. Growth was estimated turbidimetrically with a Hilger model H 810 absorptiometer fitted with a 610 mμ filter. Preliminary assays on frozen egg were consistently invalid owing to non-parallelism of the response curves. This was corrected by doubling the level of the purines adenine, guanine and uracil in the basal medium. The requirement to increase the level of these bases occurred only with the egg and was not further investigated in this present study.

Amino-acid supplementation of crude gluten

Multiple assays were prepared as for the measurement of protein R.N.V. with the extracts supplemented by one or more of the ' essential ' amino-acids. These were prepared as aqueous solutions and added as a percentage of the weight of protein (N × 5·7).

Amino-acid analysis

Gluten was analysed for total and ' available ' methionine by the microbiological method of Ford [11] employing multiple assays and growth measurement as for protein R.N.V. The total amino-acid composition was measured by ion-exchange chromatography[12] using a Technicon autoanalyser.

Total nitrogen

This was estimated by a macro Kjeldahl technique. Before performing microbiological assays it was established that no loss in total nitrogen occurred in any of the products following radiation treatment.

Statistical analysis

Tests for significance between treatments were by ' Student ' t-test, 95% confidence limits being calculated for the mean using the t-distribution and estimates of standard error. Some of the tests for significance in relation to the amino-acid supplementation studies on wheat gluten were made by Analysis of Variance.

Results and discussion

The results for the animal feeds are shown in Table I. Losses in R.N.V. occurred only with the fish meals when irradiated at 1·0 Mrad at the lower moisture content; these losses were very

Table I

Relative nutritive value of some animal feeding-stuffs after irradiation at different moisture contents

(combined results of two or more multiple assays with 95% confidence limits in parentheses)

	% Moisture	Untreated (control)	Irradiated 0·5 Mrad	% of control	Irradiated 1·0 Mrad	% of control
Fishmeal (Viobin)	9·8	81·2 (79·4–82·9)	78·9 (76·7–81·2)	97·2 (93·8–100·6)	78·8 (77·1–80·6)	97·1 (94·0–99·9)
	13·5	74·1 (73·0–75·2)	74·0 (73·2–74–9)	99·9 (97·8–101·7)	74·2 (73·3–75·2)	100·1 (98·3–102·0)
Fishmeal (Provimi 66)	9·1	56·5 (55·1–57·9)	54·6 (52·7–56·5)	96·9 (92·6–100·6)	54·0 (52·4–55·7)	95·6 (91·9–99·4)
	13·9	55·9 (54·4–57·4)	56·1 (54·5–57·7)	100·3 (96·6–104·1)	57·3 (55·5–59·1)	102·5 (98·6–106·3)
Meat and bonemeal	8·1	36·8 (35·4–38·2)	37·9 (36·8–39·0)	103·0 (98·2–107·7)	36·8 (35·6–38·0)	100·0 (92·7–107·2)
	14·9	35·3 (34·5–36·1)	34·5 (33·8–35·2)	97·6 (95·5–99·7)	34·8 (34·2–35·4)	98·6 (95·9–101·2)
Meat meal	7·0	24·8 (23·4–26·2)	24·0 (22·8–25·2)	96·8 (89·5–104·0)	24·1 (22·6–25·6)	97·1 (89·1–105·2)
	14·2	27·3 (25·9–28·7)	26·9 (25·6–28·2)	98·5 (91·7–105·3)	26·4 (25·1–27·7)	96·7 (89·9–103·5)

small and barely significant at the 5% level. The results confirm some preliminary work[13] reported on irradiated animal feeds in which available lysine was used as a measure of nutritive value; no loss was observed up to a dose of 5·0 Mrad. Other work[14] on fish and blood meals also showed no loss in available lysine following doses up to 1·0 Mrad, the highest tested. It is apparent that a radiation process designed for the purpose of elimination of Salmonellae from animal feeds will be acceptable with respect to retention of protein nutritive value.

Table II gives the results for frozen whole egg, wheat gluten and whole wheat. No losses in R.N.V. occurred with frozen whole egg ($P > 0{\cdot}05$). Whole wheat showed no change at 0·2 Mrad ($P > 0{\cdot}2$) and the loss of 6% at 1·0 Mrad ($P < 0{\cdot}01$) showed little further increase at 5·0 Mrad ($P < 0{\cdot}01$). In contrast, wheat gluten at doses of 0·2, 1·0 and 5·0 Mrad gave increasing losses ($P < 0{\cdot}01$) in R.N.V. of 5, 7 and 26% respectively; it is considered that certain

Table II

Relative nutritive value of irradiated frozen whole egg, wheat gluten and whole wheat
(combined results of three or more multiple assays with 95% confidence limits in parentheses)

Treatment	Frozen whole egg		Wheat gluten		Whole wheat	
	R.N.V.	% of control	R.N.V.	% of control	R.N.V.	% of control
Untreated (control)	93·1 (91·9–94·3)		54·6 (53·2–56·0)		51·0 (50·2–51·8)	
0·2 Mrad			52·1 (51·3–52·9)	95·4 (92·5–98·4)	51·5 (50·7–52·3)	101·0 (98·6–103·3)
0·5 Mrad	92·3 (91·1–93·4)	99·1 (97·4–100·8)				
1·0 Mrad			50·6 (49·1–52·1)	92·7 (88·8–96·5)	47·8 (46·9–48·7)	93·7 (91·3–96·2)
5·0 Mrad	91·7 (90·6–92·8)	98·5 (96·8–100·2)	40·6 (38·3–42·8)	74·3 (71·4–77·3)	47·2 (46·0–48·4)	92·5 (89·6–99·5)

constituents in whole wheat, probably starch, must exert some protective effect. In a separate experiment a dose of 0·02 Mrad caused no significant change to wheat gluten ($P > 0·5$). Measurement of the biological value of wheat gluten gave no indication of change even at 2·8 Mrad.[15]

The amino-acid composition of the untreated and irradiated (5·0 Mrad) wheat gluten measured by the ion-exchange chromatography technique on the acid hydrolysed protein, indicated small losses (<10%) of leucine, isoleucine and methionine. These losses are unlikely to account for the 26% loss in the R.N.V. of the treated protein. Irradiation may cause binding or loss of availability of certain essential amino-acids which would not be revealed by the chemical analysis. This point was elucidated by amino-acid supplementation studies of the irradiated intact protein.

Table III shows that the R.N.V. of the untreated wheat gluten was raised by supplementation with methionine ($P < 0·001$) and further improved by the addition of lysine ($P < 0·001$)

Table III

Effect of amino-acid supplementation on the relative nutritive value of untreated and 5·0-Mrad-irradiated wheat gluten
(95% confidence limits in parentheses)

Supplement*	Relative nutritive value		Irradiated as % of control
	Untreated (control)	Irradiated	
None	55 (53–57)	41 (39–43)	74 (71–77)
0·25% L-lysine	—	35 (32–38)	—
2% L-lysine	—	39 (37–41)	—
8% L-lysine	—	43 (42–44)	—
0·25% L-methionine	60 (59–61)	51 (49–53)	85 (82–88)
0·5% L-methionine	65 (64–66)	54 (52–56)	83 (79–87)
1% L-methionine	68 (66–70)	57 (55–59)	84 (80–88)
4% L-methionine	79 (77–81)	74 (71–77)	94 (90–97)
6% L-methionine	80 (78–82)	74 (71–77)	92 (88–97)
4% L-methionine + 6% L-lysine	95 (94–96)	86 (84–88)	90 (88–93)
4% L-methionine + 6% L-lysine + 1% of 8 amino-acids**	101 (99–103)	94 (92–96)	93 (91–95)
4% L-methionine + 2% L-cystine	89 (87–91)	86 (85–87)	97 (94–99)

* Amounts added calculated as % of weight of protein (N × 5·7)
** L-valine, L-leucine, L-isoleucine, L-tryptophan, L-threonine, L-histidine, L-phenylalanine, L-arginine

thus confirming the previous work by Ford.[5] The addition of methionine alone improved the R.N.V. of the irradiated gluten with respect to its correspondingly supplemented control ($P < 0.001$) indicating a loss of this amino-acid following the radiation treatment. In the presence of methionine no further improvement in the R.N.V. of the treated sample relative to the supplemented control was shown by the addition of cystine or lysine and eight more amino-acids ($P > 0.05$): such supplementation raises the R.N.V. of the test samples to an equal extent.

It is apparent from supplementation studies that the methionine in wheat gluten is the principal amino-acid affected by radiation treatment. Microbiological assays for this amino acid performed on both intact and acid-hydrolysed protein confirm that the effect of irradiation is mainly to limit availability of methionine rather than cause its chemical destruction; the results are given in Table IV.

Table IV

Methionine content of untreated and irradiated wheat gluten estimated by microbiological assay

(combined results of three multiple assays expressed as g./16 g. N; 95% confidence limits in parentheses)

	Untreated (control)	Irradiated 5·0 Mrad	% Loss†
Total*	1·7 (1·1–2·2)	1·6 (1·2–2·0)	6 (2–10)
Available**	1·5 (0·9–2·1)	1·2 (0·8–1·6)	22 (17–26)

* after acid hydrolysis ** on intact protein † significant $P < 0.01$

It can be generally concluded that the nutritive value of the protein in the range of products examined would not be affected at the doses proposed for the specific radiation applications envisaged. The biological value of the protein in other foods such as turkey, maize,[15,16] lima beans, peas, milk and beef[17] has also shown to be unchanged by radiation treatment.

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THE MINERAL COMPOSITION OF APPLES
III.*—The Composition of Seeds and Stems

By B. G. WILKINSON and M. A. PERRING

The mineral composition of apple fruit seeds and stems has been determined to supplement similar information previously obtained for the flesh of apples. The stems are similar in composition to apple tree shoots and are especially rich in calcium. The seeds are especially rich in nitrogen and magnesium. Inclusion of stems and/or seeds in homogenised samples of apple fruit could result in considerable errors in estimations of some elements, particularly calcium, nitrogen and magnesium. Potassium and sodium figures would be little affected.

Introduction

When the chemistry of apples is being considered, it is usually assumed that reference is being made to the composition of the swollen receptacle—the pulp and peel, and other fleshy tissue which makes up the acid, edible fruit body. The detached apple also includes stalk and seeds, which are usually discarded during consumption. They make up only a small proportion of the bulk of the fruit but may differ considerably in composition from the rest of the apple. Hulme[1] has shown that the concentration of nitrogen in the seeds is very much higher than that in the pulp and peel, and Lvov & Kalugina[2] find that the concentration of phosphorus in seeds is high during fruit development and storage. Hence the inclusion or exclusion of stems and seeds when analysing fruit could lead to serious errors. Also if the composition of the seeds were to be influenced by, for instance, orchard treatment to a different extent to the pulp, then misleading results might be obtained.

Therefore, for mineral analyses concerned with, for example, the effect of fertilisers on chemical composition, seeds and stems have always been removed from samples of fruit before freezing and grating,[3] which was the procedure initiated by Hulme.[4]

A further reason for excluding seeds and stems is that with the method of grating frozen tissue at −20° it may not be possible to obtain a homogeneous grated sample owing to the hard casing on the seeds and the fibrous nature of the stems. It is clear that a large portion of seed in a small sub-sample might lead to large errors in the determination of nitrogen and phosphorus.

The following analyses of stems and seeds were made to assess the errors which might occur in determining various minerals. Figures for the composition of seeds and stems also add to the picture of the distribution of mineral elements in apples which has been presented in two previous papers,[5, 6] and which were concerned with the fleshy portion of the fruit.

Experimental

Materials and methods

Seeds and stems were removed from two samples of 100 apples each when they were prepared for freezing and grating in 1959. The apples were Cox's Orange Pippin from a manurial trial at the East Malling Research Station. It has previously been shown that these samples (from treatments NPK and PKS) differed in chemical composition.[7]

The samples of seeds (30 g.) and stems (15 g.) were weighed and kept at −20° until 1964 when the whole of each sample was ashed with nitric acid.[3] Ashing of the seeds had to be carefully controlled because of the high fat content, and required a large total volume of nitric acid. A few drops of perchloric acid were added to each sample to complete the ashing.

Determinations of potassium, sodium, calcium, magnesium and phosphorus were made by methods described by Perring.[8] Sulphur (as sulphate) was determined turbidimetrically.[9]

* Part II: *J. Sci. Fd Agric.*, 1964, **15**, 752

Other elements were determined colorimetrically: iron with *o*-phenanthroline;[10] zinc with dithizone;[11] manganese by Bradfield's method;[12] and copper by Bradfield's modification[13] of the method of Somers & Garraway.[14]

In 1964 the seeds and stems were removed from two more samples of 10 apples each (known as D3/9 A and B) from another East Malling orchard, and were directly digested and analysed for nitrogen by the method of Williams.[15] Determinations of phosphorus were also made on the digests of the seed samples for comparison with the previous results.

Results

Concentrations of mineral elements in the NPK and PKS samples are shown in Table I, which also includes analytical results for the remainder of the fruit. For comparative purposes

Table I

Comparative results for samples of seeds and stems, and the remainder of the apple tissue

Samples of 100 apples. Mineral elements expressed in mg. per 100 g. fresh weight

		K	P	Ca	Mg	S	Na	Fe	Mn	Cu	Zn	Total mass of original sample, g.
1959												
Seeds	NPK	334	344	134	215	168	1·0	3·9	4·0	0·65	1·2	33
	PKS	360	363	147	252	190	1·3	4·3	2·4	0·88	1·2	32
Stems	NPK	480	64	320	18	29	20	1·7	1·6	0·24	0·46	14
	PKS	330	81	397	20	29	20	1·5	0·8	0·29	0·46	13
Flesh	NPK	193	11·3	5·1	7·2	—	2·7					12,249
	PKS	168	15·4	5·8	4·9	—	2·7					9463
1956												
Flesh	NPK	184	12·9	5·9	5·4	5·9	3·6	0·42	0·15	0·035	0·058	9912
	PKS	154	15·6	6·5	6·3	5·6	4·1	0·43	0·05	0·047	0·045	9836

results obtained in 1956, when determinations of trace elements were made on fruit from the same plots, are also included. There would of course be differences in the results of 1956 and 1959 because of seasonal factors, but the figures serve to indicate an order of magnitude. The 1956 figures for iron are almost certainly high because the fruit was grated in a machine with a steel grating disc. The 1959 seeds and stems were ashed directly as previously stated.

Table II shows the nitrogen concentrations in the seeds and stems of the D3/9 samples, and the phosphorus concentration in the seeds.

Table II

Nitrogen and phosphorus concentrations in seeds and stems from two samples of ten apples, and comparable figures for the flesh of 25 apples

(Expressed in mg. per 100 g. fresh weight)

		N	P	Total mass of original sample, g.
Seeds	D3/9 A	3,140	358	2·33
	B	2,200	366	3·72
Stems	D3/9 A	622	—	1·12
	B	460	—	1·36
Flesh	D3/9	75	—	

Discussion

The stems, seeds and flesh of apples, being of varied anatomical structure and function, are naturally different in mineral composition. The flesh consists of comparatively large cells

with only a thin layer of cytoplasm, and most of the volume of the cell is accounted for by the fruit juice, which consists mainly of organic acids and potassium giving an acid sap of pH about 3·2.

The stems on the other hand are mainly conducting tissue, and as such might be expected to show some similarity in composition with the branch system of the tree. Mason & Whitfield[16] have given an account of the distribution of mineral elements in apple trees throughout the season, and it is possible to estimate from their diagrams the approximate concentrations in September of elements in current extension growth, and in the previous year's extension growth. To obtain comparable figures it has been assumed (after Vaidya[17] that the bark and wood are in approximately equal amounts, and that shoots contain about 50% dry matter. The comparable figures for the major elements (in mg. per 100 g. fresh weight) are then as shown in Table III.

Table III

Comparison between observed composition of fruit stems and estimated composition of shoots derived from figures published elsewhere[16]

	N	P	K	Ca	Mg
Current season's shoots	400	65	250	415	35
Last year's shoots	325	45	240	590	85
Fruit stems (Mean from Tables I and II)	541	72	405	359	19

The concentrations of minerals in the fruit stems are obviously of the same magnitude as those in the shoots. For most elements the concentrations are 3–10 times higher than those in the flesh, with the notable exceptions of potassium which is only twice as high, and calcium which is 60 times as great. The small amount of calcium in the flesh of fruit compared with that in the rest of the tree (it is high also in the leaves) is of interest, because calcium in the fruit appears to be at a near-critical level as regards certain physiological disorders of stored apples.

Some average results of analyses of apple seeds of unspecified varieties have previously been given by Ramage[18] who separated the seeds into three parts, the brown outer case, the thin translucent coat of the endosperm, and the endosperm. For the endosperm (which forms the greater part of the seed) concentrations of Mg, Ca, Mn and Fe were of the same order as those shown in Table I, but K was very high giving a K/Mg/Ca ratio of about 10 : 2 : 1 compared with about 2·5 : 2 : 1 in the present results.

The amount of nitrogen in the seed is very high (probably reflecting protein content) and accounts for 2–3% of the total weight. Hulme[1] has given figures for the nitrogen content of seeds of Bramley's Seedling apples which were picked early in the season. The initial nitrogen concentrations were 490 mg./100 g. fresh weight for apples picked in June, and 910 mg./100 g. fresh weight for apples picked in early August. This compares with 2000–3000 mg./100 g. fresh weight in Cox picked in late September. This suggests a big accumulation of nitrogen in the seeds during development.

Further features of interest in the seeds are the high concentration of magnesium and the low concentration of sodium compared with those in other tissues.

From the point of view of fruit analysis, it is possible to calculate the errors incurred by including seeds and/or stems in fruit samples for grating, assuming that a homogeneous sample can be obtained. Seeds and stems amount to about 0·3 and 0·1% of the total mass of the apple. Two extreme examples can be given. If either seeds or stems were included in a sample, calcium results would be about 6% high, and so if both were included the error would be 12%. Nitrogen concentrations would be 11% and 12% high if seeds, or seeds and stems were included. The errors would vary from zero to figures of this order for different elements, and so the ratios of one element to another could be affected. Although orchard treatment appears to affect the composition of seeds and stems, errors arising from these differences are negligible. If, as seems likely, a homogeneous sample could not be obtained, errors could be extremely high when taking small sub-samples. It would however be possible to determine potassium and sodium in samples containing seeds and/or stems without incurring serious errors.

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AGENTS INDUCING THE DEATH OF CACAO SEEDS DURING FERMENTATION

By V. C. QUESNEL

The relative importance of the three agents heat, ethanol and acetic acid, responsible for the death of cacao seeds during fermentation has been investigated. Two criteria of death have been used, loss of viability and the diffusion of pigment throughout the cotyledon tissue.

During the period over which the seeds lost viability, the ethanol concentration of the pulp was relatively high (≃0·60 M) and the acetic acid concentration relatively low (~0·07 M). Twenty-four hours later, when the diffusion of pigment throughout the tissue was complete, the concentrations were 0·44 M and 0·17 M respectively.

