VITAMIN CONTENTS OF AIR-CLASSIFIED HIGH- AND LOW-PROTEIN FLOUR FRACTIONS[1]

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ABSTRACT

The thiamine content of the fine (0-17μ) fraction, obtained by air-classifying a flour laboratory-milled from soft English wheat, was similar to that of the initial flour, while those of the medium (17-35μ) and the coarse (over 35μ) fractions were respectively rather lower and rather higher. With corresponding fractions from a hard English wheat, on the other hand, the value for the fine fraction was much higher and that for the coarse rather lower. The effect of grinding the flour with pinned disks, prior to air-classification, was to reduce the thiamine content of the coarse fraction and, in the case of the soft flour, to raise markedly that of the fine fraction. With the hard flour, the high thiamine content of the fine fraction was maintained, while (as with the soft flour) the yield of this fraction was greatly increased by the grinding.

The levels of niacin in the soft flour were not markedly changed by air-classification, with or without grinding, but with the hard flour, relatively high levels were found in the fine fractions. The riboflavin contents of the fine fractions from both flours were relatively high. The pyridoxine levels in the fine fractions were markedly high with the hard, but only slightly high with the soft, flour. With pantothenic acid, while the corresponding rise in levels was marked with the hard flour, it was not shown at all with the soft.

These effects may be largely explained on the basis of the thiamine and niacin contents of scutellum, aleurone layer, and endosperm present in the flours. These values, known from previous dissection studies, indicate that the initial flours both contained about 0.05% of scutellum, while the contents of aleurone layer were 0.28% in the hard and only 0.07% in the soft. Grinding the flours with pinned disks caused the scutellum and aleurone fragments to be shattered, so that the proportion present as particles under 17 microns rose from about 10% of the total present in the flour to 55 and 100%, respectively, with the hard flour, and to 40 and 60% with the soft. As a result, the scutellum contents of the fine fractions from the ground soft and hard flours were about 0.8 and 1.2%, and the aleurone contents, 0.2 and 1.4%, respectively. Fiber contents indicated that the aleurone was present in the detached form, free from outer parts of the bran, in the fine fractions. The contents of scutellum and aleurone mentioned account largely for the increased ash content in the fine fractions, the increase being particularly high in the case of the hard flour. They also account very largely for differences in contents of pyridoxine, pantothenic acid, and riboflavin between the fractions of different finenesses, except that, with the soft flour only, protein and riboflavin contents in the endosperm appeared to be directly related.

Great interest is now being taken in the possibilities of separating flour, by means of air-classification, into different portions, of high and low protein contents respectively. Jones, Halton, and Stevens (10) have described the mechanism and the principal effects of the separation,

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which depends on the fact that, during milling, some of the interstitial protein of the endosperm breaks up into fragments less than 15 microns in length. These fragments may accordingly be separated from the bulk of the starch granules, which exceed 15 microns in size. The proportion of separable protein may be increased by means of suitable pregrinding of the flour; this has the effect of disintegrating the coarser particles of endosperm which are present to varying extents in all flours. The effect of the grinding, and hence the scope of the whole process, is greater with softer flours such as those from some types of English wheats (which are often of relatively low protein content). The effect of the protein displacement is of potential practical interest in relation to bread- and cake-making qualities of the products. Any process of protein displacement is clearly also of potential nutritional significance, and on the same score it seemed desirable to ascertain whether corresponding displacement of various B-vitamins occurred.

Materials and Methods

Flour Samples. Two samples of flour were prepared by means of the Buhler laboratory mill from soft and hard English wheats respectively. The soft wheat, of low protein content, was a mixture of types (50% Cappelle, 25% Alba (white), 25% Peko) which would conventionally be regarded as suitable for making biscuits (cookies). To minimize bran contamination, the severity of the milling process was limited; the difference in extraction rates obtained with the two wheats reflected the difference in their natural yielding capacities. The yields and color grade values of the flours were: soft, 65%, 2.6; hard, 69%, 3.2.

