SUBALEURONE ENDOSPERM CELLS OF HIGH PROTEIN CONTENT[1]

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ABSTRACT

Subaleurone and inner endosperm in the coarse (over 35 μ) air-classified fractions of flour milled from hard red winter (HRW) wheat had average protein contents of about 45 and 11%, respectively. The protein contents of subaleurone and inner endosperm, separated from coarse fraction of pin-milled HRW flour by sedimentation in nonaqueous liquids, ranged 33–54% and 8–15%, respectively. Cells of subaleurone endosperm were reduced to particles below 35-μ size less readily than were cells of inner endosperm; in consequence, subaleurone endosperm cells concentrated in the coarse air-classified fractions, and raised the protein content of the latter.

The subaleurone endosperm in wheat consists of a distinctive layer, one cell deep, situated adjacent to the aleurone cells on the outside and to the inner endosperm on the inside. In hard wheats, the layer forms a fairly complete shell around the inner endosperm (except in the regions of the scutellar epithelium and the base of the crease). In soft wheats, however, the shell is often discontinuous, and, at the points of discontinuity, endosperm cells with typical "inner endosperm" characteristics extend out to the aleurone layer.

The inner endosperm arises by rapid nuclear division of the triple-fusion nucleus (two polar nuclei plus the second male gamete), so that initially the endosperm sac is lined with free nuclei. Cell walls then form between the nuclei, and the endosperm becomes cellular (1). The subaleurone endosperm cells are formed at a later stage of development by tangential division of the aleurone cells (2).

The two types of endosperm differ in cell size and shape, size and abundance of starch granules, and level of protein content (3–8).

Subaleurone endosperm cells are generally small and cubical; those of the inner endosperm are larger and either needle-shaped (prismatic) or polyhedral (central endosperm). Microscopic measurements on sections of Manitoba grains gave ranges of 21 to 111 μ for the radial, 36 to 111 μ for the tangential dimensions of subaleurone endosperm cells, average 55 $\times$ 62 μ. Dimensions have been quoted (7) for prismatic endosperm cells 40–64 $\times$ 128–200 μ, and for polyhedral 64–120 $\times$ 72–144 μ.

Starch granules in the inner endosperm cells mostly fall into two

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size ranges: large lenticular granules, the majority within the range 15–30 μ, and small spherical granules, 0.5–7.5 μ (9). Granules of both size ranges are generally present within every cell. Cells of subaleurone endosperm generally contain starch granules within the size range 6–17 μ. Within any one subaleurone endosperm cell the granules are strikingly uniform in size and are often restricted to the outer parts of the cells (3,4,5,7), the center being occupied by a mass of protein material. These distinctive microscopic characteristics, illustrated in Fig. 1 (and also in refs. 3, 5, 7, and 10), permit identification of subaleurone endosperm cells in coarse air-classified fractions of flours. For the purpose of this paper, the term "coarse fraction" will be used to refer to the coarse fraction separated from flour by air classification at 35-μ cut size.

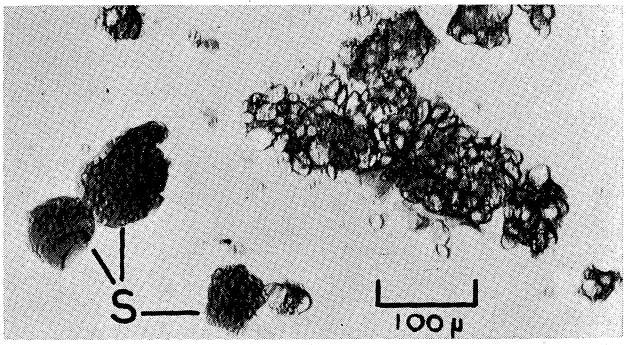

Fig. 1. Coarse air-classified fraction from pin-milled HRW flour showing subaleurone (S) and inner endosperm cells.

Attention was first directed to the subaleurone endosperm after two observations had been made:

1. The coarse air-classified fractions of flours from hard wheats milled under particular conditions had protein contents considerably higher than those of the parent flours. For example, hard red spring wheat of 14.7% protein content (14% moisture basis) milled at 22% moisture content yielded flour of 61.9% extraction rate and 14.0% protein content. This flour, when pin-milled at 13% moisture content and air-classified at 35-μ cut size, gave 22% of coarse fraction with 18.0% protein content (11).

2. Coarse fractions of relatively high protein content contained subaleurone endosperm cells in larger proportions than would have been expected if all the subaleurone endosperm in the flour had been distributed uniformly throughout all the air-classified fractions.