A study was made of the effect of temperature and various concentrations of ethanol and acetic acid on viability and diffusion of pigment in the cotyledon tissue of washed seeds. It is concluded that, in fermenting cacao, temperature is relatively unimportant in causing loss of viability and that, despite the difference in concentration, acetic acid may be rather more important than ethanol. Ethanol and acetic acid act synergistically in causing diffusion of pigment; again, temperature is relatively unimportant.

Introduction

The death of the seed in the fermentation stage of cacao curing is a necessary preliminary to the important biochemical reactions that occur within the cotyledons. It has been attributed by earlier workers to heat,[1,2] alcohol[3] and acetic acid,[4] each of these agents in turn being claimed as the most important. However, none of the earlier workers investigated the interrelation of all three factors and the present work was undertaken for this purpose. The more recent work of Platone[5] was noticed only after this investigation was substantially complete.

Two lines of approach were followed and the results are presented in two parts, the first dealing with the actual conditions of temperature, alcohol concentration and acetic acid concentration to which the seeds are subjected in a typical Trinidad sweat-box and the second dealing with the effects of heat, alcohol and acetic acid *in vitro* on the viability of fresh cacao seeds and cotyledon tissue.

The polyphenols of the cacao seed are contained in special storage cells scattered in small groups throughout the cotyledon. The polyphenols can diffuse through the cotyledon when the surrounding parenchymatous cells have lost their semi-permeability and the extent of diffusion can be used as a measure of the extent of dead tissue. When diffusion has occurred throughout the cotyledon, all the cotyledon tissue is dead and some authors have used this as a criterion of the death of the seed. On the other hand, other authors have used the loss of the ability to germinate as the criterion of death. It is obvious that these criteria are different and both have been used in the experiments reported below.

(1) Alcohol, acetic acid and heat production in a sweat-box

Experimental

General procedure

Samples of fermenting cacao were obtained from a sweat-box at River Estate, Trinidad. Between 8·30 and 9·00 a.m. the temperature of the fermenting mass was measured 9 in. below the surface and a sample removed. It was placed in a tightly stoppered jar for transport to the laboratory where working up began less than 1 h. later.

The peelings (testa plus pulp) of 20 seeds were weighed before and after drying (approximately 24 h. at 105°) for moisture determination. The peelings of 50 seeds were blended in a Waring blendor and extracted with 400 ml. acetone in three approximately equal portions and the pooled extracts made up to 500 ml. with water. This is called the pulp extract.

Volatile acid determination

A 50-ml. portion of the pulp extract was steam-distilled in a Markham still[6] after neutralisation, removal of acetone and re-acidification. Acid in the distillate was determined by titration. Volatile acid is considered to be acetic acid since only traces of any other volatile acid have been found in fermenting cacao.[7]

Volatile alcohol determination

Volatile alcohol in a 50-ml. portion of the pulp extract was oxidised overnight at 4° with a solution of potassium dichromate (2·5 g.) in concentrated sulphuric acid (1·5 ml.) and water (8·5 ml.). Unused chromic acid was removed by allowing it to react at room temperature with a small quantity of added isopropyl alcohol. Volatile acid was then determined as before, a correction being made for volatile acid already present. Volatile alcohol is considered to be ethanol.[4]

Results

The relevant results are given in Table I. Diffusion of polyphenol in the cotyledons was complete at 66 h. but at 42 h., when loss of viability was in progress, diffusion was already

Table I

Conditions in the pulp during the early part of a typical fermentation

Fermentation, h.	Temp., °c	Ethanol concn. %v/v	Ethanol concn. M	Acetic acid concn. %v/v	Acetic acid concn. M	% Germ
0	28·0	0·16	0·027	0·074	0·013	100
18	28·8	0·56	0·096	0·17	0·030	100
42	36·8	3·5	0·60	0·33	0·058	75
66	49·0	2·6	0·44	1·0	0·17	0

apparent in small areas near the periphery of some cotyledons. Thus, although conditions during fermentation change continuously, the conditions at 42 h. came close to representing the mean conditions during the period when the seeds were dying. The graph of the complete set of results,[8] suggests that the value for acetic acid concentration at 42 h. is low because of experimental error and that the true value is closer to 0·4%. This value is therefore used in the experiments described in Part 2.

(2) Effects of heat, ethanol and acetic acid on viability of cacao seeds and cotyledon tissue

Experimental

Seeds of the clone ICS 1 were washed free from pulp in a domestic washing machine[9] and divided into four lots of 50 each—three experimental lots and one control. Each experimental lot was placed in water and heated with continuous stirring to a temperature 1° below the desired final temperature. The seeds were then transferred to covered 1-l. beakers each containing 500 ml. of the test solution at the required temperature and incubated for different periods of time. Seeds for the germination test were peeled, planted out in sand and allowed to germinate. After 10 days the number of germinated seeds was recorded. The control seeds were merely peeled and planted out. Different durations of treatment were tried until the region of 50% germination had been spanned. Seeds for the diffusion test were cut open and the cut surface dipped into a 10% solution of potassium dichromate in 10% acetic acid.[4] After 10–15 min. the seeds were washed and diffusion over the cut surfaces examined. Seeds were considered to be dead when the whole surface showed diffusion.

Two difficulties were encountered, the first was a slight drop of about 1° in the temperature of the test solution when the seeds were introduced. This was accepted as inevitable and preferable to overheating the seeds in the preliminary warming-up. The actual temperature of the test solution was determined three or four times during the experiment and the mean temperature taken.

The second difficulty was the criterion of germination. As might be expected, the radicle tip was often severely affected by the treatments and became blackened and necrotic. Nevertheless, it often extended and produced secondary roots although the tap root never pierced the radicle cap. Thus, the criterion of germination was taken to be either the protrusion of the tap root or the production of secondary roots.

Results

Germination test

In the main series of experiments the test solutions were water, 4% (0·685 M) ethanol, 2% (0·35 M) acetic acid, 0·4% (0·070 M) acetic acid and a mixture of 4% ethanol with 0·4% acetic acid. These concentrations were based on the values recorded in Part 1 and on values reported from Java.[4] Experiments were made at several temperatures for each test solution; for each temperature the germination (%) was plotted against the duration of the treatment so that for each solution a family of curves was obtained. From these curves the duration of treatment required for a 50% reduction in germination at each temperature was determined and the values of these two variables were plotted against one another. The results are shown in Fig. 1.

Of the solutions tested, 2% acetic acid is the most effective for inducing death; 0·4% acetic acid is indistinguishable from 4% ethanol. It is reasonable to conclude that at any temperature below 53° (Fig. 1) acetic acid is more effective than ethanol at the same concentration, but the threshold concentration at which each begins to take effect has not been determined. Above 53° the temperature becomes of paramount importance and the seeds are quickly killed no matter in which solution they are immersed.

The graph for seeds immersed in water (Fig. 1) approaches asymptotically the value 43°. Below this temperature heat can have no direct role in causing death. Somewhere in the region

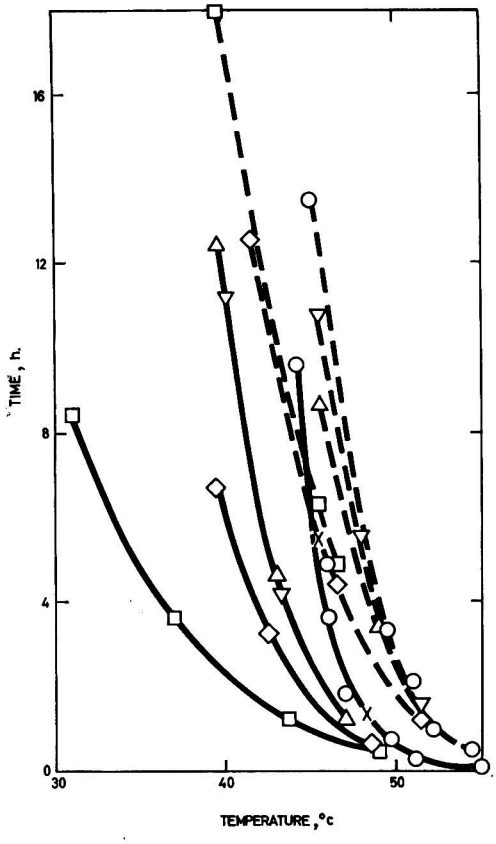

FIG. 1.—*Time–temperature relation for 50% kill of washed cacao seeds immersed in different solutions*

——— germination test ---- diffusion test
○ water □ 2% acetic acid ▽ 0·4% acetic acid
△ 4·0% ethanol ◇ 0·4% acetic acid, 4·0% ethanol
× seeds with pulp

43° to 53° heat becomes the dominant factor and it is of interest to determine at what temperature this occurs. The reciprocal of the duration of treatment which causes 50% reduction in germination may be taken as a measure of the ' rate of death ' under the particular conditions. These reciprocals were calculated from Fig. 1 and plotted against temperature to give the graphs shown in Fig. 2. It will be noted that the graph for water when extrapolated to 43° cuts the ordinate axis at 0·05. This value, which is used as a baseline for the calculations referred to in the next paragraph, was determined from a graph of the logarithms of the reciprocals against temperature which was a straight line.

From Fig. 2 the ' rate of death ' in 4% ethanol at about 46·5° is twice that in water at the same temperature. The mixture of 4% ethanol and 0·4% acetic acid at 46·5° is about as lethal as water at 48·5°. At these temperatures the ' rate of death ' from heat becomes equal to the ' rate of death ' from the other agents. Above these temperatures, heat becomes the dominant lethal agent under the particular set of conditions. It is thus clear that the temperature at which heat becomes dominant is variable and depends upon other conditions such as ethanol and acetic acid concentration.

In Trinidad fermentations, the seeds begin to lose their viability during the second day.

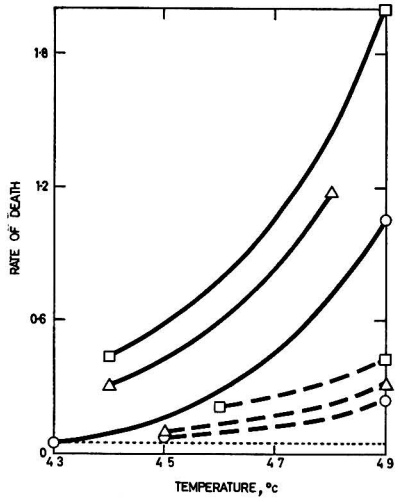

FIG. 2.—*'Rate of death' as a function of temperature for washed cacao seeds immersed in different solutions*

——— germination test - - - - diffusion test
○ water △ 4% ethanol
□ 0·4% acetic acid, 4·0% ethanol

The graph for 0·4% acetic acid (referred to in the text) is omitted from the figure in the interest of clarity; it lies just below the graph for 4·0% ethanol. Likewise, individual points, because derived and not experimentally determined, have been omitted from the graphs.

The conditions within the fermenting mass are, of course, changing continuously but the conditions at 42 h. reported in Part I may be considered average conditions, viz., ethanol concentration 3·5%, acetic acid 0·4%, temperature 37°. Are these conditions sufficient to account for death within the period of time actually observed or are other factors operative in a sweat-box? By extrapolating the appropriate curve in Fig. 1 it will be seen that under conditions close to these (4% ethanol instead of 3·5%) the time required for 50% kill is about 12 h. It may be concluded that no agents other than ethanol, acetic acid and heat need be sought. Several experiments have shown that anaerobiosis may be discounted as a contributory factor.

The effect of the pulp was tested. Seeds with pulp behaved similarly to washed seeds when immersed in water (Fig. 1), but in 2% acetic acid they took longer to die presumably because water in the pulp diluted the acid in the immediate vicinity of the seeds and slowed diffusion.

Formic, acetic and citric acids, all at 0·35 M concentration, were compared by immersing 50 seeds in each solution at 47·5° for 1½ h. The seeds in citric acid gave 56% germination—comparable with those in water; the seeds in formic and acetic acids were all dead. Thus, acetic acid is not unique in its ability to kill cacao seeds but not all acids have this ability. Citric acid, the main natural acid of cacao pulp, is completely ineffective. These results are in agreement with those of Roelofsen & Giesberger.[4] Effectiveness in this test is probably a property of the undissociated molecule and a function of size and polarity.[4,9] The pH of the pulp of fermenting cacao is no guide to its effectiveness in inducing death.

Diffusion test

The same solutions were tested and the results are shown in Fig. 1 for easy comparison with those from the germination test.

It is immediately obvious that, under any set of conditions, death as judged by the germination test occurs before death as judged by the diffusion test. In water at 50° the difference in time is 2 h. and the difference becomes smaller with increase in temperature. In 2% acetic acid at 40° when temperature as such is inoperative, the difference is about 16 h. and below 40° becomes greater. In a sweat-box the difference usually falls between these values.

Other points of interest are that by this test (*a*) 4% ethanol is more effective than 0·4% acetic acid; (*b*) the relative effectiveness of 2% acetic acid and the ethanol–acetic–acid mixture is reversed when compared with the germination test; (*c*) the temperature when heat becomes of overriding importance is about 54°.

Reciprocals of the times required for complete diffusion in the range 44°–49° have been plotted against temperature (Fig. 2) in the manner already described. In 0·4% acetic acid and 4% ethanol, temperature becomes dominant at 44° and 46° respectively (compared with 46·5° in the germination test). In the ethanol–acetic–acid mixture temperature becomes dominant at 49°. Ethanol and acetic acid act synergistically since the effect of both together is greater than the sum of their respective effects (Fig. 2). There is no synergistic action in the germination test.

Discussion

Results of early studies[1,10] of the effect of heat on viability seemed to show that cacao seeds are more sensitive to heat than has now been found. Platone's more recent results[5] indicate a lower sensitivity to heat, very probably because the temperature of the seeds was below the measured temperature. His results for the effect of ethanol and acetic acid on viability again indicate a lower sensitivity than has now been found and again the discrepancy seems to be due to differences in experimental technique. From his studies on temperature,[11] ethanol production[12] and acid concentrations[13] in Venezuelan fermentations, Platone concluded that loss of viability begins as a result of rising ethanol concentration but that the major effect is due to acetic acid.

Roelofsen & Giesberger,[4] using the diffusion test, studied the effects of acids and ethanol at the temperatures obtaining in sweat-boxes during the first 36 h. They found that 0·5% acetic acid did not cause death within 24 h. but that 1% acetic acid did, and that 4% ethanol did not cause death within 36 h. Since the temperature of their sweat-boxes never rose above 39° in the first 36 h. these results seem compatible with those recorded here. Their conclusion that the primary cause of death is neither the hydrogen ion nor the acetate ion but the undissociated acetic acid molecule, is also supported by the present results.

In the sweat-box studied (Part 1) the conditions under which the seeds lost viability were, in general terms, high ethanol concentration (~3·5%), low acetic acid concentration (~0·4%) and low temperature (~37°). Since heat begins to have a direct effect only above 43° and becomes dominant at still higher temperatures, it can have played little or no part in causing death in this sense. At concentrations of 4% and 0·4% respectively, ethanol and acetic acid are equally effective. In the sweat-box, however, over the period when the seeds were dying ethanol was decreasing in concentration and acetic acid increasing so it is reasonable to conclude that acetic acid was more important.

Typically, in Trinidad sweat-boxes the seeds lose viability during the second day before the temperature reaches 43°. Furthermore, polyphenol diffusion in the cotyledon tissue is complete before the temperature reaches 48°, often before the temperature reaches 43°. The conclusion is drawn that, in Trinidad, heat plays no direct role in causing loss of viability and a very minor role, if any, in causing the death of the cotyledon tissue.

Published results for West Africa are available in summary form only,[14] but more detailed observations were kindly supplied by Dr. T. A. Rohan (personal communication). These show that in large heaps (4000 lb.) the seeds die at the centre before the temperature reaches 43° and at the top before the temperature reaches 46°. In small heaps (600 lb.) the seeds are not dead when the temperature reaches 43° but die before the temperature reaches 48°. The statements apply to both criteria of death. In large heaps the temperature rises quickly on the surface but slowly in the centre so that 43° may be reached at the surface in 36 h. but in the centre only in 84 h.[14]

In Java, the seeds die (diffusion test) after 30–35 h. of fermentation but the temperature does not rise to 43° until the 44th hour. Roelofsen & Giesberger[4] considered acetic acid to be the primary cause of death. They recognised the relative unimportance of heat in causing death while overlooking the importance of ethanol.

It may now be concluded that heat as an agent of death is important only when the temperature rises rapidly, i.e., in small heaps and at the surface of larger heaps in West Africa and possibly in the trays of the Allison & Rohan method.[15] Even in these circumstances it does not seem to be the dominant factor in causing death. In sweat-boxes and in the centres

of large heaps where the temperature rises relatively slowly heat is unimportant as an agent of death. Under these circumstances acetic acid is the main agent causing loss of viability whereas acetic acid and ethanol act synergistically in causing diffusion of polyphenols in the cotyledon tissue.

Although a high temperature is thus not necessary for the death of the seed, it is still considered important[16] in fermentation because of its effect on the enzymic reactions necessary for the development of good flavour.

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NUCLEOTIDE DEGRADATION DURING THE EXTENDED STORAGE OF LAMB AND BEEF

D. N. RHODES

Beef and lamb meat from various muscles was irradiated (0·4 Mrad) and the autolysis of nucleotides followed during storage for 70 days at 2°c. The irradiation prevented bacterial spoilage during this period but had no effect on the nucleotide content or on the enzymic activity. Inosine monophosphate was completely hydrolysed to hypoxanthine in 30–40 days in fillet, neck and shoulder meat of beef but more slowly in topside. The rate of autolysis was much lower in lamb leg. The disappearance of inosine monophosphate did not correlate with changes in organoleptic acceptability in these meats.

Introduction

Extensive studies have been made of the degradation of the nucleotides present in the muscle of carcass meats and of fish during normal chill storage. During rigor, adenosine tri- and diphosphates are degraded by the action of tissue ATPase and myokinase to adenosine monophosphate which is rapidly deaminated to inosine monophosphate (IMP); thus the reactions of interest during storage of meats are almost exclusively the subsequent autolysis of IMP. IMP is comparatively stable in post-rigor muscle but is hydrolysed to give inosine, then hypoxanthine and eventually, through the action of bacterial oxidases, xanthine. The disappearance of IMP, therefore, progresses with time and is less rapid at lower temperatures and the measurement of the nucleotide itself or of its degradation products has been suggested as a means of estimating the age of meats[1,2] or fish[3,4] and hence of predicting the remaining storage life. At the same time, since many authors have commented on the meaty flavour and the flavour-intensifying properties of IMP[5,6] its disappearance in the early stages of storage has been correlated, e.g. in fish, with the simultaneous loss of fresh flavour.

In conflict with this hypothesis, studies in this laboratory on the extension of the storage life of fish and carcass meats at temperatures between 0° and 5° by treatment with pasteurising doses of ionising radiation have shown[7] that the meats undergo almost no change in acceptability, as judged by tasting after cooking, over prolonged storage periods and that the storage changes in the flavour of irradiated fish do not correlate with the disappearance of IMP.[7] In the present work more extensive studies of the degradation of the nucleotides in meat have been made.