Air-classification of the flours, into fractions consisting of particles lying between specified sizes, was effected by using a No. 132 Mikroplex Spiral Air Classifier on the lines described by Jones *et al.* (10).

Grinding of certain flour samples prior to air-classification was performed with a No. 4 Kek pinned-disk grinder (described by Jones *et al.*, 10), the shaft of which was driven at 1720 r.p.m. (giving a disk speed of about 11,000 r.p.m.).

Flour color grade values were determined by means of the Kent-Jones and Martin Color grader (11). *Ash* was determined on a 5-g. sample incinerated overnight at 600°C. in a silica dish. *Nitrogen,* determined by the Kjeldahl-Gunning-Arnold method, was converted to protein by the factor 5.7. Other determinations were made as follows: *thiamine,* by a thiochrome method based on the work of Ridyard (13); *fiber, riboflavin,* and *niacin* by the methods recommended for flour by the Analytical Methods Committee of the Society of Public Analysts (14,15); *pantothenic acid* by an adaptation of the method described by

Barton-Wright (2) in which Takadiastase was used in place of the chick liver enzyme and phosphatase; *pyridoxine*, by a modification of the method of Atkin *et al.* (1), in which the medium prescribed by Jones and Morris (9) was used, the agar being omitted. This method is essentially that described by Clegg and Hinton (3), differing only in the inoculation technique.

Results

Results obtained on variously treated subsamples of the soft flour are shown in Table I and on those of the hard flour in Table II. In each case, in test 1 the unground flour was separated by means of air-classification into three fractions, denoted as fine, medium, and coarse, consisting of particles lying between the size limits shown. The yields and protein contents in test 1 of Table I are in accordance with those reported as typical for a soft flour by Jones *et al.* (10), who also showed that with hard flours (ordinarily milled), as in test 1 of Table

TABLE I

BEHAVIOR OF SOFT ENGLISH FLOUR,[a] WITH RESPECT TO PROTEIN AND VITAMIN SHIFTS DURING AIR-CLASSIFICATION, WITH AND WITHOUT PRIOR GRINDING

FLOUR OR FRACTION	PARTICLE SIZE	YIELD	PROTEIN	THIAMINE	NIACIN	RIBO-FLAVIN	PANTO-THENIC ACID	PYRI-DOXINE
	μ	%	%	γ/g	γ/g	γ/g	γ/g	γ/g
	Test 1: Unground flour							
Initial flour		100.0	7.0	0.9	5.1	0.32	3.1	0.5
Fine	0–17	13.0	13.4	0.9	...	...	...	...
Medium	17–35	33.0	3.4	0.6	4.5	0.17	2.2	0.3
Coarse	over 35	54.0	7.8	1.1	...	...	...	...
	Test 2: Coarse fraction from test 1 ground with pinned disks, then classified							
Fine	0–17	11.0	14.7	1.9				
Medium	17–35	21.0	5.1	0.9				
Coarse	over 35	22.0	6.4	0.9				
	Test 3: Initial flour ground with pinned disks, then classified by process used in tests 1 and 2							
Fine	0–17	25.0	14.5	1.4	5.9	0.46	3.0	0.6
Medium	17–35	62.0	3.9	0.7	4.7	0.19	2.1	0.4
Coarse	over 35	13.0	5.9	0.7	...	...	...	...
	Test 4: Flour ground as in test 3, then classified with an additional stage							
1st Fine	0–7	7.0	22.5	2.0	6.7	0.63	3.0	0.8
2nd Fine	7–17	18.0	11.4	1.2	...	...	...	...
Medium	17–35	62.0	3.9	0.7	...	...	...	...
Coarse	over 35	13.0	5.9	0.7	...	...	...	...

[a] Values are expressed on a 14% moisture basis. Yields are expressed with respect to 100 parts of initial flour.