It was concluded that cells of subaleurone endosperm were less readily reduced than were inner endosperm cells during roller milling

and impact milling, and hence became concentrated in the coarse air-classified fractions. The observations also suggested that the protein content of subaleurone endosperm cells was probably somewhat higher than the values previously reported for dissected "outer endosperm" of wheat.

The fact that the protein content of coarse air-classified fractions is often higher than that of the parent flour had been reported by others (e.g., 12) and attributed to a concentration of harder and larger particles of endosperm from a particular (but unspecified) part of the kernel (13), or to a concentration of prismatic and peripheral (subaleurone) endosperm cells which have higher protein content than the central endosperm (14).

Free starch granules and particles of free protein derived from reduced endosperm are mostly less than 35 μ in size, whereas whole endosperm cells and substantial fragments of cells (unreduced endosperm) are mostly upwards of 35 μ. Classification of flour at this cut size thus separates reduced endosperm from unreduced. The proportion of the flour below 35 μ, termed the "degree of reduction" (15,16, 17), indicates how much of the flour is in a form suitable for protein shifting by means of air classification at smaller cut sizes.

This paper presents results of attempts to measure the reducibility (to below 35-μ size) during impact milling, and the protein content, of the subaleurone endosperm cells of hard red winter wheat in comparison with those of inner endosperm cells.

Experimental

Air Classification of Straight-Run Flours. Straight-run flours (66–70% extraction rate) of Manitoba wheat and of U.S. Hard Red Spring (HRS), Hard Red Winter (HRW), Soft Red Winter (SRW), and Soft White (SW) wheats, milled at 16.3, 14.4, 14.7, 14.3, and 14.9% moisture contents, respectively, as previously described (11), were pin-milled and separated into nine fractions by air classification on a Bahco machine at cut sizes 10, 13, 17, 22, 28, 35, 44, and 55 μ in succession. Protein and moisture were determined on the flours and fractions.

Milling and Air Classification of HRW Flour. In addition, flour of 71.5% extraction rate was milled in the laboratory from HRW wheat of 13.7% protein content (14% m.b.). The first four breaks were milled with the wheat at 20% initial moisture content; the fourth-break tails were then dried to 14.5% moisture content, the first- to fourth-break releases to 13% moisture. Two further breaks were milled, but only the flour sifted from the fifth- and sixth-break grinds was retained. Besides the six break flours, five reduction flours were produced: com-

bined sizings (semolinas) flour from first- and second-break sizings; combined middlings flour from first- and second-break middlings, and the reduction middlings from first- and second-break sizings; sizings (semolina) flour from the third-break sizings; first and second middlings flours from the combined third- and fourth-break middlings, and the reduction middlings from third-break sizings. Flour yields (shown in Table I) are expressed as percentages of the total products of milling, dry-matter basis.

Protein content of the straight-run flour was 12.7%. The six break and five reduction flours were kept separate; a portion of each was air-classified at 35-μ cut size, and moisture and protein contents of the flours and fractions were determined, also the subaleurone endosperm contents of the coarse fractions.

TABLE I

CHARACTERISTICS OF FLOUR STREAMS FROM LABORATORY MILLING OF HRW WHEAT

Blending Group and Flour Stream	Whole Flours		Air-Classified Fractions					
			0–35 μ		Coarse (Over 35 μ)			
					Whole Coarse Fraction		Endosperm of Coarse Fraction	
	Yield	Protein Content	Proportion	Protein Content	Proportion	Protein Content	Protein Content	Subal. Endosp. Content
	%	%	%	%	%	%	%	%
Break Flours								
C–1st break	5.3	11.3	24	7.7	76	12.4	12.1	4
C–2nd break	5.1	11.5	32	7.7	68	13.3	12.9	7
B–3rd break	6.1	14.3	24	8.9	76	16.0	16.0	12
A–4th break	4.6	18.0	24	11.3	76	20.1	20.1	27
A–5th break	1.7	19.9	37	12.7	63	24.2	24.3	39
A–6th break	1.1	21.1	48	14.0	52	27.7	28.3	54
Reduction Flours								
D–1st & 2nd break sizings	8.4	10.9	24	8.2	76	11.8	11.6	5
D–1st & 2nd bk. midd.	27.5	11.1	19	9.0	81	11.6	11.5	4
B–3rd break sizings	1.4	13.1	22	9.1	78	14.2	13.7	10
B–3rd & 4th bk., 1st midd.	7.1	13.9	19	10.5	81	14.7	14.6	12
B–3rd & 4th bk., 2nd midd.	3.2	15.5	26	11.1	74	17.1	16.9	20
Total break	23.9	14.4	28	9.5	72	16.3	16.2	15.3
Total reduction	47.6	11.8	20	9.2	80	12.5	12.4	6.7
Straight run	71.5	12.7	23	9.3	77	13.9	13.7	9.6