Experimental

Meat was obtained at a local slaughterhouse at known times after killing, placed in plastic bags and immersed in ice and water. The temperature was maintained at 0° at all times before, during and after irradiation until samples were placed at 2° for storage. In the experiment with neck of beef, the initial sample was cut from the carcass immediately and extracted after 60 min. from the death of the animal.

Samples of meat (100 g.) were sealed under vacuum in pouches of a plastic film of low oxygen permeability (Metathene X, Metal Box Co.) and irradiated with γ-radiation in the Spent Fuel Rod Assembly (Atomic Energy Authority, Harwell) at a dose of 0·4 Mrad. Storage at 2° was started within 24 h. of irradiation.

Methods

The dose of radiation given (0·4 Mrad) has been found sufficient to eliminate those species of bacteria normally responsible for meat spoilage. Storage life of 12 weeks at 2° in anaerobic packages has regularly been achieved without bacterial growth occurring and, when the surviving microorganisms eventually grew, they were yeasts or souring bacteria.[7] Thus, during the 70-day storage periods involved in the present work, no bacterial activity leading to the enzymic oxidation of hypoxanthine to xanthine was anticipated and, since the method used[8] would not distinguish between these two purines, the results are reported as hypoxanthine.

Samples of meats (25 g.) were homogenised for 5 min. with 50 ml. of 0·6N-perchloric acid in a top drive homogeniser (no different results were obtained with 1- or 2-min. treatments). The homogenate was filtered, 50 ml. of the extract brought to pH 6·5–7·0 with 30% potassium hydroxide solution and the volume adjusted to 75 ml. The precipitate of potassium perchlorate was spun down and the supernatant solution decanted. To 0·5 ml. of the neutralised supernatant were added 2 ml. of water, 2 ml. of phosphate buffer (pH 7·6, 0·25 M) and 0·5 ml. of xanthine oxidase solution (a 250-fold aqueous dilution of the L. Light & Co., Colnbrook, preparation). The mixture was incubated for 30 min. at 37° and the uric acid formed measured photometrically at 290 mμ in 1 cm. cuvettes. Calibration was established by putting hypoxanthine solutions (0–100 μg. final amounts) through the whole procedure. Blanks for enzyme and tissue extract were measured and deducted. A highly reproducible linear relationship between extinction and hypoxanthine taken, up to 40 μg., was obtained with a slope of 0·0190 extinction units/μg. in a 1-cm. cell.

The *total nucleotide* content of the meat was estimated by freezing and thawing samples and extracting as described above. The light absorption at 250 mμ was then measured on the extract

before and after treatment with a basic ion-exchange resin (Dowex 2 X 8).[8] The total nucleotide content was calculated from the difference in extinction as IMP which absorbs at this wavelength (milli-molecular extinction coefficient 12·30).[9]

Results

Effect of irradiation on the hypoxanthine content of beef

Two experiments were made in which adjacent 25-g. samples of a beef muscle were irradiated to doses of approximately 1 and 3 Mrads before extraction. The meat used had been held at 2° for 4 weeks and its hypoxanthine content was high. Table I shows that the purine present was unaffected by these doses of gamma radiation.

Table I

Immediate effect of irradiation on the hypoxanthine content of beef muscle

Dose Mrads	Hypoxanthine content, μg/g. wet meat	
	Expt. I	Expt. II
0	609	—
1	604	598
3	611	600

Nucleotide autolysis during storage

Beef.—The hypoxanthine content of neck muscle taken at 60 min. from death was too low to be measurable and the curves for all muscles have, therefore, been started from zero at time of death.

During storage at 2° the hypoxanthine content of beef neck, fillet and shoulder meat increased during the first 20 days and then attained a steady value after 40 days (Fig. 1). Taking the asymptotic value as a measure of the total nucleotide present, the decrease in nucleotide was exponential with time and the slopes were almost identical for the three tissues.

Samples of topside meat from two animals were stored in the same way, but the rate of hydrolysis of the nucleotides was very much slower in both cases (Fig. 2). The content of total nucleotides, however, did not differ greatly from that in the other joints (Table II).

Lamb.—The formation of hypoxanthine was followed in lamb legs obtained from three animals (Fig. 3). Although the total concentration of nucleotides was not greatly different from those in the beef muscles (Table II), the rate of autolysis was very much slower.

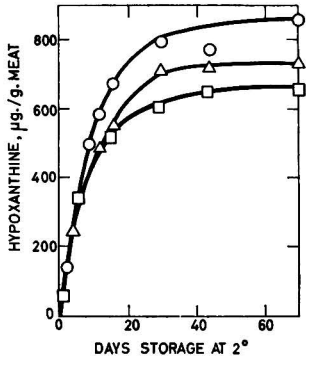

FIG. 1.—*Production of hypoxanthine in irradiated beef* (0·4 *Mrad*) *during storage at* 2° *in the absence of bacterial spoilage*
○ fillet steak □ neck △ shoulder

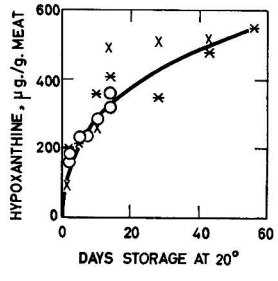

FIG. 2.—*Production of hypoxanthine in topside of beef during storage at* 2° *in the absence of bacterial spoilage*
○ unirradiated * × irradiated

Table II

Total nucleotide content of meats estimated as IMP by light absorption of extracts at 250 mμ or by the maximum level of hypoxanthine formed in autolysis

		Nucleotide content (as hypoxanthine)	
		Direct method	Enzymic method
		(μg/g. wet meat)	
Beef	topside	834	—
	neck	742	660
	shoulder	702	740
Lamb	leg	592, 535	—

Effect of radiation pasteurisation on the autolytic reaction

One joint of topside was cut into 1·5 cm. thick slices and alternate pieces were taken to make duplicate sets of samples. All were packed as before, one set was irradiated to a dose of 0·4 Mrad and both sets were stored at 2°. The untreated samples remained unspoiled by bacterial action (as judged by odour) until the 20th day. During this period the degradation of the nucleotides occurred equally rapidly in both sets of samples (Fig. 2) indicating that the pasteurising dose had no measurable effect on the activity of the enzymes concerned.

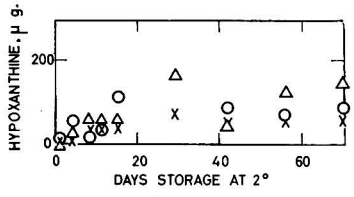

FIG. 3.—*Production of hypoxanthine in irradiated lamb* (0·4 Mrad) *during storage at 2° in the absence of bacterial spoilage.*
(Leg meat from three animals)
○ unirradiated ✱ × irradiated

Determination of total nucleotides

Estimation of total nucleotides, based upon the measurement at 250 mμ and a millimolecular extinction of 12·30 for IMP gave values in fair agreement with those derived from the formation of hypoxanthine during storage (Table II). The direct determination could be high because of the presence of acidic tissue components absorbing in the ultra-violet for which no blank correction could be devised, while the asymptotic level of hypoxanthine could be low because of inhibition of the enzymes (possibly by the action of tissue proteases) during the long storage or destruction of the purine nucleus by other reactions.

Discussion

The present results confirm the findings of Solov'ev[1] and of Lee & Webster[2] that the increase in hypoxanthine resulting from the autolysis of IMP is rapid and linear during the normal storage life of meats at chill temperatures. In this respect, the situation is similar to that in fish.[4] The use of radiation to eliminate interference from bacterial growth at dose levels which had no effect on the autolytic activity in the early stages at least, has permitted the study of the reaction to a stage of completion in beef muscles. This confirmed that the previous workers with meat were dealing only with the early stages of autolysis and has demonstrated that the rate of autolysis of the nucleotides in fish is very much more rapid than that in beef and even more so than that in lamb.

It has been shown by many experiments in this laboratory[7] and elsewhere[10] that the organoleptic acceptability of beef or lamb is unaffected by pasteurising doses of radiation (0·4 Mrad) and that treated meats, stored for 10 weeks at chill temperatures, are indistinguishable from a frozen control held at −20° when submitted to a taste panel in blind comparison tests. During such

storage it is now established that the IMP present in beef joints has been completely destroyed by degradation to hypoxanthine. These observations, therefore, do not support the view that IMP plays any major role in the appreciation of the flavour of meats, either as a flavour precursor or potentiator, and measurements of its disappearance, in meat at least, cannot be expected to correlate with changes in quality. This is not the case with fish where, during the normal 10–14 days of useful storage life of commercial fish, the changes in flavour from fresh to spoiled, and in IMP content from initial to zero, are simultaneous. It would appear, however, on the basis of the results on meat that this correlation does not indicate any causal relationship between IMP content and flavour.

The marked difference in the rate of nucleotide hydrolysis in beef and in lamb may be due to a species effect or may reflect the physiological age of the animals. However, since the rate of autolysis in the leg muscles of the beef animal was slower than in the other tissues examined, the very slow rate in lamb leg may not be typical of all other parts of the carcass. A more detailed study of the hydrolysis of nucleotides in meat is in progress.

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PROPERTIES OF ANIMAL DEPOT FAT IN RELATION TO DIETARY FAT

By OLLE DAHL* and KAI-ÅKE PERSSON

The quality of the depot fat in ruminants is only slightly affected by the dietary fat. Non-ruminants like pigs, however, deposit ingested unsaturated fatty acids selectively. This may give rise to a depot fat with a higher iodine value than the dietary fat.

Introduction

Fat synthesised by land mammals consists essentially of mixed glycerides of oleic, palmitic and stearic acid. In the fat depots of non-ruminants the synthesised fat is laid down with the dietary fat, evidently without being subject to significant changes prior to the deposition. That is not the case with ruminants. In their bodies the unsaturated dietary fat is first hydrogenated to give a saturated or less unsaturated fat before assimilation and deposition, the hydrogenation being mainly effected by the rumen microflora.

Ruminants

From what has been said above there is little reason to discuss the depot fat of ruminants as related to the dietary fat. Several investigators have demonstrated that the fat ingested does not appreciably affect the properties of the depot fat of ruminants, owing to the biohydrogenating mechanism in these animals.[1–5] Analyses of beef fat have revealed, however, some seasonal variations, e.g. in the iodine value, which is slightly increased during summer and autumn.[6] This is due to 'leakage' of some octadecatrienoic acid from the grass lipids into the carcass fat.[7] In rapidly formed fat, like milk fat, more of the character of the dietary fat is reflected. Calves, before entering the stage of rumination, do not hydrogenate unsaturated dietary fat and, consequently, lay down such fat like non-ruminants.[8]

The colour and vitamin A content of beef fat are considerably influenced by the feed and values for these are significantly higher in summer and autumn than during the rest of the year.[9]

Non-ruminants

Horses

Horses are herbivorous non-ruminants having different feeding and working conditions during summer–autumn and winter–spring. During the former period pasturing predominates, and octadeca-dienoic and -trienoic acids present in small amounts in grass lipids are deposited. When kept in stables, horses receive a lot of cereals. These contain some oil, but the main component is starch, which is converted to relatively solid fat. As farm horses work less during this period, more synthesised (solid) fat is deposited. Table I lists some data pertaining to the seasonal changes of various horse depot fats. According to these investigations highly significant seasonal differences exist in the iodine value, which is higher in autumn than in spring, and is attributable to a substantially higher content of linolenic acid in the autumn fats.[7] The saponification equivalents differ only slightly. Characteristic differences, in most cases highly significant, exist between the iodine values and saponification equivalents for different depot fats.

As with beef fat, but to a greater degree, the colour and the vitamin A content of horse fat exhibit considerable seasonal changes,[9] the vitamin A content of horse fat during the summer–autumn period being equally as high as that of summer butter. Seasonal changes of iodine value, refractive index, density and titer have been established on commercial horse fats for a number of years.[6]

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Table I

Horse depot fats: characteristics for autumn and spring

Average figures on samples from 14 horses killed at the end of October 1957 and 15 horses killed in the beginning of April 1958, respectively. Breed: Ardennes. Sex: mares and geldings. Grade: expr and 1. Age: 9–19 years. Dressed weight: 375–530 kg.

W = wither fat, S = subcutaneous fat from the rump end, L = leaf fat (inside the belly), M = mesenteric fat

Depot fat	Iodine value		Saponification equivalent	
	Autumn[a]	Spring[b]	Autumn[c]	Spring[d]
W	90·3	83·9	280·3	279·3
S	87·6	82·0	280·0	279·5
L	84·2	77·4	279·6	279·2
M	87·0	83·3	280·5	280·4
Mean	87·3	81·7	280·1	279·6

(All differences were highly significant except for *a* S–M; *b* S–M and W–M; *c* W–S, W–M and S–L; and *d* W–S, W–L and S–L)

Pigs

Pigs are omnivorous non-ruminants. Generally there are no significant seasonal changes with regard to their feeding and exercise, but the quantity and composition of the ration fed by different breeders may sometimes vary appreciably. Foodstuffs containing large amounts of oil (e.g., some marine products and oil seeds) have a considerable influence on the quality of pig depot fat, not only with respect to consistency but also taste and keepability (for references, see Hilditch[10]). If the oil is rancid, the depot fat may be discoloured. The quality of poultry carcass fat is also strongly influenced by the dietary fat, as has been demonstrated in feeding experiments with turkeys.[11–13]

As, normally, pigs do not starve, the preformed fat will be accumulated partly or wholly in the fat depots and—contrary to general belief—the feed given some period prior to slaughter is not the only factor to affect the quality of pork.

Because of the accumulation of dietary fat, even small quantities of oil, for instance those contained in barley fed as basal diet, will exert an influence on the properties of the depot fat. In Table II are shown results from recent feeding experiments with groups of bacon pigs fed on potato flour, barley, barley and/or oats, i.e. basal diets containing different proportions of oil.[14, 15] In all these experiments the main source of protein was skim milk.

From Table II it can be seen that the content of linoleic acid in back fat and leaf fat runs parallel to the oil content of the basal food. The contents of trienoic and tetraenoic acids in the depot fats were low and were practically the same in all cases. However, as was shown by Beadle *et al.*,[16] pigs and also rats fed liberal amounts of flaxseed are able to deposit large quantities of octadecatrienoic (linolenic) acid as well.

Table II

Characteristics of depot fats from pigs fed various basal diets: (A) potato flour (B) barley (C) barley + oats (1:1) (D) oats.

All groups received skim milk as main protein feed

Average data from 8 pigs in the groups A, C, and D, and 16 pigs in group B.
For further details, see [14, 15]
I.V. = iodine value
Keep. = keepability in days at 55°

Basal feed	Basal feed				Back fat			Leaf fat		
	Lipids,† %	I.V. of lipids	% in lipids of Linoleic	% in lipids of Linolenic	I.V.	Linoleic acid	Keep.	I.V.	Linoleic acid	Keep.
A*	0·31	104·1	35·0	6·3	57·7	2·0	9·2	48·8	1·4	24·0
B	1·8	121·9	54·5	8·1	57·5	7·3	15·5	48·0	6·4	22·3
C	3·6	113·2	38·9	7·3	57·7	9·8	6·8	49·2	8·8	11·5
D	5·3	110·2	33·6	7·0	67·0	15·6	3·4	56·8	13·2	6·8

* Including 15% dried beet pulp, 2% solvent extracted soybean oil meal and 3% dried yeast.
† Excluding unsaponifiable matter.

It is also evident from Table II that the iodine value of the depot fats is not influenced by the diet, unless the ingested amount of oil becomes large, as in the case of feeding with oats. This maintenance of a practically constant iodine value in cases of a variable but low or moderate supply of oil in the diet might be achieved in the body by regulation of the amount of synthesised oleic acid going to the fat depots. The keeping qualities of the fat—for determination, see reference[14]—are negatively correlated with the percentage of oil in the feed and with the proportion of linoleic acid in the depot fats with one exception, viz., back fat from pigs fed potato flour. We have no explanation for this; maybe this fat is devoid of antioxidants or contains some pro-oxidant.

Evidently any level of oil in the diet will affect the quality of pig fat. This is true for back fat, leaf fat and also mesenteric fat.[17,18]

Whether the fatty acids contained in the oil of the diet other than octadeca-dienoic and -trienoic acid, i.e. essentially oleic acid and small amounts of palmitic and palmitoleic acid, are also deposited, has not been demonstrated. Recent experiments have given evidence, however, for a preferential deposition of polyunsaturated fatty acids. Thus, when pigs were fed excessive amounts of fat, depot fats with even a higher iodine value than the dietary fat resulted, indicating a selective deposition of unsaturated fatty acids. The results obtained by Février,[19] who fed pigs a dry feed mixture containing about 14% of lard, and those obtained[20] on depot fats from pigs fed large amounts of chicken offals (viscera) with a content of about 15% of fat, must be interpreted in this way. Calculation of the quantities of fat consumed by the pigs revealed that the dietary fat exceeded the weight of the total fat in the carcass. The details are given in Table III. From this Table it is evident that the unsaturated fatty acids

Table III

Results of feeding extremely large quantities of lard and chicken fat, respectively, to pigs[19,20]

	Extra fat consumed[1]		No. of	Back fat[4]	
	Quantity (calc.)	Iodine value	pigs in the experiments	Quantity (calc.)	Iodine Value
Lard[2]	37 kg	49·9	10	25 kg	75·5
Chicken fat[3]	35 kg	71·0	6	21 kg	83·5

[1] Only lard and chicken fat. Smaller quantities of fat supplied by cereal, meat meal, and other feed ingredients are not included.
[2] Constituting 13·9% of the dry feeding mixture.
[3] Fed as chicken offals (viscera), on an average 1·8 kg per pig per day and containing 15% fat.
[4] The back fat makes 80–85% of the total fat. Thus, the total quantities of body fat may be calculated as 30 and 25 kg., respectively.

will be enriched in the back fat. Further experiments have shown that this enrichment refers to all depot fats, not only in back fat.[20] These results are principally in agreement with those obtained by Hilditch & Pedelty[21] on depot fats from pigs subjected to starvation and those made on fats from pigs fed on a restricted diet.[22]

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FUMIGATION OF AGRICULTURAL PRODUCTS

XX.*—Prolonged storage of cereals fumigated with methyl bromide

By R. E. BLACKITH† and O. F. LUBATTI

Cereal seeds of various varieties which had been fumigated at one of four moisture contents were kept for 6 years. The germination capacity of the samples was determined initially and again after 6 months, 3 years and 6 years of storage. At the lowest moisture contents good survival of fumigated and of unfumigated seed was noted, but varietal differences appeared which had not been found during the earlier stages of the tests. Rye remained viable for 3 years when stored dry, but lost its viability after that period.