II, the yield of the fine fraction is lower, and the difference between its protein content and that of the initial flour (expressed as a proportion of that of the initial flour) is less than with soft flours.

In test 2, with both the soft and the hard flour, the coarse fraction from test 1 was ground (as described under "Materials and Methods") and subsequently air-classified. This operation gave a further yield of proteinaceous fine material, a result consistent with those obtained in the tests, numbered 3 in each series, in which the flour as a whole was ground prior to classification. The effect of the grinding on the yields and protein contents of the fractions from the soft flour, as reflected in the differences between the results of tests 1 and 3 of Table I, is closely similar to that reported by Jones *et al.* (10). In experiments with the grinder in these laboratories, it has been noticed that its effect is to increase the combined yields of the two finer fractions, and to decrease correspondingly the yield of the coarser fraction, by roughly the same amount with most types of flour, whether hard or soft. Thus, in the present case, the yields of the coarse fraction in tests 1 and 3 on the harder flour (Table II) showed a fall of 35 parts due to the grind-

TABLE II

BEHAVIOR OF HARD (ATLE) ENGLISH FLOUR WITH RESPECT TO PROTEIN AND VITAMIN SHIFTS DURING AIR-CLASSIFICATION, WITH AND WITHOUT PRIOR GRINDING

FLOUR OR FRACTION	PARTICLE SIZE	YIELD	PROTEIN	THIAMINE	NIACIN	RIBOFLAVIN	PANTOTHENIC ACID	PYRIDOXINE
	μ	%	%	γ/g	γ/g	γ/g	γ/g	γ/g
	Test 1: Unground flour							
Initial flour		100.0	8.8	0.9	6.5	0.27	1.5	0.6
Fine	0–17	3.0	13.0	1.8	13.1	0.49	2.1	1.2
Medium	17–35	15.0	4.3	0.7	5.4	0.19	1.6	0.4
Coarse	over 35	82.0	9.1	0.7	...	...	...	...
	Test 2: Coarse fraction from test 1 ground with pinned disks, then classified							
Fine	0–17	15.0	14.4	1.6				
Medium	17–35	27.0	6.3	0.6				
Coarse	over 35	40.0	9.3	0.4				
	Test 3: Initial flour ground with pinned disks, then classified by process used in tests 1 and 2							
Fine	0–17	20.0	15.0	2.2	13.9	0.51	2.4	1.3
Medium	17–35	33.0	5.8	0.7	4.8	0.20	1.3	0.4
Coarse	over 35	47.0	8.3	0.5	...	...	...	...
	Test 4: Flour ground as in test 3, then classified with an additional stage							
1st Fine	0–7	9.0	17.8	3.1	19.4	0.57	2.7	1.9
2nd Fine	7–17	13.0	12.5	1.4	...	...	...	...
Medium	17–35	33.0	5.6	0.7	...	...	...	...
Coarse	over 35	45.0	8.3	0.5	...	...	...	...

ing, the corresponding fall with the softer flour (Table I) being 41. Since, initially, the soft flour was much less coarse than the hard, the end result is that the ground product from the soft flour contains a higher proportion of fine material than that from the hard.

With both flours, test 4 was carried out similarly to test 3, except that an additional very fine separation (giving a "1st fine fraction") was made. The actual levels of protein in the fine fractions from both soft and hard flours (after grinding) were similar, except in the case of the 1st fines of test 4, where the hard flour did not give so high a level as the soft, no doubt because its protein tended to shatter less finely during grinding. In keeping with our experience with other flours, the spread between the protein contents of the fine and medium fractions, following grinding, was rather less with the hard than with the soft flour.

Thiamine Contents. Although the soft and hard flours are similar initially in thiamine content, they differ markedly in respect to its distribution among the fractions of different particle sizes. With the unground soft flour, the thiamine content of the fine fraction is similar to that of the initial flour, but that of the coarse is rather higher. With the hard flour, on the other hand, the thiamine content of the fine fraction is much higher than that of the initial flour and that of the coarse is rather lower.