The 11 flour streams were grouped (in rational proportions) into four blends for pin-milling (see text table below). Each blend was pin-milled (one pass) and air-classified at 35-μ cut size, and the protein and subaleurone endosperm contents of the coarse fractions were assessed.

Blending of Flour Streams from Milling of HRW Wheat

Blend	Flours
A	fourth to sixth break
B	third break, and reduction flours from third and fourth break stocks
C	first and second break
D	reduction, from first and second break stocks

The laboratory model of the Kek pinned-disk mill ("Minikek"), used for fine-grinding the flours, had a 6-in.-diam. fixed plate and a 5⅛-in.-diam. rotor driven at about 19,740 rev./min. The Minikek pin mill, and the Bahco centrifugal air elutriator, used for air classification, have been described previously (11).

The term "reducibility" is used in this paper to refer to the percentage of the over-35-μ material in an initial (roller-milled) flour that is broken to below 35-μ particle size in a regrinding process; it is calculated as $100(1 - y/x)$, where x and y are the percentages of coarse fraction in the initial and reground flours, respectively, as determined by air classification (18).

Microscopical Determination of Subaleurone Endosperm Content. Subaleurone endosperm content of the coarse air-classified fractions was determined by a microscopical particle-counting method.

Microscopical preparations were made in 0.5% Congo Red in 65% sucrose solution and examined at ×60 magnification. All the particles seen in two or three independent traverses of the cover glass (1.6 cm.^{2}) were classified into one or more of the following categories: subaleurone endosperm, inner endosperm, aleurone cell contents, germ (embryo and scutellum), bran, cell wall, free starch. The total non-endosperm constituents rarely exceeded 5% of the fraction. The size of each particle was visually assessed as a multiple or a fraction of that of an average-sized subaleurone endosperm cell (about 60 μ^3). The numbers of particle units in each category were expressed as percentages of the total. Between 500 and 1,000 particle units were counted per slide. The results reported are mean values for two to five slides per sample.

Accuracy of Endosperm Particle Counts. A sample of 22% subaleurone endosperm content (total endosperm basis) was made by blending one part of a coarse fraction (density 1.36) consisting of 97% of subaleurone endosperm and 3% of inner endosperm, with four

parts of a coarse fraction (density 1.49) consisting of 96.1% of inner endosperm, 0.3% of subaleurone endosperm, and 3.6% of nonendosperm constituents. Five slides of this blend were examined, counting between 460 and 1,160 particle units per slide. The subaleurone endosperm content was assessed as 25, 25, 22, 23, and 25%, average 24% of the total endosperm.

Separation of Subaleurone Endosperm from Inner Endosperm. By a sedimentation technique (19,20), samples of subaleurone endosperm and of inner endosperm were separated from coarse air-classified fractions of pin-milled HRW flour, blends A–D described above. These samples contained little or no free interstitial protein, which had been removed in the air-classification step.

Carbon tetrachloride (density: 1.594 g./ml. at 20°) and benzene (density: 0.875 g./ml. at 20°) were blended to give mixtures with particular densities between 1.30 and 1.50. Density was determined with a pycnometer and corrected for temperature.

Portions of the coarse fractions were defatted in petroleum ether, dispersed in liquid of appropriate density, and centrifuged for 15 min. at 1,500 rev./min. (=350 *g*). The floating and sinking portions were recovered, examined microscopically, and re-treated successively at other densities as required until fractions of either subaleurone endosperm or inner endosperm practically free from particles of the other constituent had been obtained. Portions with density <1.39 were subaleurone endosperm containing <5% of inner endosperm; portions with density >1.44 were inner endosperm containing <2% of subaleurone endosperm (on whole-sample basis). Small particles of interstitial protein in the fractions with density <1.39 were removed by air classification at 28-μ cut; detached starch granules and particles of aleurone cell contents (with relatively high density) were removed from the fractions of density >1.44 by sedimentation at 1.50 density (19). Protein and composition (by particle counting) of all the fractions were determined.