Introduction

Prolonged storage of seeds generally results in a very slow deterioration unless the moisture content is excessive, or the seed is one of those species which store poorly. Fumigation may also render moist seed less easy to store for short periods, although there is a generally useful maxim, that if seed is dry enough to store it is dry enough to fumigate. Few experiments have been performed to examine the joint effects of fumigation and of prolonged storage. This paper reports the fate of the seed whose tolerance of fumigation was originally reported by Lubatti & Blackith.[1] The seed was treated in the winter of 1955–56 with one of three doses (concentration × time products 0, 600 and 1200 mg.h./l.) of methyl bromide and at each of four moisture contents (8, 11, 14, 18%). It was tested for germination immediately after fumigation and again after 6 months' storage at 20°. Subsequently, the seed was retested after 3 years of storage and again after 6 years, at 20° throughout. The maize seed was retested only after 6 years of storage. As in the earlier experiments, these germination tests were conducted according to the recommendations of the International Seed Testing Association.

Results

The results are detailed in Table I.

Maize (Hybrid W.268)

Maize withstands prolonged storage remarkably well. When fumigated at 8% moisture

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content, the seed still shows over 95% germination after 6 years' storage, whatever the dosage of fumigant initially employed. Indeed, there are no important changes in the germination of the 6-year-old maize seed as compared with the germination after 6 months' storage.[1]

Table I

Percentage germination capacity (means of 400 seeds tested on each occasion) of fumigated, stored cereals

Dosage of methyl bromide in mg.h./l.

Cereals	Moisture content, %	Controls (unfumigated seed) Storage for		(Concentration × time product) 600 Storage for		1200 Storage for	
		3 years	6 years	3 years	6 years	3 years	6 years
Wheat (Peko)	8	93·0	91·3	92·0	93·3	93·0	88·8
	11	89·5	87·0	94·5	93·0	85·0	90·0
	14	88·5	90·5	24·3	25·0	25·5	20·8
	18	31·8	14·5	6·8	0	1·0	0
Wheat (Atle)	8	95·0	93·0	93·0	92·5	96·0	92·0
	11	91·8	85·0	92·0	88·5	63·5	58·0
	14	92·8	88·3	40·5	35·8	41·8	30·5
	18	87·0	0	0·3	0	3·5	0
Oats (Star)	8	36·5	85·0	85·8	80·0	82·0	76·0
	11	84·5	82·0	82·5	77·0	69·3	69·0
	14	82·5	65·0	60·3	46·8	36·5	23·3
	18	0·5	0	7·3	0	1·3	0
Oats (Blenda)	8	98·0	96·8	98·5	96·5	96·3	97·0
	11	99·3	97·8	94·5	94·5	97·5	93·0
	14	96·0	96·0	89·0	86·0	75·5	55·3
	18	48·8	7·8	3·0	0	1·8	0
Barley (Procter)	8	96·5	95·3	97·3	97·5	97·3	95·3
	11	96·5	94·0	95·8	94·0	96·3	92·0
	14	95·0	93·8	81·5	81·3	81·8	85·0
	18	0	0	52·8	17·8	0	0
Barley (Herta)	8	97·5	93·5	99·0	93·8	98·3	94·5
	11	99·3	96·5	99·3	92·0	81·3	83·8
	14	95·5	84·7	75·5	67·8	75·3	70·3
	18	4·3	0	0	0	0	0
Rye (Winter)	8	91·8	28·0	98·3	27·8	97·3	35·7
	11	99·0	27·5	93·0	13·5	72·3	9·3
	14	69·5	4·3	39·0	0·8	44·8	0
	18	23·3	0	23·8	0	23·5	0
Maize (W268)	8	—	94·8	—	96·5	—	96·8
	11	—	94·0	—	65·5	—	48·0
	14	—	73·0	—	32·3	—	2·7
	18	—	0	—	0	—	0

Rye (var. Winter)

After 3 years, the fumigated rye seed showed only minor deterioration, all the seed fumigated at 8% moisture content, and that fumigated at 11% moisture content but given the less severe treatment of 600 mg.h. per l., gave better than 90% germination. Rye seed stored at 14% or 18% moisture for the 3 years showed substantially reduced germination whether or not it had been fumigated. However, after 6 years, a sharper reduction of germination had taken place, and no sample, even the unfumigated seed stored at 8% moisture content, had better than 40% germination.

Oats (var. Blenda)

Blenda oats withstand prolonged storage very well; little deterioration had taken place after 6 years' storage that had not occurred after 3 years. All seed fumigated and stored at 8% or at 11% moisture content remained with at least 93% germination over the whole period.

Oats (var. Star)

Whether fumigated or not, this variety did not stand up to prolonged storage as well as Blenda. Serious deterioration had set in after 3 years, even in those samples kept at the lowest moisture content, and these losses of germination capacity were accentuated after 6 years.

Wheat (var. Peko)

Peko wheat retains its germination capacity well, the losses of germination after only 6 months' storage[1] being little greater after 3 years. No clear picture of further deterioration could be seen after 6 years' storage.

Wheat (var. Atle)

The results for Atle wheat closely follow those for Peko, although the deterioration of heavily fumigated samples of Atle stored for 6 years at 11% moisture content is greater than in Peko wheat.

Barley (var. Procter)

Little deterioration can be detected after 3 years, when all samples stored at 8% or 11% moisture content retained better than 95% germination; however, a small loss after 6 years brought some samples stored at 11% moisture content to a germination capacity of 92%. At higher moisture contents the losses after 3 years' storage were already of the order of 20% in fumigated samples.

Barley (var. Herta)

The preservation of germination in this variety follows that of Procter fairly closely, except in those samples fumigated at the highest dose, and stored at 11% moisture content, where losses approaching 20% were noted.

Discussion

In general, the stored seeds retained their germination well, considering the relatively high and constant temperature (20°) at which they were stored. None, however, survived the 6-year period with reasonable germination when stored at 18% moisture content, and only Atle wheat survived the 3-years period at this moisture content with better than 80% germination. At the other extreme, the dryest seeds stored at 8% moisture content, retained better than 90% germination in all cases except for Star oats, rye, and a heavily fumigated sample of Peko wheat, over the full 6 years. The rye seed retained its good germination at this moisture content for 3 years but failed afterwards, to a surprising extent. The varietal differences, which were not clearly marked in the early stages of the experiments,[1] became more marked later on. Only Blenda oats and Procter barley could be confidently stored at 11% moisture content after the high dose of methyl bromide had been applied, if the storage period was likely to extend over several years.

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WHEAT PROTEINS. I.—Fractionation and Varietal Variation of Endosperm Proteins of *T. vulgare*

By C. B. COULSON* and A. K. SIM

A method of starch gel electrophoresis has been established for the fractionation of wheat proteins which allowed the resolution of up to 32 components.

A study of the endosperm proteins of several varieties of *T. vulgare* has shown distinct differences in the composition of fractions of low electrophoretic mobility. No satisfactory correlation was achieved between these components and the rheological characteristics of the flour, and there appeared to be no obvious relationship between closely related varieties. There is, however, some evidence to suggest that some of these fractions may reflect the morphological characteristics of the variety and may be influenced, at least to a small extent, by environmental factors. In contrast, fractions of intermediate electrophoretic mobility are similar for all varieties and are probably distinctive of both the species and variety.

Introduction

Attempts to explain the characteristic differences in the rheological properties of flour from different wheat varieties have so far been relatively unsuccessful. It is generally accepted that these differences are probably attributable, at least in part, to differences in protein composition and are not necessarily related to such factors as total gluten content or the proportion of gliadin to glutenin as were formerly proposed.

Many attempts have been made in the past to compare the protein constituents of different wheat varieties, but in the main, methods of fractionation lacked sufficient resolving power or non-ideal conditions made results difficult to interpret with certainty.

A significant advance in the electrophoretic separation of gluten proteins was reported by Jones *et al.*[1] who, using the Tiselius free boundary techniques, showed that symmetrical patterns could be obtained by the use of relatively low protein concentrations in conjunction with an aluminium lactate–lactic acid buffer system of low pH and low ionic strength.[2] Elton & Ewart[3,4] first reported the application of this buffer system to the electrophoretic separation of cereal proteins in starch gel. Since then, the successful fractionation of flour and gluten proteins in starch[4–10] and polyacrylamide[11–13] gels has been reported by a number of workers. Recent investigations have suggested that the protein composition of wheat flour and isolated gluten is somewhat more complex than had hitherto been suspected.[8,9]

The present report describes an effective simplified method of electrophoresis and deals with the detailed fractionation of the water-soluble endosperm components of a large number of modern wheat varieties in an attempt to elucidate differences in protein composition and some of the factors which may influence it. Aqueous extracts were choseh for investigation for reasons described in an earlier report.[14]

Experimental

Starch gel electrophoresis

Starch gel moulds of two sizes were used as shown in Fig. 1. The smaller gel allowed the separation of four samples simultaneously and required no cooling. For comparative work however, it was more suitable to employ the larger 9 in. × 8 in. gel as shown. Adequate cooling was obtained by allowing a constant stream of tap-water to pass through the baffled, brass base-plate. Up to nine samples could be accommodated in each gel.

In all separations 10% gels (Connaught Starch-Hydrolysed, Connaught Medical Research Laboratories, Toronto, Canada) were used and were prepared in an aluminium lactate–lactic acid buffer containing 0·5M-urea. Samples were positioned in the gel using the filter paper insertion method of Smithies[15] (Whatman No. 3, 1/2 in. × 1/4 in.).

Separations were carried out for 3 h. at an external potential of 400 V. (9 in. × 8 in. gel).

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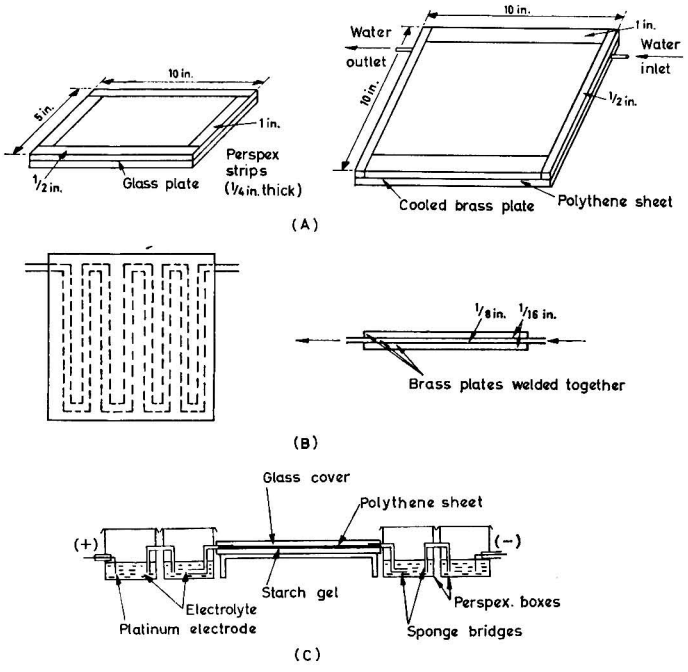

FIG. 1.—*Starch gel electrophoresis apparatus etc.* (A) Starch gel moulds (B) cooling system (C) electrophoresis apparatus.

Direct current was supplied from a stabilised power pack capable of delivering 1500 V. d.c. and 200 mA. The external potential was measured with an Avometer connected between the platinum electrodes. The voltage gradient was measured by inserting platinum probes at either end of the gel.

After electrophoresis, the Perspex sides of the mould ($\frac{1}{2} \times \frac{1}{4} \times 10$ in.) were replaced by similar strips $\frac{1}{8}$ in. thick and the gel sliced horizontally with a thin cheese-wire (0·0068 in.) conveniently held in a hack-saw frame. A sheet of Polythene was placed over the top half of the gel, which was then gently peeled from the bottom half, while the gel mould was held in a vertical position. The top and bottom halves of the gel were transferred to staining dishes by means of the supporting Polythene sheets.

The addition of urea (0·5M) to aluminium lactate buffers before gel preparation was found to increase the rigidity of the gels and had a band-sharpening effect without any obvious alteration of the protein fractionation. Concentrations of above 0·5M, however, led to greatly extended gel setting times and caused some precipitation of the protein-components during the separation.

Gels were stained in a solution of Nigrosine (0·5 mg./ml.) in glacial acetic acid/water/methanol (10:50:50 v/v/v) for at least 12 h. Under these conditions, the patterns were immediately visible after removal of the staining solution. Washing was however continued in water for a further 5–6 h. to reduce the intensity of the background stain. Gels stained in a similar solution of Naphthalene Black 12B required extended washing times, particularly with water. The patterns could be rendered visible after shorter periods of staining (5–6 h.) but some of the minor components were extremely weakly stained.

The use of glacial acetic acid, methanol or the usual protein-precipitating agent (e.g., trichloroacetic acid, 10%) in the washing solution tended to reduce the intensity of the patterns, particularly with Naphthalene Black.

Gels immersed in water were photographed as follows:

Illumination:	Two 275-W lamps (Photolita No. 1, Philips Ltd., London)		
Camera:	M.P.P. Technical	Film:	FP3 (5 in. × 4 in.) (Ilford Ltd.)
Exposure:	1/10 sec. at *f*8.	Developer:	ID 11 (Ilford Ltd.) for 10 min.

A commercial automatic recording, double-beam reflectance densitometer with integrator (Messrs. Joyce, Loebl & Co. Ltd., Newcastle-upon-Tyne; ' Chromoscan ' 1961) was used for producing graphical scans (with peak integration values) of starch gel patterns.

Preparation of protein solutions

All varieties of *Triticum vulgare* were obtained from the previous year's harvest. Samples were ground by hand and the endosperm material isolated by sieving (100 mesh); 2·0 g. of the isolated flour was dispersed in distilled water for 30 min. in a top-drive homogeniser (M.S.E. Ltd., London). The mixture was centrifuged for 15 min. at approx. 3000 *g*, followed by centrifugation of the supernatant for a further 30 min. at approx. 25,000 *g* (0°C). Protein solutions extracted thus contained approx. 2 mg. of protein per ml, and in most cases were used immediately without further concentration.

β-Amylase activity in protein extracts was found to cause significant degradation of the starch gel during electrophoresis, thus obscuring most of the slow-moving components (cf. Elton & Ewart[4]), To inactivate the enzyme, solutions were placed in a boiling water bath for 2 min. before electrophoretic separation. Effective inhibition was also achieved by the addition of mercuric chloride solution (1%) to the protein extracts, but in some cases this resulted in the precipitation of protein material.

The protein composition of different varieties of Triticum vulgare

Aqueous extracts of the varieties of *T. vulgare* shown in Table I were compared by starch gel electrophoresis. In addition, samples of *T. vulgare* cv. Champlein, Viking and Jufy, obtained from two different sources were examined in an attempt to check the reproducibility of the varietal patterns already obtained. Also closely related varieties of *T. vulgare* of known pedigree (Table II) were examined in the same way.

Effect of plant selection on protein composition

New varieties of wheat may be introduced either by cross-breeding between two existing varieties or by plant selection and development from a single variety. In most cases the former method

Table I

Varieties of wheat studied

Variety	*Source*	*Character*
Viking	English	Soft, winter
Champlein	English	Soft, winter
Gabo	Australian	Hard, spring
Russian variety	—	Hard, winter
Manitoba variety	—	Hard, spring
Als	English	Soft, winter
Professor Marchal	English	Hard, winter
N 59	English	Hard, winter
Chieftain x Tenmarq	American	Hard, winter
Capelle Desprez	English	Soft, winter
Jufy	English	Soft, spring
Kaw	American	Hard, winter
Minister	English	Soft, winter
Maître Pierre	English	Hard, winter
Nord Desprez	English	Soft, winter
Vilmorin 27	English	Soft, winter
Glasnevin Rosa	English	Soft, winter
Lutescens-329 x *A. glaucum* (' gliadin ' only)	Russian	Hard, winter
Lutescens-329 (' gliadin ' only)	Russian	Hard, winter

Table II

Closely related varieties of wheat studied

Variety	*Pedigree*
Jufy I	Jubiligem x Fylgia
Phoebus	Jubiligem x Fylgia
Jubiligem	Vilmorin 23 x Iron III
Fylgia	Aurore x Extra Kolben II
Fylgia II	Aurore x Extra Kolben II
Skandia	Fylgia x Crown
Welcome	Jubiligem x Wilma
Dominator	Jubiligem x Atle
Leda	Jubiligem x Zanda
Als	Danish land variety (control)

would be expected to lead to distinct changes in the protein constituents even although the varieties are of the same parent forms. This was indeed verified with the exception of two varieties mentioned below. It was of interest therefore to examine the protein composition of varieties developed by plant selection, to determine if changes in only the morphological characteristics were also reflected by alteration of the protein constituents.

Aqueous extracts of the following varieties were examined:

(A) Square head's Master; (B) Square head's Master 13/4 [plant selection from (A)]; (C) Wilhelmina; (D) Wilma [plant selection from (C)].

Results

The protein composition of different varieties of T. vulgare

Starch gel electrophoresis patterns of the aqueous soluble protein components of various varieties of *T. vulgare* can conveniently be divided according to electrophoretic mobility into a number of groups as shown in Fig. 2.

The most distinctive feature of all varieties was the similarity in protein composition of region F within the range of intermediate electrophoretic mobility. All patterns appeared to be very similar in this region, showing the same protein distribution observed for *T. vulgare* in previous investigations.[15] This pattern would seem to be typical of the species.

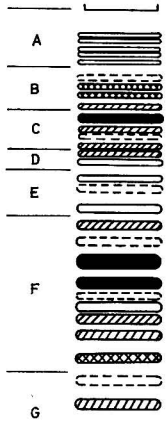

FIG. 2.—*Diagram of starch gel electrophoresis pattern of water-soluble wheat endosperm proteins (pH 5·6)*

Distinct differences between varieties were however observed in the region of low electrophoretic mobility (Fig. 2, A to D). These differences were most marked in region A where the number of components varied between 1 and 6. Region C appeared to be similar in many of the samples, although differences in component concentration were observed from the densitometric scans. The number of components in region D appeared to vary between 1 and 2. Minor differences in protein pattern were also observed in region B, but these were essentially of a quantitative nature.

Densitometric scans of protein fractions are shown in Fig. 3.

The protein composition of the same varieties of T. vulgare *obtained from different sources*

Of the samples of *T. vulgare* examined, all identical varieties obtained from different sources appeared to contain an identical complement of water-soluble protein components as judged by starch-gel electrophoresis. Densitometric scans of protein patterns indicated that the relative concentrations of individual fractions of the same variety were also very similar. Extracts of two such samples of Professor Marchal are shown in Fig. 3 (G and L).