The effect of grinding is to reduce the thiamine content of the coarse fraction and — in the case of the soft flour — to raise markedly that of the fine fraction. With the hard flour, the high thiamine content of the fine fraction is maintained, while (as with the soft flour) the yield of this fraction is greatly increased by the grinding. The inference is that some vitamin-rich tissue, present, initially, mainly in the coarse fraction, has been broken down relatively finely by the pinned-disk grinding process.

The indication from the results is that any association of thiamine with endosperm protein must, if it exists, be minor in degree, the major effect on the relative thiamine contents of the fractions being due to the behavior of a vitamin-rich nonendosperm tissue. With the soft flour, the evidence points to scutellum as the tissue in question. Stevens (16) has shown in these laboratories that laboratory-milled straight-run white flour may contain 0.3% scutellum. Table III shows that the content of scutellum (calculated from the analytical data[4] in the preceding tables), in the various fractions from the soft flour, ranged from 0.26 to 1.12, that in the initial flour being 0.44%. In particular,

[4] In conjunction with the basic values shown in the footnote to Table III: Simultaneous equations, with aleurone and scutellum contents denoted as x and y respectively, were solved by using the data for niacin and thiamine in that footnote, in conjunction with the niacin and thiamine values for the various

TABLE III

CONTENTS OF SCUTELLUM AND ALEURONE LAYER, CALCULATED[a] FROM THIAMINE AND NIACIN CONTENTS IN CERTAIN FRACTIONS OF TABLES I AND II, AND THEIR EFFECTS ON RIBOFLAVIN CONTENTS

FLOUR OR FRACTION[b] AND DESCRIPTION	ALEURONE	SCUTELLUM	RIBOFLAVIN		
			From Aleurone	From Scutellum	On Scutellum- and Aleurone-free Basis
	%	%	γ/g	γ/g	γ/g
	Soft flour (Table I)				
Initial flour	0.07	0.44	0.01	0.06	0.25
FF from:					
Ground; 7μ; test 4, 1 fine	0.27	1.12	0.02	0.14	0.47
Ground; 17μ; test 3, fine	0.17	0.75	0.02	0.10	0.34
MF from:					
Unground; test 1, medium	nil	0.26	nil	0.03	0.14
Ground; test 3, medium	0.01	0.40	...	0.05	0.14
	Hard flour (Table II)				
Initial flour	0.28	0.45	0.03	0.05	0.19
FF from:					
Unground; 17μ; test 1, fine	1.25	0.86	0.13	0.11	0.25
Ground; 7μ; test 4, 1st fine	2.15	1.64	0.22	0.21	0.14
Ground; 17μ; test 3, fine	1.35	1.20	0.13	0.16	0.22
MF from:					
Unground; test 1, medium	0.12	0.30	0.02	0.04	0.13
Ground; test 3, medium	nil	0.32	nil	0.04	0.16

[a] The following basic values were taken (ref. 12), in γ/g:

	In aleurone layer	*In endosperm*	*In scutellum*
Niacin	670.0	4.5	38.0
Thiamine	16.5	0.2	156.0
Riboflavin	10.0	0.7	13.0

[b] FF = fine fractions; MF = medium fractions.

the calculated scutellum contents for the fine fractions from test 1 (not shown in Table III) and from test 3 were 0.45 and 0.75%; and here independent confirmation was available, based on the finding by Daniels (4) that MHQ (methoxyhyroquinone) glycosides are highly concentrated in the germ of the wheat grain, the contents in germ and endosperm being respectively about 4,000 and 12γ per g. Determinations on the fine fractions from test 1 and test 3 gave respectively 12 and 24γ per g. If it is assumed that the value 4,000 applies equally to scutellum and to whole germ, the difference between the values just given would correspond to a difference of 0.3% in scutellum content. This is in good agreement with the difference between the

flour samples shown in Tables I and II. In framing the equations, the proportion (%) of endosperm in the samples was assumed to be 100 — x — y.