Grain Sections. Microtome sections of wheat grains, 10 or 20 μ thick, were stained and mounted in Orange G-glycerol (60 parts of a 0.1% solution of Orange G dye buffered to pH 2.2 with McIlvaine's buffering reagents, plus 40 parts of glycerol). Orange G stains the protein; the relative intensity of color shows the sites of high and low protein concentrations.

Protein Content. Protein content (N × 5.7) was determined by macro or micro Kjeldahl methods and is expressed on 14% moisture basis. Nitrogen content of the total endosperm in coarse air-classified fractions was calculated by subtracting from the determined nitrogen

content of the whole fraction the nitrogen contributed by nonendosperm constituents. For this purpose, the following nitrogen contents were used: 3.8% for aleurone cell contents (19,21), 4.7% for germ, and 0.7% for bran (21). In the case of fractions separated by sedimentation after fat extraction, allowances for the nitrogen contribution of aleurone cell contents and germ were made at 4.75 and 6.0% nitrogen content, respectively (19,21), and the calculated nitrogen content of the endosperm was corrected to full-fat basis.

Results

Successive Air-Classified Fractions of Flours. Protein content and cumulative yields of the successive fine fractions obtained by air classification at 10-, 13-, 17-, 22-, 28-, 35-, 44-, and 55-μ cut sizes are shown in Fig. 2 as histograms, reading from left to right. The parent flours were pin-milled straight-run flours from Manitoba, HRS, HRW, SRW, and SW wheats. The continuous curves show protein contents of the cumulative fines, the broken lines those of the parent flours.

The pattern of these data resembles that of similar data given in numerous publications (e.g., 12,13,14,22,23,24). All the hard wheat flour fractions coarser than 35 μ had protein content higher than that of the parent flour; protein content of the soft wheat flour fractions coarser than 44 μ was similarly high, but the coarse fractions were present in relatively small quantities.

Flour Streams from Milling of HRW Wheat. Table I shows the yields and protein contents of the flour streams obtained in the laboratory milling of HRW wheat, the proportions and protein contents of the fractions air-classified at 35 μ, and the protein and subaleurone endosperm contents of the endosperm in the coarse fractions.

The range of endosperm protein contents in the coarse fractions was 11.5 to 28.3%; the range of subaleurone endosperm contents (total endosperm basis) 4 to 54%. These values, graphed in Fig. 3, are closely related. The linear regression of endosperm protein content (y) on subaleurone endosperm content of the total endosperm (x) was: $y = 10.6 + 0.344\ x$. This relationship can be interpreted as indicating that the two types of endosperm (inner and subaleurone) in the coarse fractions had average protein contents of about 11 and 45%, respectively, in the particular sample of HRW wheat milled.

It was concluded that the increasing protein content of the coarse fractions in the later break and reduction flours could be largely attributed to a concentration of subaleurone endosperm of high protein content in these fractions.

Table I also shows that the protein contents of the finer (0- to 35-μ)