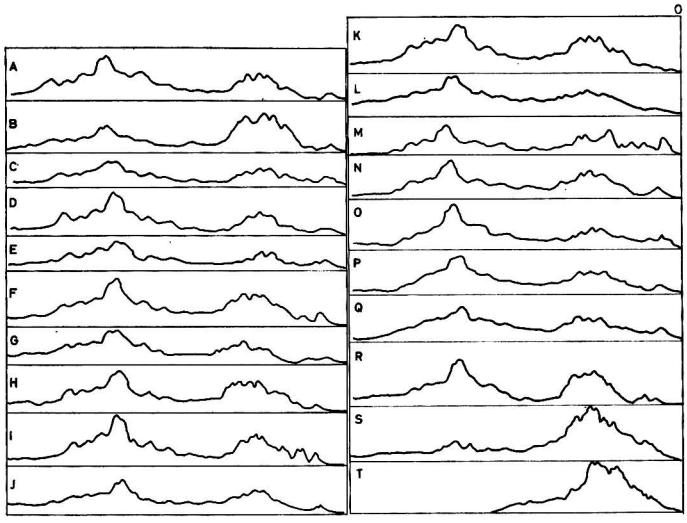

FIG. 3.—*Starch gel electrophoresis of the water-soluble endosperm proteins of various varieties of* T. vulgare (A) Viking (B) Champlein (C) Gabo (D) Russian (E) Manitoba (F) Als (G) Professor Marchal (H) N59 (I) Chieftain x Tenmarq (J) Capelle (K) Jufy (L) Professor Marchal (M) Kaw (N) Minister (O) Maître Pierre (P) Nord Desprez (Q) Vilmorin 27 (R) Glasnevin Rosa (S) Lutescens 329 x *A. glaucum* (' gliadin ') (T) Lutescens – 329 (' gliadin ')

The protein composition of closely related varieties of T. vulgare

As already shown, the distribution of protein components of intermediate electrophoretic mobility was very similar for all aqueous extracts. Distinct differences were again observed in the region of low mobility (A to D), particularly in regions A and D. There appeared to be no obvious similarities in the protein patterns of most closely related varieties (cf. Fig. 4).

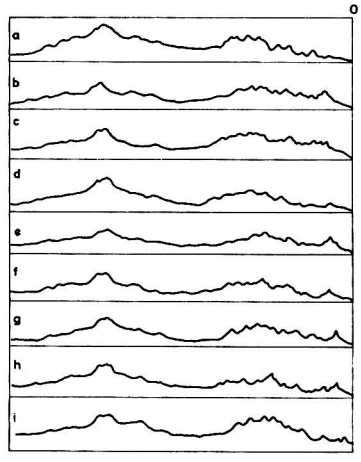

FIG. 4.—*Starch gel electrophoresis of the water-soluble endosperm proteins of closely related varieties of* T. vulgare (a) Jufy (b) Phoebus (c) Jubiligem (d) Fylgia (e) Skandia (f) Welcome (g) Dominator (h) Leda (i) Als

In the case of Fylgia and Fylgia II however, electrophoresis patterns of the water-soluble protein components were identical, although both varieties were known to have originated from separate crosses of the same parents. In contrast, the corresponding patterns of Jufy I and Phoebus, also obtained from separate crosses of the same parents, were distinctly different in the region of low mobility (A to D).

The effect of plant selection on protein composition

Aqueous extracts of varieties of *T. vulgare* obtained from the same cross, but developed by plant selection, appeared to contain an essentially similar distribution of protein components, as judged by starch-gel electrophoresis.

Some minor quantitative differences were noted among the slow-moving components of region B.

Discussion

These results would seem to indicate that the rheological properties of flour are not directly reflected in terms of protein composition, at least by those components resolved by starch gel electrophoresis. This is hardly surprising however, since it is likely that many non-protein constituents, e.g., lipids and carbohydrates, also have a direct influence on gluten and flour rheology and indeed may mask much of the protein influence.

The work has however indicated some of the other factors which may influence the protein composition of the endosperm, and it is hoped that this approach may eventually lead to a more realistic classification of this complex system.

The most obvious feature of all varieties of *T. vulgare* investigated was the similarity of components of intermediate electrophoretic mobility (Fig. 2, F). It has been shown in a previous report[15] that these components probably reflect the genetical characteristics of the species, and this would seem to be the pattern typical of all varieties of *T. vulgare*.

Significant differences in protein patterns were confined to regions A to D (Fig. 2), in particular regions A and D. Components in region A varied from 1 to 6 even amongst the closely related varieties. Chieftain x Tenmarq and Kaw were distinctly different from other varieties in this region. We have already shown that this group of components probably correspond to the ' adhering ' (Haft) proteins of Hess[9] and are therefore closely associated with the starch granules and possibly with phospholipids.

Region D appeared to contain 1 or 2 components and this, too, varied between closely related varieties. Regions B and C showed least variation and with region D, they corresponded to the generally accepted ' gliadin ' fraction of the endosperm or the ' wedge ' (Zwickel) protein of Hess.[9] These components would appear to represent the true storage proteins of the endosperm[16] (see later paper; Part II of this series) and being major components, presumably contribute in part to gluten rheology.

It is of interest to note the striking similarity in protein composition of varieties Fylgia and Fylgia II, particularly as these two varieties possess very similar morphological characteristics. They were produced by the same breeder by separate crosses of the same parents (Fylgia was produced 15 years before Fylgia II). The morphological similarity is accidental and was presumably the reason for retaining the name in the later variety. It is tempting to suggest, however, that these protein fractions may reflect, at least in part, the morphological rather than the rheological characteristics of the wheat—Fylgia is a soft wheat and Fylgia II is a hard wheat. Jufy I and Phoebus, also from the same parent forms and bred about the same time, showed no such similarities either in protein composition or morphology.

This proposition is not substantiated by examination of varieties derived by plant selection. Here the patterns were relatively unchanged except for minor concentration differences in region B. It should be noted, however, that this procedure does not necessarily alter many of the original characteristics of the variety.

Only minor quantitative differences were noted between identical varieties obtained from different sources. Koenig *et al.*[17] also reported minor quantitative changes in varieties grown in different environments. It is not possible to conclude whether these patterns will be effected

significantly and qualitatively by wide variations in climate and soil conditions, since all varieties examined here were grown in Scotland, presumably under relatively similar conditions. It may be significant however, that Chieftain x Tenmarq and Kaw, both of American origin, differed significantly in region A from all other varieties.

Acknowledgments

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BROMINE RESIDUES IN OIL SEEDS AND OIL MEALS AFTER FUMIGATION WITH ETHYLENE DIBROMIDE*

By EUGENIA ALUMOT and M. CALDERON (with Ch. Genige)

Fumigation of oil seeds (sunflower, soya-bean, groundnuts and cotton) with ethylene dibromide (EDB) was performed at two concentrations in order to establish the effect of seed structure and oil content on the sorption and reaction of EDB. Amounts of the fumigant sorbed were markedly increased by the presence of fat in the seeds. Hulls generally inhibited sorption. Amounts of bound bromine were not affected by the presence of fat and were proportional to the initial concentration of EDB in the seeds. No bound bromine was found in the oil fraction of the seeds. About one month's airing was needed to free the seeds of sorbed EDB.

* Contribution from the National and University Institute of Agriculture, Rehovoth, 1964 series, No. 801-E.

Introduction

It is known that fumigation with EDB results in residues of two forms—free EDB and bound, probably inorganic, bromide. From the results of a previous work[1] it was suggested that the protein fraction of the seed is responsible mainly for the bound residues, whereas fat acts as a solvent and its presence causes sorption of greater amounts of the fumigant. Since the previous work was done on cereal grains, it was deemed of interest to study the process of sorption and airing of EDB in oil seeds in order to establish whether the seed structure and/or oil content affect the amounts of sorbed and bound bromine. Some suggestions about the mode of action of EDB on oil seeds were found in annual reports of the Central Food Technological Institute of Mysore, India.[2] The authors stated that ethylene dibromide was absorbed and retained by the oil fraction of the seeds, in contrast to methyl bromide, which was bound to the protein fraction.

Methods and materials

Fumigation experiments

The laboratory-scale fumigation experiments were performed in 20-l. fumigation containers, filled up to 3/4 of their volume with the seed samples. The samples were packed in duplicate, in cloth bags each containing 0·5 kg. of seeds.

The EDB dosages applied were 100 and 300 g./m.^{3} which are higher than are normally used in commercial fumigation. The fumigant was applied in liquid form by pipetting through a hole in the lid into a plug of cotton placed on the top of the material to be fumigated. In preliminary experiments, it was found that EDB evaporated completely from the cotton plug after the first 24 h. of fumigation. The fumigation time was 72 h. at a temperature of 22–24°.

Samples were withdrawn for bromine analysis immediately after the containers were opened at the end of the fumigation period. The oil seeds subjected to fumigation were: sunflower, soya-bean, groundnuts and cotton, containing 26·0, 18·0, 43·0 and 23·0% of oil, respectively.

In order to examine the effect of seed structure and fat content on sorption, sunflower, soya-bean and groundnuts were fumigated in three forms: as whole seeds, as dehulled seeds before fat extraction, and as meals after fat extraction. Cotton seeds were fumigated as whole seeds and as meal.

Airing

The fumigated material was spread over perforated metal trays and exposed to the air. Bromine determinations were performed at different times in order to establish the end point, i.e., when there was no further decrease of bromine content.

Bromine determination

The methods of Mapes & Shrader[3] (hydrolysis by monoethanolamine), and of Olomucki & Bondi[1] (hydrolysis by 2% alcoholic potassium hydroxide) were used. Recoveries of 95–101% were obtained with both methods when known amounts of EDB in alcoholic solution were analysed

It is known that, by the monoethanolamine method, total bromine (EDB bromine and bound bromine) is recovered, while potassium hydroxide hydrolysis yields half the EDB bromine and all the bound bromine. By use of both methods, a calculation of organic (EDB) and inorganic residues may be made separately.

The reliability of this calculation was confirmed by the fact that the final bound bromine content, determined at the end of the airing period, did not differ from calculated values obtained from analysis made during airing. These results seem to indicate that the residual bromine content does not change greatly during airing, as was reported for cereals.[4]

In the course of this work the method of Kennett & Huelin,[5] consisting of the determination of EDB after steam distillation with benzene, was tried. The results obtained with several samples agreed well with those obtained by the two methods mentioned above.

In view of the results obtained it seems that the simplest method for establishing an airing curve of EDB is the Kennett–Huelin method, followed by determination of bound bromine at the end of the airing period, i.e., when no free EDB is present.

Results

The airing curves of soya-bean, sunflower and groundnuts are presented in Figs. 1, 2 and 3, respectively. In the case of sunflower and groundnuts, the results are presented at the high concentration of EDB (300 g./m.^{3}). The airing curves at 100 g./m.^{3} differ from the above only in being proportionately lower (as in the case of soya-bean).

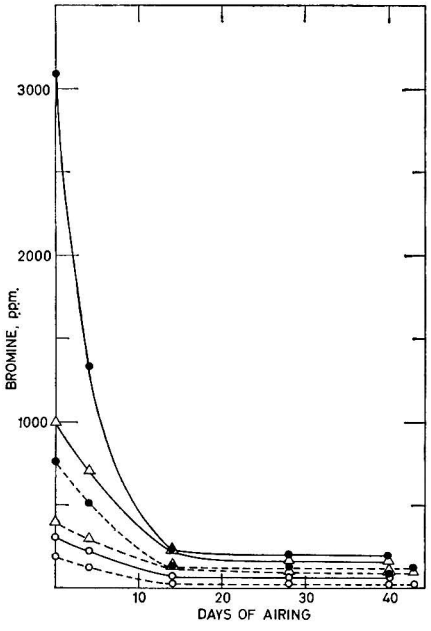

FIG. 1.—*Bromine content of soya-beans during airing [fumigation for 72 h. with* 100 (- - - - -) *and* 300 *g./m.*^{3} (————) *of EDB].*

● Flakes △ oil meal ○ whole seeds

It appears from the Figures that the amount of EDB sorbed by oil seeds before airing (i.e., the points on the ordinates of Figs. 1–3) depends on seed structure and is enhanced by the presence of fat. Whole soya-beans and sunflower seeds (Figs. 1 and 2) contained relatively small amounts of bromine, whereas whole flakes or dehulled seeds sorbed much more. In meals, after fat extraction, the sorption decreased. Amounts of bound bromine did not differ as greatly as the

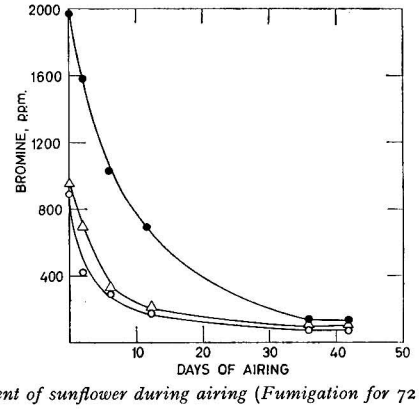

FIG. 2.—*Bromine content of sunflower during airing (Fumigation for 72 h. with* 300 *g./m.*^{3} *EDB)*

● dehulled seeds △ meal ○ whole seeds

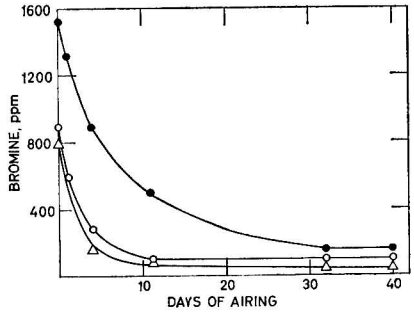

FIG. 3.—*Bromine content of groundnuts during airing (Fumigation for 72 h. with 300 g./m.³ EDB)*
● dehulled seeds △ meal ○ whole seeds

amounts of EDB sorbed. In order to determine whether bound bromine is connected with oil fraction, several samples of fumigated soya-bean flakes (after airing) were extracted with ether and bromine determinations were carried out in the oil fraction and in the residual meal. No bound bromine was found in the oil, whereas the extracted meal contained bound bromine in amounts proportionally higher than before extraction, because of loss of 18% of fat from the samples. For example, 270 and 175 p.p.m. of bromine were found in two samples of flakes compared with 225 and 144 p.p.m. respectively before fat extraction.

In the experiments with groundnuts (Fig. 3), dehulled seeds sorbed more EDB than seeds with hulls, but the differences were smaller than between whole seeds and flakes of soya-bean. In order to check the possibility of EDB sorption by the hulls, the latter were fumigated separately. Sorption of 880 p.p.m. at 100 g./m.³ and 2500 p.p.m. at 300 g./m.³ was found. These amounts disappeared rapidly during airing, leaving 50 p.p.m. and 100 p.p.m., respectively, of bound bromine after 2 weeks.

The results obtained with cottonseed (whole seeds and meal) are presented in Fig. 4. The amounts sorbed by the meal are similar to those obtained with sunflower and groundnut meal, but whole seeds sorbed much less bromine, indicating that the cottonseed hulls are less permeable to EDB penetration than are the hulls of groundnut and sunflower seeds.

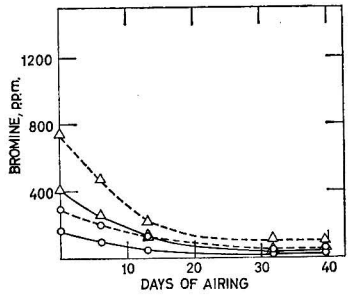

FIG. 4.—*Bromine content of cotton during airing [fumigation for 72 h. with 100 (—) and 300 (– – –) g./m.³ EDB]*
△ meal ○ whole seeds

Discussion

The results presented indicate that two factors, acting in opposition, are responsible for the amounts of EDB sorbed by oil seeds: the morphological structure of the whole seed (with hulls) prevents sorption, and the presence of fat enhances it. The highest amounts of bromine were sorbed by dehulled, fat-containing seeds, and the lowest by whole seeds, with meals giving intermediate results. Only in the case of groundnuts were the amounts sorbed by whole seeds slightly greater than those sorbed by defatted meal, indicating the possibility of EDB sorption by hulls.

It was shown that groundnut hulls are indeed able to sorb considerable amounts of bromine, probably owing to their structure and great surface.

The influence of fat, acting probably as solvent for EDB, on the sorption of the fumigant confirms earlier results obtained with cereals.[1] The presence of fat caused a marked (sometimes threefold) increase of sorbed fumigant in flakes and dehulled seeds as compared with fat-extracted meals.

Concerning bound bromine, it seems that its concentration depends chiefly on the amounts of the initially sorbed EDB, independently of the reasons that had caused this sorption. The presence of fat affects the amounts of bound bromine only indirectly, in all cases when increased amounts of free EDB were sorbed owing to the presence of fat. It was postulated on the basis of previous results[1] that the bound bromine results from reaction of EDB with the protein fraction of the seeds, but now it is found that the amounts of bound bromine are not proportional to the amount of protein in the sample (compare results of protein-rich meals with those of fat-containing samples). If a chemical reaction occurs between EDB and seed constituents, it depends not only on the presence of characteristic chemical groups but also on fumigation conditions responsible for the initial sorption of EDB.

An important point concerning the safe use of commodities fumigated with EDB is the determination of an airing period after which only traces of free EDB would be present. Previous experiments[1] showed that this period is practically uninfluenced by the mode of airing and is the same for laboratory experiments and for bulk storage. It is now found that, similarly to previous results with cereals[1], about one month of airing is needed to free the fumigated seeds from almost all sorbed EDB.

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ELASTICITY COEFFICIENTS AND TEXTURE OF COOKED DRIED PEAS

By D. E. C. CREAN and D. R. HAISMAN

The elasticity coefficient of peas can be estimated from the distance they bounce when dropped vertically on to an inclined plane. The rebound range is related to the texture of the peas, and the standard deviation of the rebound range is a good measure of variation in the texture of the peas in a batch.

Introduction

In the canning of dried peas, the texture of the cooked peas is one of the most important aspects of quality which has to be considered in determining the heat process necessary above the bacteriologically safe minimum. The texture of the peas is generally assessed by a tasting panel but can be measured by any of a variety of instruments, such as the tenderometer,[1] maturometer,[2] shear press[3] and the hardness meter,[4] which measure the force required to pierce, compress or shear the peas. A good correlation between maturometer readings and texture scores assessed by a tasting panel has been found and, in general, the mechanical instruments correlate well with one another. These instruments all measure the average texture in terms of the yield point under a shear stress of a batch of peas varying in size from the 143 peas used in the maturometer, upwards, and unless a great number of measurements are made, they give little indication of the range of texture to be found amongst the peas in any batch. To this extent they provide less information than a tasting panel, although they are quicker and more convenient to use.

It has been suggested that the elasticity coefficient of peas might provide a good measure of their texture,[5] and as it is a physical property which can be measured rapidly for individual peas, it could also be used in assessing the variation of texture within a large batch. Preliminary tests measuring the distance cooked peas bounced after dropping on to a plate set at 45° to the vertical showed a promising correlation between resilience and texture as measured with a maturometer. A trial apparatus was constructed to investigate the method further.

Experimental

The apparatus used for the measurements is shown diagrammatically in Fig. 1. A can of peas emptied on to the slowly moving belt A is sorted into single file by the gates B. The peas fall off the belt at C on to the glass plate D, set with the centre 105 cm. vertically below C, and are deflected into the partitioned collector, E, 25 cm. below the point of impact of the peas on the plate. The slope of the glass plate (α) can be adjusted to increase or decrease the range of the peas, so accommodating samples of different average elasticity coefficients. An efficient collecting box is made by looping polythene sheet over cords stretched at intervals of 7·6 cm. across a steel frame.