For example, the equations for the fine fraction (designated in Table III, in line 4 below the headings as "test 3, fine"), which was separated from the ground soft flour at 17 microns, were:

$$670x + 38y + 4.5 \quad (100 - x - y) = 590 \qquad (1)$$
$$16.5x + 156y + 0.2 \quad (100 - x - y) = 140 \qquad (2)$$

from which x is 0.17, and y is 0.75, as shown in Table III.

values of 0.45 and 0.75 calculated from the thiamine contents.

With the harder flour the small amount of the fine fraction initially present contains a relatively high concentration of scutellum, and this concentration is raised more markedly by grinding than it is with the soft flour. Other tests (not fully reported here) on a still harder type of wheat (Svenno) showed the following scutellum contents:

	%
Initial flour (Svenno)	0.64
Fine fraction from unground flour	2.3
Fine fraction from ground flour	2.3

No doubt owing to the relative hardness, and exceptional resistance to reduction, of Svenno wheat endosperm, scutellum enters the flour during milling to a rather greater extent than with the other wheats. In this case, although the pinned-disk grinding process did not raise the concentration of scutellum in the fine fraction, it increased the extent of shattering of the scutellum in the total flour fourfold (since the yield of the fine fraction was increased fourfold).

Niacin Contents. Tables I and II show a further marked differentiation between soft and hard flours in respect to the levels of niacin in the fine fractions — which are much higher with the hard than with the soft flours. Niacin has been shown (5) to be highly concentrated in the aleurone layer, approximate figures for the contents in aleurone layer and endosperm being, respectively, 670 and 5γ per g. The figures in Tables I and II suggest, therefore, that aleurone layer is present to a much greater extent in flours milled from hard wheats than in those from soft, and that it is largely reduced to a finely divided form by the special grinding process. Table III shows the contents of aleurone layer in the present samples, calculated (as described under "Thiamine Contents") from the available data for niacin and thiamine. These reach the rather surprisingly high levels of 1 to 2%, or even more, in fine fractions from the hard wheat, whereas with the soft wheat they are only of the order of 0.2 to 0.3%.

Consideration of the figures further suggests that, with the hard flour, the application of the special grinding process causes all the aleurone layer present to be shattered to such an extent that it enters entirely into the fine fraction (under 17 microns). Thus:

	Sample	Initial Flour (Atle)	Fine Fractions: From Unground Flour	Fine Fractions: From Ground Flour
		%	%	%
a)	Content of aleurone	0.28	1.25	1.35
b)	Yield of fraction	100.0	3.0	20.0
	a × b/100	0.28	0.038	0.27

Calculations on similar lines show that with the soft flour only about half the relatively small amount of aleurone layer present is caused by the special grinding process to enter the fine fraction.

Furthermore, the high content of aleurone layer under discussion must refer to detached aleurone layer—free from the outer layers of the grain (to which it remains attached when present in the by-product of milling known as bran). This is evident from consideration of the figures in Table IV for fiber and ash, determined on certain of the samples of Tables I and II. From the work of Hinton (7), the ash contents of aleurone layer and scutellum may be taken, respectively, as approximately 16 and 7%. On this basis the ash contents, shown in Table IV, support the conclusions already drawn (from the vitamin figures) as to the relative contents of scutellum and aleurone layer in the various fractions.