Results

The speed of the deflected pea and the angle at which it leaves the plate are both functions of its elasticity. The greater the elasticity, the further is the range on rebound, and the peas collected in each compartment of E represent fractions with successively increasing elasticity coefficients. Each batch of peas put through the apparatus is separated into fractions whose distribution was characteristic for that particular batch. The number of peas in each compartment is counted, and, for the purpose of comparison, the average range and the standard deviation from the average are calculated from the tabulated data. Both the average range and the standard deviation are expressed in units corresponding to the compartments in which the peas are collected, 0 indicating zero range and 14 the maximum range. The standard deviation from the average range is taken as a suitable measure of the spread of the peas over

all the compartments, and is referred to as the ' spread '. The results were found to be quite reproducible; over a series of twelve replicate determinations the coefficient of variation of the average range was 2% and the standard deviation of the ' spread ', which varied from 1·002 to 1·816, was 0·105.

To test the relationship between the range of the peas on rebound and the texture measured by conventional means, the peas were collected from each compartment and their texture measured on a maturometer. The averaged results of three experiments are shown in Fig. 2. The maturometer reading increased with the rebound range of the peas, the difference between successive fractions diminishing over the higher ranges.

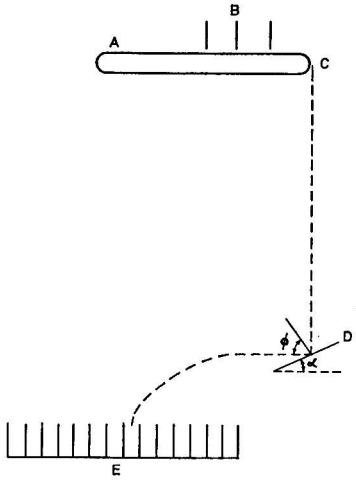

FIG. 1.—*(left) Apparatus for measuring the rebound range of cooked peas*

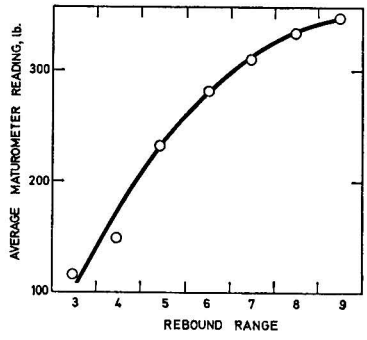

FIG. 2.—*Relationship between texture and rebound range*

Nineteen samples of peas, representing fractions collected from three batches of cooked peas, were assessed for texture by a panel of three and by maturometer measurements. The panel scored the peas from 1 to 10, where 1 denoted very soft and 10 very hard peas. The panel score correlated well with both the maturometer readings and the rebound range (correlation coefficients of 0·91 and 0·82 respectively).

Although the reproducibility of the test for peas from the same batch had been shown to be satisfactory, it did not necessarily follow that the individual fractions which were collected were completely homogeneous. After separation of a batch of peas into different fractions, each fraction was put through the apparatus again. It was found that in fact the fractions were not homogeneous, the second test sub-dividing each fraction again into a range of fractions with a peak corresponding to the original fraction (Table I).

The peas, particularly the soft peas, were deformed to some extent by the bouncing procedure, and this probably explains the differences in the average ranges, which are greater than was indicated by the reproducibility test. Nevertheless, the results show that more than one factor determines the range of the peas after bouncing, and as peas are irregular in shape it can probably be assumed that the apparent coefficient of elasticity varies along each axis of the peas. Thus the range depends to a certain extent on which facet of the pea impinges on the plate, and each fraction collected actually contains peas with diverse elasticity coefficients.

Because the separation is not perfect, so that a pea having any particular elasticity coefficient may fall into one of a number of compartments, it was found that the relationship between rebound range and texture was influenced by the average texture of the batch. The spread of the peas from the modal compartment was sufficient to affect the average texture of the peas in every other fraction. Thus if the average texture of a batch of peas was hard, all

Table I

Repetition of the fractionation procedure

Peas tested	Average range	Standard deviation from average
Original batch (884 peas)	5·6	1·832
Peas range 3	3·6	1·147
Peas range 4	4·7	1·450
Peas range 5	6·0	1·603
Peas range 6	6·7	1·500
Peas range 7	7·6	1·671
Peas range 8	7·7	1·690
Sum of the fractions on the second test	6·4	

the fractions collected from that batch were harder than the analogous fractions collected from a batch of soft peas, although the gradation in hardness from the low to the high fractions was preserved in both cases. This is illustrated in Fig. 3 which shows three texture–range curves obtained from the same batch of peas after being cooked for three different lengths of time.

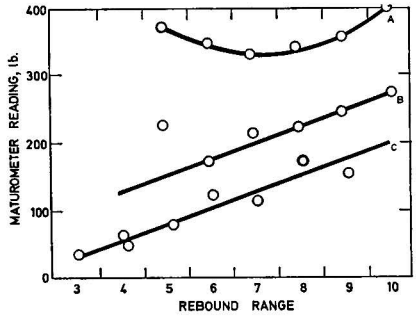

FIG. 3.—*Relationship between texture and range for the same batch of peas cooked for different times*

A cooked 2 min.; B cooked 5 min.; C cooked 10 min.

The weighting of the outlying compartments by the scatter from the preponderant fractions makes the rebound range an unreliable measure of both texture and elasticity coefficient. The experiments have shown that the error is quite large for the small fractions at either extreme of the distribution pattern, where peas with the same rebound range can have quite different textures. However, the error is much smaller for the larger fractions surrounding the average range, and by restricting the data to these fractions a consistent relationship between rebound range and texture can be obtained. The results for nine batches of peas using this method are shown in Fig. 4.

The rebound range provides a convenient empirical measure of elasticity and texture for the practical assessment of quality. Average values of the elasticity coefficient can be estimated from the rebound range by calculating the rebound range of perfect spheres, having known coefficients of elasticity, from the geometry of the apparatus.

Thus, rebound range $= t\,v\cos(\phi + \alpha - 90)$ where

$$t = \left[-v\sin(\phi + \alpha - 90) \pm \sqrt{v^2\sin^2(\phi + \alpha - 90) + 2gh}\right]/g$$

$$v = u\sqrt{\sin^2\alpha + e^2\cos^2\alpha}$$

ϕ = the angle at which the pea leaves the plate (see Fig. 1) $= \cot^{-1}(e\cdot\cot\alpha)$

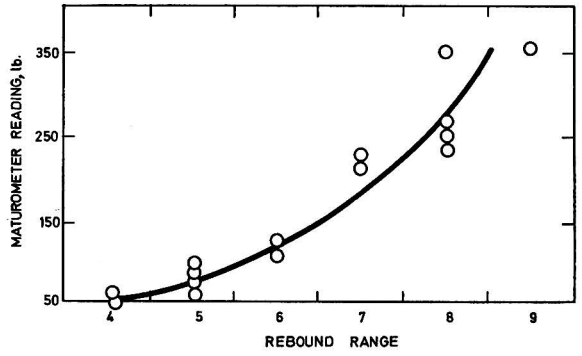

FIG. 4.—*Relationship between texture and range of the larger fractions only*

α = the angle of the plate to the horizontal

u = the velocity of the pea just before hitting the plate

e = the coefficient of elasticity of the pea

h = the height of the point of impact above the collecting box

and g = the gravitational constant.

The average elasticity coefficient of each fraction of cooked peas was interpolated from this calculated calibration curve. Values for the nine batches of peas used in the previous experiment are plotted against maturometer readings in Fig. 5.

The maturometer compresses and then pierces the peas with blunt pins, and actually measures some function of the shear strength of the peas. When the peas are relatively soft, the shear strength is almost directly proportional to the elasticity coefficient of the peas, but as the shear strength increases there is a progressively lower increase in the elasticity coefficient, and when the maturometer readings are over 300 lb. the shear strength appears to increase independently of the elasticity coefficient.

It has been shown that the efficiency of the apparatus in sorting cooked peas into fractions according to their elasticity coefficient is limited by the natural scatter arising from the asymmetry of the peas. Despite this defect in selectivity, the method still provides a good measure of the variation of texture in a batch of peas.

Thirteen commercial samples of canned dried peas were examined by a quality inspection panel for evenness of texture. The panel assessed the peas by spooning through them, testing

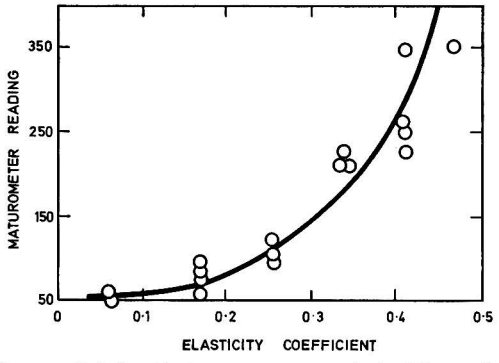

FIG. 5.—*Relationship between texture and elasticity coefficient of the larger fractions only*

the response of individual peas to pressure exerted by the spoon, and chewing the peas at random. Each batch was scored between 1 and 10, 1 representing extreme variation in texture from very soft to very hard, and 10 representing complete homogeneity of texture. The peas were then fractionated on the basis of their elasticity coefficients in our apparatus. The ' spread ' was calculated for each batch from the distribution histogram. The two methods are compared in Fig. 6, and show good agreement, having a correlation coefficient of −0·735 (significant at the 1% confidence level).

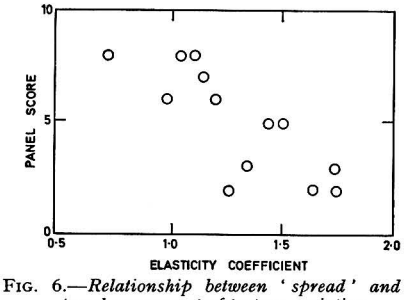

FIG. 6.—*Relationship between ' spread ' and a panel assessment of texture variation*

A bad practice sometimes encountered in the industry is the bulking of dried peas of different ages and origins. This usually leads to extreme non-uniformity of texture in the canned peas. When samples of this type are fractionated, the histogram usually has two peaks and a very large spread. A typical example is shown in Fig. 7, which was obtained when two

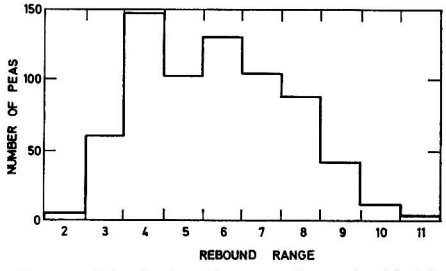

FIG. 7.—*Distribution histogram for a mixed batch of peas*

batches of peas, which had average textures of 5·2 and 7·0, and respective spreads of 1·57 and 1·71, after a standard process, were mixed and then subjected to the same process. Two peaks are shown, and the spread was increased to 1·85.

Conclusions

When cooked peas are bounced off an inclined surface, the horizontal distance they rebound is a function of their elasticity coefficient and is related to their texture as measured by the maturometer or a tasting panel. Batches of peas can be separated into a number of fractions with gradually increasing coefficients of elasticity. The separation is not perfect, as even rheologically identical peas have a natural distribution pattern arising from their asymmetry, so that the elasticity coefficients and texture of all the fractions from any particular batch tend to be influenced by scatter from the larger fractions. Nevertheless, the fractionation is sufficiently efficient to provide a good indication of the distribution of rheological properties within any batch. The standard deviation of the rebound range is a measure of the variation in texture in a sample of cooked peas, and correlates well with a tasting panel assessment.

Acknowledgment

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The Fruit and Vegetable Canning and Quick
Freezing Research Association,
Chipping Campden, Glos.

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THE OXIDATION OF ASCORBIC ACID AND ITS IMPROVER EFFECT IN BREAD DOUGHS

By PETER MEREDITH

The breadmaking improver action of ascorbic acid has been determined by measuring its effect on load–extension curves of fermenting doughs. By mixing in nitrogen it is shown that air is involved in the reaction and that the oxidation product dehydroascorbic acid, can function equally well as an improver in nitrogen or air. The improver and oxidation reaction are partly inhibited by sodium diethyldithiocarbamate but not by versene. The oxidation–reduction system re-uses ascorbic acid during fermentation. Oxidation catalysts present in flour are not limiting in the ascorbate improver reaction. Bromate accentuates ascorbate improvement so that a mixture of bromate and ascorbate is more effective than the sum of their separate actions. Ascorbate and dehydroascorbate have both an immediate improver effect and an effect which increases with time.

Introduction

With the advent of faster breadmaking processes, L-ascorbic acid (AsA) seems likely to increase in importance as a bread improver in those countries where other fast acting improvers are not permitted. Melville & Shattock[1] working in this laboratory postulated that AsA in dough was oxidised by atmospheric oxygen to dehydroascorbic acid (DHA), that this oxidised form was the actual improver and that the oxidation of AsA was mediated by an oxidase present in flour. Such an oxidase has been observed by Szent-Gyorgyi[2] in plant extracts. It was pointed out by Crook[3] that Szent-Gyorgyi had also observed the effect of a reductase system mediating the reduction of DHA by glutathione. There has been a recent revival of interest in this reductase system[4–6] as possibly applying to the dough system. Tsen[4] has pointed out that AsA may be oxidised by bromate. A mixture of the two has already been used in baking studies in Australia[7] as an improver superior to AsA alone.

The oxidase system as an explanation of the improver action of AsA has been no more than a strongly supported postulate. The purpose of the present work is to demonstrate directly the role of atmospheric oxygen and to attempt to assess by inhibition studies the part played by an oxidase system. The rate of the reaction has also been investigated. Increase in resistance of dough to stretching or mixing has been used to assess improver action.

Experimental

Only one flour has been used in most of the work; a bakers' flour milled to 78% extraction from New Zealand wheats. The protein content was 10·4%, ash 0·65% and moisture 13·8%.

DHA was prepared as the crystalline methanol complex[8] by a modification of the method of Kenyon & Munro.[9] The hygroscopic crystals, m.p. 102–104° (uncorrected), were stored over sulphuric acid. The complex was assumed to contain one molecule of methanol.

A crude enzyme preparation was made from cauliflower florets by extraction into M/15 phosphate buffer, pH 5·9, and precipitated twice by saturation with ammonium sulphate. A similar crude enzyme preparation was made from flour, soluble at 30% saturation with ammonium sulphate but precipitated at 100% saturation. The activity found by reaction in solution was such that the extract of 300 g. of flour would catalyse the oxidation of 0·5 mg. of AsA in 5 min. at 27° and pH 6·0 by atmospheric oxygen.

Doughs were mixed, usually for 5 min. in a Farinograph operated at 60 r.p.m., using a plated 300-g. bowl. Each mix consisted of 300 g. of flour with 150 ml. of liquor containing 9 g. of salt, 6 g. of sugar and 7·5 g. of compressed yeast.

When anaerobic conditions were required, mixing was carried out in a rapid current of water-saturated nitrogen. Flour was evacuated and replaced with nitrogen three times then stored overnight under nitrogen. Dough liquor was vigorously bubbled with nitrogen for 10 min.

Additives were included in the dough liquor at the following concentrations, p.p.m. flour basis: potassium bromate 30, potassium iodate 10, ascorbic acid 30, dehydroascorbic acid–methanol complex 35·1, sodium diethyldithiocarbamate (DDC) 100, oxine (8-hydroxyquinoline) 100, Versene (ethylenediaminetetra-acetate) disodium 100. The amount of enzyme solution added to each dough was sufficient to oxidise in solution 5 mg. of AsA in 10 min. at 27°.

The doughs were allowed to ferment for varying periods (reaction time) in closed containers at 27° then rounded by hand, moulded on a 'National' three-roll moulder and impaled in Brabender extensograph holders. After exactly 45 min. (relaxation time) the dough specimens were stretched on a Simon Extensimeter modified[10] to take the Brabender dough holders. The extensimeter had also been modified to give 1·45 cm./sec. rate of extension and 1 chart unit per g. load sensitivity; the curve drawn then approximates to an enlargement of that from a Brabender Extensograph.

From the load–extension curves thus obtained, the resistance of the dough when the hook had travelled 9·25 cm. was determined. Differences in this resistance at constant extension have been used as a parameter of improving action at any particular reaction time. Values presented in the figures are for differences between two doughs, a 'test' and a 'control'. All experiments have been duplicated from separate mixings of dough and the studies with DDC have been repeated with another similar flour and a fresh sample of inhibitor.

Extensograph[11, 12] and Alveograph techniques[13, 14] have been used previously to demonstrate an increase in strength of doughs by AsA treatment.

Results and discussion

A comparison of improver effects in yeasted and non-yeasted doughs (Fig. 1) showed that fermentation had a much larger effect on improvement by AsA than on improvement by iodate or bromate. It is assumed that utilisation of the available oxygen by yeast competes with the oxidation of AsA to DHA. The duration of mixing has a much more drastic effect on AsA improvement than on iodate or bromate improvement in unyeasted doughs (Fig. 2), which again may be explained by the involvement of atmospheric oxygen. This effect was observed by Sandstedt & Hites[15] in their baking experiments.

The role of oxygen can be more directly demonstrated by a comparison of doughs mixed in presence or absence of air (Fig. 3), when improvement by AsA is seen to be dependent on the atmosphere. Bromate improvement was slightly increased by mixing in nitrogen (not illustrated). As expected, the postulated oxidation product (DHA) was an improver even when mixing was carried out in nitrogen (Fig. 3).

Since an oxidase is postulated as intermediary in the oxidation of AsA, the effects of a chelating agent, DDC, which inhibits ascorbate oxidase activity by removal of prosthetic copper, were

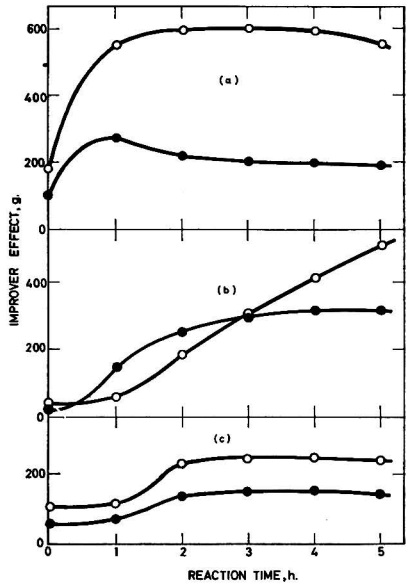

FIG. 1.—*Effect of fermentation on improvement by* (*a*) 30 *p.p.m. ascorbic acid,* (*b*) 30 *p.p.m. potassium bromate,* (*c*) 10 *p.p.m. potassium iodate.*

Doughs were mixed in air. ○ without yeast. ● with yeast.

observed (Fig. 4 and Table II). Inhibition of the improver effect of AsA was considerable and immediate. There was also inhibition of the improver effect of DHA, though not immediately. That DDC could inhibit the improver effect of DHA may be taken to imply re-use of AsA, with DHA being reduced to AsA and re-oxidised by air. It must be stressed that at no time was improver action in dough completely inhibited.

A crude extract of flour showed ascorbate oxidase activity which in solution was completely inhibited by DDC but not by Versene (Table I). Crook[3] showed that a dehydroascorbate reductase preparation was not inhibited by DDC. The dehydroascorbate reductase system of

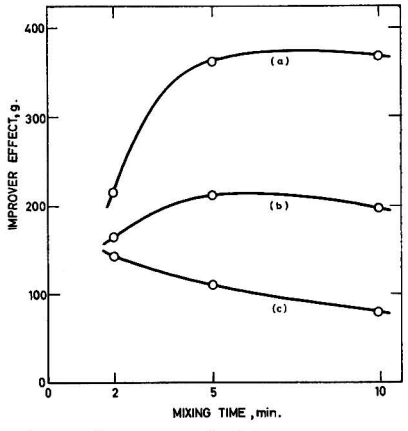

FIG. 2.—*Effect of varied mixing time on improvement by* (*a*) 30 *p.p.m. ascorbic acid,* (*b*) 30 *p.p.m. potassium bromate,* (*c*) 10 *p.p.m. potassium iodate*

Reaction time 2 h. Doughs were mixed in air, without yeast.

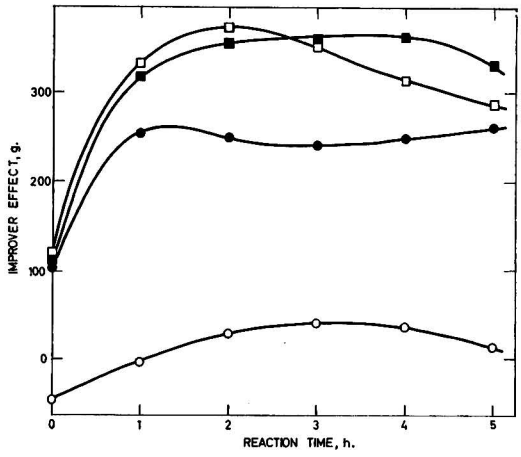

FIG. 3.—*Effect of air on improvement by ascorbic acid* (○) *and by dehydroascorbic acid* (□) *in fermenting doughs*
○ □ doughs mixed in nitrogen. ● ■ doughs mixed in air.

flour is scarcely affected by either DDC or Versene (Table I). The reductase activity was much greater than the oxidase activity, as has been found for many plant extracts,[16] so that reductase activity is not likely to be limiting in the improver action.

The inhibition of improver effect by DDC calls for comment. Kuninori & Matsumoto[12] found no inhibition at zero reaction time and only a small inhibition after 3 h. reaction, using 33 p.p.m. DDC in doughs. On the other hand Proskuryakov & Auerman[14] showed a large inhibition by DDC of oxidation of AsA by flour suspensions. Their concentration of DDC was probably 500 p.p.m. (flour basis). The present work (Table II) showed both an inhibition of AsA improvement by 100 p.p.m. of DDC in the dough and, at the same time, an improving effect of DDC itself on the dough. This inhibition and improvement may be of similar magnitude so that little effect may be observed unless proper controls are used. Kuninori & Matsumoto show only one control relaxation curve for each reaction time, although they demonstrated in their extensograms a large improver effect by 500 p.p.m. DDC. It can be suggested that a complexing

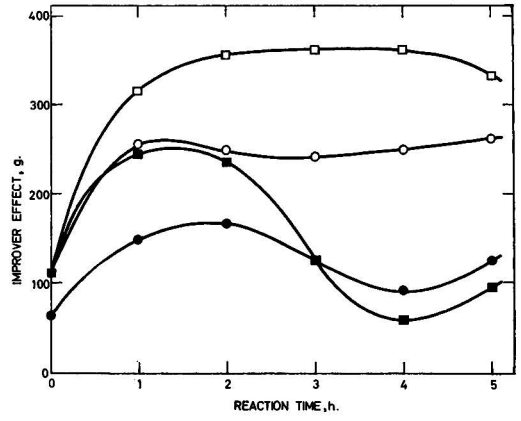

FIG. 4.—*Effect of DDC on improvement by ascorbic acid* (○) *and by dehydroascorbic acid* (□) *in fermenting doughs mixed in air.*
○ □ without DDC ● ■ with DDC and corrected by a control containing DDC.

Table I

Effect of chelating agents on ascorbate oxidase and dehydroascorbate reductase activities of flour, measured in solution

(Oxidase activity of 20 g. flour acting on 1 mg. of ascorbic acid in 2 ml. of solution pH 6·0 at 20° for 10 min.
Reductase activity of 6 mg. of flour acting on 0·5 mg. of dehydroascorbic acid and 2 mg. of glutathione in 2 ml. of solution pH 6·2 at 27° for 10 min.)

	Oxidase	Reductase
	Ascorbate oxidised, μg.	Ascorbate formed, μg.
No addition	71	28
Versene, 100 p.p.m.	60	25
DDC, 100 p.p.m.	0	25

Table II

Effect of chelating agents on fermenting doughs, mixed in air

The units are load (g). supported by dough at 9·25 cm. extension. Columns (*a*) and (*b*) are for different flours.

Chelator	Reaction time, h.	Inhibition of effect of 30 p.p.m. of AsA by chelator		Improver effect of chelator alone		Residual effect of AsA in presence of chelator	
		(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
DDC	0	60	96	0	9	60	84
	2	80	74	135	16	170	296
	4	160	192	145	57	90	78
Oxine	0		−10		76		190
	2		58		43		312
	4		14		26		256
Versene	0		−11		18		191
	2		−15		−1		385
	4		33		−14		237

agent such as DDC may decrease the availability of certain metal ions normally inhibiting yeast growth or fermentation.

Although the addition of oxidase to AsA-treated dough had been demonstrated by Kuninori & Matsumoto[12] to have no beneficial effect, the experiment has been repeated with ascorbate oxidase prepared from cauliflower florets. There was a small increase in improver effect after 2 h. but not sufficient to warrant further consideration. There is evidently enough oxidising catalytic power present in flour for the AsA improver effect.

Sandstedt & Hites[15] and Maltha[11] showed that dehydro-D-ascorbic acid had no marked improver action. This specificity for the laevo form suggests that the reaction of DHA with flour is entirely enzyme catalysed. Also Yamaguchi & Joslyn[17] showed that the reaction of DHA with glutathione in solution was predominantly enzymic in their preparation from peas. The observations of Sandstedt & Hites and of Maltha that D-glucoascorbic acid is not an improver do not allow us to decide whether the oxidation of AsA to DHA is entirely or only partly enzymic. Maltha[11] concluded that there was a thermostable oxidation catalyst in addition to the enzyme. The presence of many trace metals in flour and bread was established by Sullivan[18] and these could be catalytic though we would expect most to be inactivated by DDC. Versene will inhibit the catalytic effect of copper whilst leaving the activities of ascorbate oxidase and phenolase unimpaired,[19] the oxidase being, of course, a protein containing strongly bound copper.[20] Thus a comparison of inhibition by DDC and by versene of AsA improvement gives a measure of the enzyme-catalysed and metal-catalysed reactions. The experiments (Table II) showed little inhibition of AsA improver action by Versene, suggesting that most of the inhibition by DDC is

inhibition of enzyme activity. The degree of inhibition by DDC observed in these experiments is of the same order as that observed by Proskuryakov & Auerman[14] in flour suspensions. The remaining improvement by AsA in the presence of DDC is unexplained. Maltha[11] suggests that his ' heat-stable ' factor may be an organic-metal complex.

In these experiments oxine has occupied a position intermediate between Versene and DDC; alone it had an improver effect, but there was little inhibition of the AsA improver effect. Oxine produced a marked grey colour of the doughs which increased with duration of fermentation.

When doughs were prepared in nitrogen, the mixture of bromate and AsA was much more effective than bromate alone, whilst AsA alone had virtually no effect. Thus the increase in resistance after 1 h. reaction was for AsA 0, for bromate 120, for AsA plus bromate 235. Presumably bromate has oxidised AsA to DHA. As relatively anaerobic conditions prevail in dough, this synergistic combination of bromate and AsA (see [4]) serves as an innocuous rapid-acting improver for short fermentation processes. It is of course well known that AsA and bromate react chemically in a spectacular and possibly dangerous manner.[21]

Just how fast AsA acts as an improver in dough cannot be measured by the extension technique described. When doughs are mixed in a Farinograph, allowed to rest and remixed, the improver action can be measured as a stiffening of the dough compared with a control. Such measurements were taken for varying times up to 45 min. and extrapolated to zero time as shown in Fig. 5. As expected, iodate gave an almost immediate improvement with no increase with time and bromate gave no immediate improvement but there was an effect increasing with time. AsA gave some immediate effect and an increase with time, while DHA gave about twice the immediate effect of AsA and an increase with time.

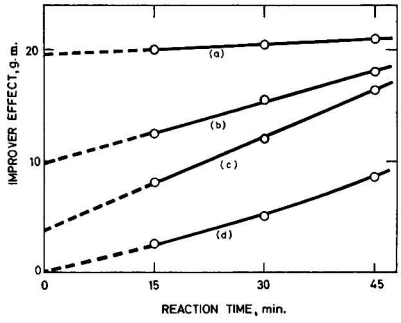

FIG. 5.—*Extrapolation to zero reaction time of improvement of non-fermenting doughs by (a) 10 p.p.m. potassium iodate, (b) 35 p.p.m. dehydroascorbic acid, (c) 30 p.p.m. ascorbic acid (d) 30 p.p.m. potassium bromate*

Summary

To summarise, it is demonstrated that AsA requires air or bromate to produce improvement of dough. The improvement is both immediate and time dependent. Neither the oxidative nor the reductive catalysis stages of the reaction sequence is limiting for dough improvement under our conditions. While the reductive phase is probably entirely enzymic, the oxidative catalysis is only partly enzymic. The non-enzymic oxidative catalysis is not inhibited by metal-chelating agents.

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EFFECTS OF TYPE OF POTASH AND RATES OF NITROGEN, PHOSPHATE AND POTASSIUM IN FERTILISERS ON GROWTH, YIELD AND QUALITY OF POTATO CROPS

By R. HENDERSON

Eleven experiments were made in Scotland to compare the effects of fertiliser sulphate and muriate of potash on the growth, yield and quality of potato crops. Potassium bicarbonate was also tested in nine of the experiments. There were two rates of nitrogen and potash in all cases and of phosphate in six.

In general, sulphate gave more seed, less ware and more total crop than muriate, indicating that both the number and the size of tubers were affected by the exchange, number more than size. There were large differences between sites in the exchange effect, but the reasons for this were not clear.

Sulphate generally gave a drier potato with higher content of dry matter and higher specific gravity than muriate, and also a mealier product when cooked. Intersite differences in quality were very large.

The higher nitrogen rate reduced seed and total yields but the higher phosphate rate increased yield. The higher potassium rate reduced the weight of seed but increased ware and total yields.

Introduction

Gething in 1959,[1] in discussing sulphate or muriate of potash for potatoes, suggested that differences in favour of sulphate might have increased with intensification in fertiliser practice since the last war. He noted that there was no published post-war experimental evidence about use of the two types of potash on potatoes, and added that field trials had been started in England in 1958 to get such evidence. He reported that there was a much larger average yield difference between sulphate- and muriate-treated crops in the 1958 trials than was obtained in pre-war work. This result led to further investigation, including a series of experiments carried out in Scotland in co-operation with the three Colleges of Agriculture from 1959 to 1962. There were eleven successful experiments in the series and these are described.

Experimental

The object of the work was to determine the main and interaction effects of varying the type of potash and the rates of nitrogen, phosphate and potash application on the growth, yield and quality of the potato crop. The primary concern was a comparison of muriate and sulphate of potash at different rates of application. The N and P variables were included principally to see if they affected this comparison. Bicarbonate was used as a third potash type in all experiments except those in 1959. It was hoped this might help to explain the results of comparisons between the other two types.

The treatments were factorial combinations of potash type and nutrient rates. The fertilisers used to supply nitrogen and phosphate were urea (46% N) and monoammonium phosphate (11·8% N and 55% P_2O_5) in 1959, and Nitro-chalk 21 with monoammonium phosphate (12% N and 60% P_2O_5) in all other years. Fertiliser grades of muriate, sulphate and bicarbonate of potash (60, 50 and 46% K_2O respectively) were used throughout. Details of the nutrient rates and of the factors included in each experiment are given in Table I. A yard strip at each end and usually one drill at each side of every plot were discarded before recording observations. In certain cases the side discards comprised three drills. Details of the experimental designs and of the plot dimensions are included in Table I.

All the experiments were made on commercial farms, nine in East Scotland—from East Lothian to Aberdeenshire—and two in West Scotland (Dunbartonshire). All were sited in fields growing potatoes in the course of the farm rotation. In eight cases the crops were grown primarily for production of seed; the other three were ware crops. Husbandry was the same as in the rest of the field, except for manuring, planting and, in some cases, harvesting. Varieties and purposes of the crops are given in Table I.

All the soils were medium in texture and were considered moderately to highly fertile. Chemical analyses of soil samples, taken from each site before the treatments were applied, showed that the pH was generally suitable except possibly in the case of experiment 60/28, where—at 7·0–7·5—it was rather high. Available phosphate varied around medium levels except at the site of 60/28, where most plots were very high or extra high. Available potash varied considerably at most sites; in certain cases the range was from very low to extra high. The most general level was slightly on the low side of medium, except at sites 59/25, where most plots were moderate to high, and 59/26 where most were low or very low.

Sites were usually marked out and soil samples taken soon after the fields were ridged. The fertilisers were applied by hand along the bottom of the drills. They were lightly raked into the soil except at the sites of 60/27, 61/14 and 61/15; rain began before this could be done at

Table I

Experimental variables, designs, plot sizes and crop type

Experiment No.	Nutrient rates in lb. per acre N	P_2O_5*	K_2O	Potash types	Experimental designs	Plot sizes (no. of drills × length in ft.) Overall	Recorded	Crop Variety	Purpose
59/25	56 & 112** and	45 & 90	90 & 180	Muriate and	2^4 in 6 randomised blocks of 8 plots each. NPKT interaction wholly confounded	4 × 72	2 × 66	Majestic	Seed
59/26	66 & 102			sulphate	with block differences	4 × 72	2 × 66	Majestic	Seed
60/27				Muriate	3×2^3 in 12 randomised blocks	6 × 33	4 × 27	Majestic	Seed
60/28	56 & 112	45 & 90	90 & 180	sulphate	of 6 plots each. NP, NK,	6 × 33	4 × 27	Majestic	Seed
61/14				and	PK, NPT, NKT and PKT	6 × 20	4 × 14	Arran Pilot	Seed
61/17				bicarbonate	interactions partially confounded with block differences	6 × 23½	4 × 17½	Redskin	Ware
60/30					3×2^3 in 6 randomised blocks	6 × 33	3 × 27	Majestic	Seed
61/15	56 & 112	60	90 & 180	,,	of 6 plots each. NK and	6 × 23½	4 × 17½	Redskin	Ware
61/16					NKT interactions partially confounded with block differences	6 × 25	3 × 19	Majestic	Seed
60/29	56 & 112	80	112 & 224	,,	ditto	6 × 33	3 × 27	Redskin	Ware
62/10	56 & 112	60	90 & 180	,,	3×2^3 in 4 randomised blocks of 12 plots each	6 × 20	4 × 14	Majestic	Seed

Notes on Table I

** A mistake was made in calculating urea requirements for treatments combining single nitrogen with double phosphate, and double nitrogen with single phosphate, in experiments 59/25 and 59/26. Plots receiving these treatments got 66 and 102 lb. of N per acre respectively. Other plots got the correct rates of 56 and 112 lb. of N/acre.

* Where only one P_2O_5 rate is given, this was applied to all plots.
Drill widths were 28 in. in all cases except No. 59/25 (27 in.) and No. 60/27, 61/17 and 61/15 (26 in.)

the last two of these sites. Commercial seed, provided by the farmers, was planted as soon after fertiliser application as possible. Ridges were then split to cover the seed.

Conditions were dry at and for some time after planting for both experiments in 1959 and for 60/28. In all other cases there was ample moisture in the soil at planting time and later. The soil was very wet at planting in the case of experiment 61/15, where heavy rain fell before and just after the fertilisers were applied.

Growth was moderate to good at all sites except 60/28, where moisture was short and pH rather high, 60/29, where weeds were plentiful, and 61/17 where seed was badly infected with skin spot disease. At this last site, emergence and growth were slow and most uneven and some seed failed to develop in most of the plots. Blight affected all crops quite seriously in 1960 and 1962 but was not so severe in the other years. Haulms were destroyed, by spraying or pulverising, at all seed-producing sites between early August and early September. Crops were usually lifted 4–6 weeks afterwards, by hand in some cases and by machine in others. Very poor weather delayed lifting until mid November for 60/28.

Emergence counts were made at a few sites and top growth was scored for vigour at some. The numbers of plants per plot were counted just before lifting in the case of 61/17, where growth had been affected by skin spot disease.

Soon after being lifted, all crops were riddled through $1\frac{1}{4}$ and $2\frac{1}{4}$ in. riddles at all seed-producing sites, $1\frac{1}{4}$ and $1\frac{3}{4}$ in. riddles at two ware sites and $1\frac{3}{4}$ in. riddle only at the third ware site—60/29. Diseased and damaged tubers were discarded during riddling. At this stage, too, samples of about 4 lb. of ware-sized potatoes each were drawn from individual plot yields.

At the request of The Edinburgh School of Agriculture, the Food Science & Plant Health Division of the Ministry of Agriculture, Fisheries and Food agreed to carry out tests of the eating quality of these samples, in view of their general interest in the factors affecting eating quality in potatoes. Every plot was sampled for the tests in both 1959 experiments, but, in 1960 and 1961, only plots which received sulphate or muriate of potash with single rates of both nitrogen and phosphate were sampled. No samples were taken in 1962. A trained and experienced taste panel allotted scores in 1959 for disintegration, colour, discoloration, humidity, mealiness, consistency, grain structure, flavour and off-flavour. In 1960, tasting methods were again used to assess disintegration, colour, humidity, mealiness, consistency and off-flavour. (The tasting procedure was that described by Harries.[2,3]) In 1961, since it had become apparent that mealiness was the only characteristic affected by exchanging the types of potash, laboratory examinations were made of moisture content, specific gravity, tenderometer characteristics and of the microscopical appearance of the cell structure and contents. Specific gravity determinations were made by placing tubers in solutions of common salt. The specific gravity of the tuber was taken to be equal to that of the brine solution in which it just sank.

Yields were weighed in every experiment and tuber numbers were counted in three (61/14, 61/17 and 62/10). The two crop fractions containing the largest and the next largest tubers were weighed separately in every trial. In a few cases chats and damaged tubers were also weighed. For 62/10 the total crop was weighed as lifted, before riddling. Tubers were counted in the fractions containing the largest and next largest potatoes.