TABLE IV

ASH AND FIBER FIGURES FOR CERTAIN OF THE SAMPLES LISTED IN THE PRECEDING TABLES, IN RELATION TO CONTENTS OF ALEURONE LAYER AND SCUTELLUM

	Type of Flour Initially			
	Soft		Hard (Atle)	
	Initial Flour (Table I)	Ground, Fine Fraction (Table I, Test 3)	Initial Flour (Table II)	Ground, 1st Fine Fraction (Table II, Test 4)
Contents of:				
Niacin, γ/g	5.1	5.9	6.5	19.4
Ash, %	0.40	0.45	0.40	0.95
Fiber, %	0.07	0.05	0.08	0.04
Aleurone layer, %	0.07	0.17	0.28	2.15
Scutellum, %	0.44	0.75	0.45	1.64
Ash contribution from:				
Aleurone layer, %	0.011	0.027	0.045	0.342
Scutellum, %	0.031	0.053	0.031	0.115
Both above, %	0.042	0.080	0.076	0.457
"Net" ash content, %	0.358	0.370	0.324	0.493

It is evident from Table IV that the relatively very high ash content of the 1st fine fraction from the hard (Atle) wheat is mainly attributable to its high contents of aleurone layer and scutellum. More than one-third of its ash content is contributed by aleurone layer. The low value for its fiber content implies that the aleurone layer is unaccompanied by the other layers of the bran (which have a high fiber content). The "net" ash contents of 0.32–0.37% (for the initial flours and the fine fraction from the soft wheat) are such as might reasonably be expected for flour produced by the reduction of substantially pure endosperm.

Other Vitamins. Riboflavin: It is evident from Tables I and II that the contents of riboflavin respond very differently from those of both thiamine and niacin to fractionation by particle size, to the special grinding treatment, and to change in type of wheat. At first glance it might be thought that the trends shown by the fractions from the soft and from the hard flour are much alike. However, in some fractions a considerable part of the riboflavin content is due to the proportions of scutellum and of aleurone layer they contain, and if this is allowed for, as in Table III, the trends of the "net" riboflavin contents (i.e., the contents in the endosperm alone) are seen to differ markedly as between the two wheat types. The fractions from the soft wheat show a fairly regular increase in riboflavin content with increase in protein content, but with the hard wheat there is no real indication of this. The reason for this difference is not clear.

Pantothenic Acid and Pyridoxine: Calculations on the same lines as those set out for riboflavin in Table III (using the values for the vitamin contents of different parts of the grain collated by Moran, 12) show that, with the hard flour, the major part of the increased contents of pantothenic acid and pyridoxine in the fine fractions is attributable, as in the case of riboflavin, to their increased contents of scutellum and aleurone. However, some residual trend with increasing protein content of endosperm appears; thus:

Reference to Sample in Table II	*Protein*	*"Net" Pantothenic Acid*	*"Net" Pyridoxine*
	%	γ/g	γ/g
Test 3, medium	5.8	1.26	0.33
Initial flour	8.8	1.32	0.40
Test 1, fine	13.0	1.46	0.56
Test 3, fine	15.0	1.66	0.55
Test 4, 1st fine	17.8	1.56	0.78

With the soft flour, however, this trend does not appear; virtually the whole of the increase in pyridoxine content shown by the fine fractions is attributable to increased contents of scutellum and aleurone; on the other hand, the fine fractions do not show an increased content of pantothenic acid.[5] A possible explanation of this difference in behavior between the soft and hard wheats would be that the values used in the calculation, for the pantothenic acid and pyridoxine contents of aleurone, and possibly of scutellum, apply well to the soft wheat but require some adjustment in the case of the Atle wheat. The residual trend noted above in the endosperm of the Atle samples would

[5] It is, however, curious that the content of pantothenic acid in the initial flour should be twice as high as in the hard flour.

disappear, on the assumption that the pantothenic acid content of the aleurone layer in Atle wheat were 59 instead of the value of 45γ per g. (12) assumed for the soft English. The same consideration would apply to the pyridoxine values if the content in the aleurone of Atle wheat were 54 instead of 36, or alternatively if, say, those in the aleurone and scutellum were 45 and 35 instead of 36 and 23 respectively. The case of the riboflavin can hardly be explained in this way, however, because the adjustment required to the value assumed for the content in the scutellum of the soft English wheat would appear to be much too great.

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