Quality and yield data were analysed statistically. The quality analyses were done for sites individually and in groups, while yields were analysed for each site separately. Something more than the normal analysis of variance was required for experiments 60/29 and 61/17. In the former case, yields were consistently greater in the four plots to the east of each block than in the remaining two plots for no apparent reason. This increased error and probably distorted the estimates of treatment effects. It was suggested that co-variance analysis on the lines described by Federer & Schlottfeldt[4] and discussed by Outhwaite & Rutherford,[5] might help to control the effects of these yield gradients, and this was done successfully. An ' x ' variate of $+2$ was allotted to each of the two plots at the lower end of the gradient in each block, and one of -1 to each of the remaining plots. In experiment 61/17, where skin spot had been found, it was thought that the variation in plant numbers per plot would add to error, possibly quite substantially. Co-variance analysis was tried here too, using plant number as the ' x ' variate; the attempt was abandoned, however, when it emerged that certain treatments had

slightly—but significantly—affected plant numbers. It might be added that these effects were small in comparison with those of the disease.

Results

Plant emergence and top growth

Emergence was slower with muriate than with sulphate of potash at site 59/26. Top growth early in the season was better with sulphate than with muriate at sites 59/25 and 59/26; this difference became less apparent later. Higher rates of nitrogen and potash were often associated with slower, early top-growth than were the lower rates. These differences also became less marked later, and in the case of nitrogen were reversed later still. Mid-season top-growth was markedly more vigorous at higher than at lower phosphate rates.

At certain sites there were marked differences in top growth between crops treated with bicarbonate of potash and those receiving the other two forms of potassium. The former grew noticeably more strongly in 1960, but very much less strongly at two sites in 1961. At one of these (61/14) haulms in bicarbonate-treated plots were much less vigorous, died back more quickly and showed signs of what was taken to be magnesium deficiency to a far greater extent, than haulms in plots receiving other types of potash.

Potato eating quality

Mealiness was the only characteristic examined in the tests of the 1959 and 1960 samples which showed any marked effect of exchanging sulphate and muriate of potash. The effect was not consistent. At one site in 1959 potatoes grown with muriate of potash were mealier, disintegrated more in cooking and discoloured more after cooking than those grown with sulphate. At the other site in the same year, sulphate gave a mealier potato than muriate. A similar situation occurred in two of the 1960 sites. Differences in almost all the characteristics studied were much greater between sites than between one and the other type of potash.

The 1961 tests showed that tuber moisture content and specific gravity were affected by the exchange of sulphate and muriate of potash. Potatoes grown with sulphate tended to be drier than those grown with muriate, but this was not equally apparent at all sites, nor was it so with raw and cooked samples. Tuber specific gravity tended to be higher with sulphate than with muriate, but there were large variations in specific gravity between sites and even between tubers within single samples. Average moisture contents are given in Table II. Table III

Table II

Average moisture content (%) of potato samples

Sample state	Potash type	Experiment No. 61/14	61/15	61/16	61/17
Raw	Sulphate	82·17	78·17	82·68	79·63
	Muriate	82·80	79·47	83·35	80·35
Cooked	Sulphate	80·84	81·79	81·97	81·07
	Muriate	81·90	82·20	82·10	81·40

Table III

Average concentrations (%) of brine solutions in which tubers just sank

Potash type	Experiment No. 61/14	61/15	61/16	61/17
Sulphate	9·27	10·55	9·03	10·88
Muriate	8·84	10·13	8·51	10·69
Mean	9·05	10·34	8·77	10·79

gives average concentrations (%) of brine solutions in which tubers just sank, which show how tuber specific gravity varied. The only clear effect of potash type exchange shown by the tenderometer tests was that sulphate gave a harder mash than muriate at one site. Microscopical examination did not reveal any clear-cut reason for this difference.

Tuber yield

Yield is considered here mainly in terms of crop fractions differentiated by riddling. The fractions containing the largest size tubers are called ' ware ' and all containing the next largest are called ' seed ' regardless of the gauge of the riddle which separated them or of the main purpose for which the crops were grown. Weights of seed and ware are treated separately for every experiment except 60/29, for which only ware is considered. Numbers of seed and ware tubers are considered separately and together where counts were made. Total crop weight is considered for every experiment, but the term varies a little in application. It means the whole crop as lifted for 62/10; the collective weight of seed, ware and chats for 59/25, 59/26 and 60/30; the sum of ware and all other sound tubers for 60/29; and the sum of seed and ware for all other experiments.

Treatment interactions were generally infrequent in these experiments. The results given in Tables IV–VI are therefore limited to the mean yields, average differences between treatments, significant two-factor interactions ($P \leqslant 0.05$) and standard errors per plot. The functions used for comparing the types of potash are the difference between sulphate- and muriate-treated crops (S — M), and the difference between the average of sulphate- and muriate-treated crops and

Table IV

Principal differences between treatment mean yields at seed-producing sites (in tons of tubers per acre)

Expt. No.	General mean yield	$N_2 - N_1$	$P_2 - P_1$	$K_2 - K_1$	S − M	$\frac{S+M}{2} - B$	Significant two-factor interactions ($P \leqslant 0.05$)	Standard error of one plot
				SEED				
59/25	10·6	0	−0·03	+0·23	+0·93**	—	KT_1 + 0·83*	±1·109
59·26	7·4	+0·06	+0·40*	+0·25	+0·54**	—	—	±0·609
60/27	11·8	−0·84***	+0·72***	−0·70***	+1·03***	−0·79***	—	±0·834
60/28	6·2	−0·44**	+0·35*	+0·13	+0·44*	+0·18	—	±0·673
60/30	10·6	−0·07	—	−0·63*	+0·32	−0·23	—	±0·883
61/14	9·9	−0·10	+0·29	−0·38	+0·01	+0·74**	—	±1·015
61/16	9·1	−0·46	—	+0·11	+0·49	+0·30	—	±0·852
62/10	8·8	−0·75**	—	−0·09	+0·60*	+0·03	KT_2 + 0·59*	±0·814
				WARE				
59/25	5·0	−0·72**	+0·26	+0·14	−0·56*	—	—	±0·768
59/26	2·5	−0·38	+0·09	+0·25	−0·16	—	—	±0·651
60/27	8·0	−0·06	+0·21	+0·59**	−0·42*	+0·84***	—	±0·702
60/28	1·4	+0·04	+0·21	+0·24	−0·35*	+0·13	—	±0·567
60/30	5·3	+0·41	—	+0·50	−0·15	+0·26	—	±0·775
61/14	2·8	+0·34*	+0·17	+0·41**	−0·10	+0·81***	KT_2 + 0·43**	±0·582
61/16	2·8	+0·36	—	+0·36	+0·01	+0·03	—	±0·650
62/10	3·6	+0·02	—	−0·03	−0·39	+0·34	KT_1 + 0·59*	±0·595
				TOTAL YIELD				
59/25	16·5	−0·79	−0·02	+0·43	+0·31	—	KT_1 + 0·99*	±1·495
59/26	10·2	−0·29	+0·51**	+0·48*	+0·41*	—	—	±0·616
60/27	19·8	−0·89***	+0·93***	−0·11	+0·61*	+0·05	—	±0·906
60/28	7·7	−0·40	+0·57*	+0·37	+0·09	+0·32	—	±0·919
60/30	18·0	+0·51	—	−0·25	+0·25	−0·02	—	±1·076
61/14	12·7	+0·24	+0·47	+0·02	−0·09	+1·55***	—	±1·191
61/16	11·9	−0·10	—	+0·47*	+0·50*	+0·33	—	±0·577
62/10	14·5	−0·61*	—	−0·11	−0·43	+0·35	NK − 0·56* NT_1 − 0·74* KT_2 + 0·78**	±0·820

In column 8, T_1 and T_2 represent $S - M$ and $\frac{S+M}{2} - B$ respectively

*, ** and *** = statistical significance at the 5%, 1% and 0·1% points respectively

bicarbonate-treated ones $[(S + M)/2 - B]$. These were chosen because they best served the main purpose of the experiments and were orthogonal.

Most experimental factors affected yield at about two-thirds of the sites at which they were tested. Most of the effects were less than 1 ton of tubers per acre, but some were above this level. There was considerable inter-site variation in all effects except that of additional phosphate which was remarkably uniform. The relation between effects on seed and ware also varied substantially, from one factor to the other. It was most clear and consistent in the case of exchanging sulphate and muriate of potash. Interactions were generally infrequent, though less so between potash rate and type factors than otherwise.

Additional nitrogen tended to reduce yield, especially of seed and total crops. Ware was increased at two sites. There were some interactions with potash type and rate factors at site 62/10.

Yields were consistently and uniformly increased by additional phosphate. This was particularly true of seed and total crops. There were interactions with additional nitrogen and with potash type factors at one site, but tuber numbers only seemed affected.

Table V

Principal differences between treatment mean yields at ware-producing sites (in tons of tubers per acre)

Expt. No.	General mean	$N_2 - N_1$	$P_2 - P_1$	$K_2 - K_1$	S − M	$\frac{S+M}{2} - B$	Significant two-factor interactions ($P \leqslant 0.05$)	Standard error of one plot
				SEED				
61/15	1·1	+0·07	—	0	+0·07	+0·07	—	±0·216
61/17	1·0	−0·05	+0·08	0	+0·12*	−0·08	—	±0·178
				WARE				
61/15	11·7	+0·46	—	+0·33	−0·30	+0·93*	—	±1·141
61/17	9·7	+0·51*	+0·62**	−0·17	+0·02	+0·39	—	±0·943
60/29	7·2	+0·63	—	+0·72	+0·82	+0·86	—	±0·840
				TOTAL YIELD				
61/15	12·8	+0·53	—	+0·33	−0·22	+1·00*	—	±1·277
61/17	10·7	+0·46*	+0·71**	−0·18	+0·14	+0·31	—	±0·937
60/29	9·9	+0·91	—	+0·66	+0·68	+0·57	—	±0·992

Table VI

Principal differences between treatment mean numbers at various sites (in thousands of tubers per acre)

Expt. No.	General mean	$N_2 - N_1$	$P_2 - P_1$	$K_2 - K_1$	S − M	$\frac{S+M}{2} - B$	Significant two-factor interactions ($P \leqslant 0.05$)	Standard error of one plot
				SEED				
61/14	115·2	−2·0	+4·2	−4·9	−0·4	+4·3	—	±12·02
61/17	24·1	−1·7	+1·8	−0·3	+4·4**	−3·0**	PT_1 − 2·7*	± 4·40
62/10	106·8	−8·3*	—	−1·3	+4·6	+4·6	NT_1 − 8·5* KT_1 − 9·8* KT_2 + 7·5*	±11·13
				WARE				
61/14	11·3	+2·3***	+1·9**	+1·7**	−0·9	+3·0***	KT_2 + 1·9**	± 2·34
61/17	69·4	+2·0	+4·5**	−2·0	+3·1	−1·5	—	± 6·37
62/10	17·7	+0·3	—	−0·1	−2·1	+1·6	KT_1 + 2·6*	± 3·33
				SEED + WARE				
61/14	126·5	+0·4	+6·1*	−3·1	−1·3	+7·3*	—	±12·44
61/17	93·4	+0·3	+6·3**	−2·3	+7·5**	−4·5*	NP − 4·4* PT_2 + 4·8*	± 8·59
62/10	124·4	−8·0**	—	−1·4	+2·4	+6·1*	NT_1 − 7·0* KT_1 − 7·2* KT_2 + 8·3**	± 9·73

(For meaning of T_1 and T_2 in column 8 and *, **, *** see Table IV)

Additional potash tended to reduce seed yields and increase ware and total. As with additional nitrogen, however, there were considerable variations in the effects from site to site. There were several interactions with the potash type factors and these suggested that additional sulphate and muriate were rather more likely to increase yield than was additional bicarbonate.

The effects of exchanging sulphate and muriate of potash were notably consistent in direction but variable in size. Sulphate generally gave higher seed and total yields than muriate, but smaller ware crops. The effects on seed tended to be greater than those on ware. Interactions occurred, particularly with additional potash, but these showed no special trends. The exchange affected seed yield by as much as $1\frac{3}{4}$ tons per acre at site 59/25; this was the largest effect to be found in these experiments.

Exchanging bicarbonate and other forms of potassium affected yields at only half the sites, bicarbonate usually tending to give the lower yields. This was especially so at the higher rate at two sites, and also in terms of ware rather than seed.

Discussion

The exchange of sulphate and muriate of potash affected tuber yield in these experiments as much as, and generally more consistently than, the rate variables. In general, the effects on seed and ware fractions were opposite in direction—sulphate favouring seed and muriate ware, the effects on seed being generally the greater. Total yield tended to be affected in the same direction as seed. In an earlier series of experiments, Dickins *et al.*[6] found that—on the average—the exchange effects on yields of medium and large tubers were opposite in direction, as in the series reported here, but equal in size. Total yield was unaffected.

It is evident that the exchange of sulphate and muriate of potash influenced tuber size in both series—muriate giving the bigger potato—and further that this influence on size contributed proportionally more to the exchange effect on yield in the experiments of Dickins *et al.*[6] than at the seed sites. This may have been because tubers were generally bigger—fewer passed the $2\frac{1}{4}$ in. riddle—in the earlier series. Dickins *et al.*[6] noted that the exchange affected the number as well as the size of tubers, sulphate giving more than muriate. This is also apparent from the yield results reported here, although—unfortunately—the counts made are too few and too inconsistent to afford useful direct evidence. It is evident, too, from the yields at the seed sites, that the difference in numbers between sulphate- and muriate-treated crops contributed more to the exchange effect on yield than the difference in tuber size. In the experiments of Dickins *et al.*,[6] however, the two factors—size and number—contributed equally to the effect on yield.

Of the nutrient rate factors in these experiments, the effect of nitrogen on tuber number and size approximated most closely to the effect of potash exchange, but these nitrogen effects do not seem to be typical. They are akin to those found by Levington Research Station[7] at excessive rates, but not at low and moderate levels of application, and are different from those described by Garner[8] and Russell & Garner.[9]

The phosphate- and potash-rate effects were similar to those described by Garner[8] and Russell & Garner,[9] and to those found by Levington Research Station.[7] Phosphate mainly affected tuber number, while potash chiefly influenced the size of the tubers. The mechanism of these fertiliser effects on tuber number and size is not discussed here, but one aspect may be mentioned which has arisen repeatedly in considering these effects. It concerns the extent to which changes in number determine those in size. It would seem likely that there is a general difference in this between the exchange factor and nutrient rate variables. More particularly, it may be speculated that the exchange effect on size is largely due to its effect on number, whereas nutrient rate effects on size appear substantially independent of those on numbers. This suggestion may gain some support from the marked variation between fertiliser factors in relative effects on number and size.

Variation in the sulphate–muriate exchange effect on yield has been discussed by several authors.[1,6,9,10] There was clearly much variation in the exchange effect in the experiments reported here, but the cause is not clear—except in general terms. The overall mean effect of the exchange on total yield is very similar to those quoted by Russell & Garner[9] and Cowie,[10] so that the present evidence does not support the suggestion made by Gething[1] that the exchange

effect might have been increased by the general intensification in fertiliser use in recent years. The exchange effect was little altered by doubling the nitrogen and phosphate rates. Doubling the potash rate was more effective, but no real trend emerged. This paucity of interaction can hardly be regarded as conclusive, however, since the ranges of rates were very limited. Much variation in the exchange effect seems to have been due to inter-site factors, but there is no satisfactory way of identifying those primarily involved. For example, the heavy rain between fertiliser application and planting could have been associated much more convincingly with the absence of exchange effects at the 61/14 and 61/15 sites had it not been for the substantial effects of the exchange of bicarbonate and other potash at these sites. Overall differences in the proportion of the effects on the main crop fractions and in the effects on total yield have already been noted, between the experiments of Dickins *et al.*[6] and those at the present seed sites. It has also been pointed out that there are parallel differences in tuber size generally between the series. This suggests the possibility that the exchange effect may be influenced by the general level of tuber size in the crop and, further, that such major size determinants as seed size, seed spacing and haulm destruction may interact with the exchange factor and so vary its effect.

A general point which arises from the discussion is that the terms used in expressing total yield usually adopted in published accounts before Dickins *et al.*[6] are inadequate for describing the effects of exchanging sulphate and muriate of potash on potato yields. Fraction yields must also be included, particularly as one or other of these is often more important and valuable than the rest of the crop.

The results of the eating quality tests agree with previous findings, such as those of Harrap,[11] that sulphate of potash tends to give a drier, mealier potato of higher specific gravity than muriate of potash, although the effects were not large and did not always occur. The exchange of the two types of potash had no marked effect on any of the other characteristics of quality which were considered in these experiments. From the amount and type of variation in these characteristics, between sites, it appears that managerial and environmental factors had considerably more influence on tuber quality than the fertiliser variables.

There is a general tendency for the other types of potash to outyield bicarbonate. This is difficult to explain. Quidet *et al.*[12] found that bicarbonate often gave better results than sulphate or muriate. There seems no good reason, on general grounds, why the contrary should have been the case in the present experiments. The fact that the exchange effects were particularly large at sites 61/14 and 61/15, where rain fell soon after the fertilisers were applied and before they were covered, is especially difficult to explain. No reason has been found for the very marked differences in haulm growth between plots receiving bicarbonate and those treated with the other types of potash at site 61/14. It had been thought that the inclusion of bicarbonate in the experiments might simulate the supply of ' anion-free ' potassium to the crop and so provide a standard by which to distinguish individual effects of the sulphate and chloride ions. This was not pursued. The functions adopted for comparing the three types of potash were unsuitable for the purpose and the basis for the idea began to seem more open to question than had appeared at first. Certain of the effects ascribed to bicarbonate of potash by Garaudeaux & Beaucorps[13] seem to be due to the anion; application of this salt does not then simulate the supply of ' anion-free ' potassium and the comparisons envisaged do not reflect the effects of the sulphate or chloride ions.

The single-nitrogen and double-phosphate rates were generally preferable at seed-producing sites, and there is a suggestion that the double levels of both were the better at ware sites. At half the seed producing sites, the single-potash rate was preferable to the double; at the other half, the double rate was the better. There is a slight indication that the double rate was preferable, too, at ware sites.

The preference for the lower nitrogen rate, at seed producing sites, is in keeping with the findings of Simpson & Crooks,[14] but the present evidence suggests that a slightly higher general level of phosphate application than that advocated by these authors may well be justified for seed production and that a considerably higher level of potash application is not infrequently required.

Conclusions

The results of these experiments show that it is necessary to consider the main commercial fractions of the crop in choosing the type of potash to use in potato fertilisers. In terms of effects on these fractions the choice of sulphate or muriate of potash was sometimes of greater practical importance than were large increases in rates of applying nutrients. Sulphate was generally the best type of potash for seed production, but the evidence was inconclusive in regard to ware production. Although sulphate tended to give a drier potato than muriate, the exchange had generally smaller effects on potato quality than inter-site factors. The exchange effects on yield varied, but no more so than those of doubling N and K rates. Specific sources of variation could not be identified. It was not possible to say why the exchange had no effect on yield at a few sites. More detailed investigation would be justified of the exchange effects on tuber number and size.

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