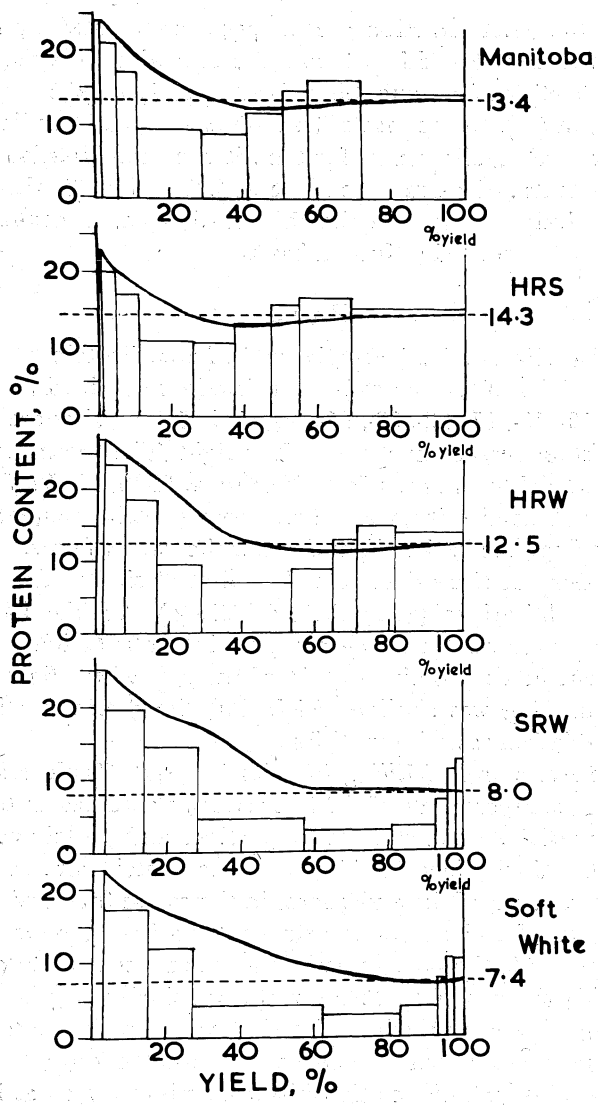

Fig. 2. Protein content and cumulative yield of successive fine fractions obtained by air classification of pin-milled straight-run flours of hard and soft wheats. For further explanation, see text.

fractions, consisting mainly of reduced endosperm, were lower than those of the coarse fractions, being of the order 8–11% protein except in fifth- and sixth-break fines. Microscopical examination of the finer fractions of the fourth- to sixth-break flours showed the presence of a few particles (in the 17- to 35-μ size range) derived from subaleurone

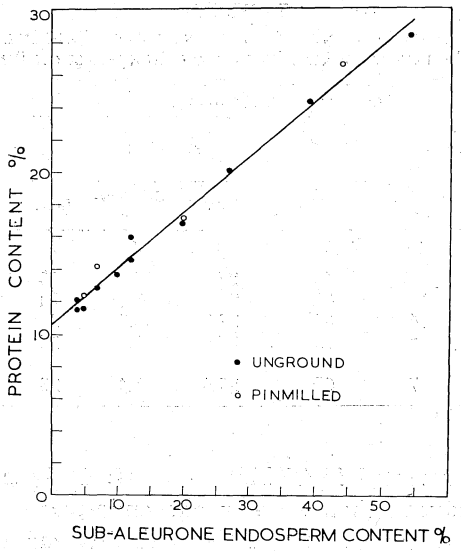

Fig. 3. Relation between protein and subaleurone endosperm contents of endosperm in coarse air-classified fractions of flour streams milled from HRW wheat.

endosperm, whereas the finer fractions of all other flour streams appeared to be almost free from such particles.

The weighted average subaleurone endosperm content of 9.6% in the endosperm of the coarse fraction of the straight-run flour is equivalent to 9.4% in the whole coarse fraction, and to 7.2% (i.e., 9.4 × 0.77) in the total straight-run flour. The latter figure would be increased to 7.5% if provision were made for a contribution from the fourth- to sixth-break finer fractions. In comparison, Kent and Jones (7) estimated that the subaleurone endosperm content of 73%-extraction Manitoba flour milled on a four-break system was 4%, with 8% in the break flour and 3% in the reduction flour. They calculated that the Manitoba wheat grain contains about 11% of subaleurone endosperm by volume.

Reducibility of Inner and Subaleurone Endosperm. To assess the reducibility of the two types of endosperm, estimates of subaleurone and inner endosperm contents of the coarse fractions of the HRW flour blends A–D before and after pin-milling were compared. The results, shown in Table II on whole flour blend basis, show that 65 to 70%, average 67%, of the inner endosperm in the coarse fraction of the unground flour blends was reduced by pin-milling to below 35-μ size, whereas only 46 to 63%, average 51%, of the subaleurone endosperm was similarly reduced.

Two conclusions regarding reduction of endosperm were drawn

TABLE II

COARSE[a] ENDOSPERM CONTENT OF UNGROUND AND OF PIN-MILLED FLOURS OF HRW WHEAT, AND REDUCIBILITY OF ENDOSPERM ON PIN MILL

FLOUR BLEND	COARSE ENDOSPERM CONTENT						REDUCIBILITY OF ENDOSPERM ON PIN MILL	
	Unground Flour			Pin-milled Flour				
	Total	Subaleurone	Inner	Total	Subaleurone	Inner	Subaleurone	Inner
	%	%	%	%	%	%	%	%
A	68	22	46	27	12	15	46	68
B	76	10	66	25	5	20	51	70
C	70	4	66	22.5	1.5	21	63	69
D	78.5	3.5	75	27.5	1.5	26	56	65
Straight run	76	7	69	26.4	3.4	23	51	67

[a] Over 35 μ.

from the data presented in Tables I and II: 1) Inner endosperm is more readily reduced than subaleurone endosperm. 2) Inner endosperm of low protein content is more readily reduced than inner endosperm of higher protein content.

These conclusions were generalized as follow: 1) Relative ease of reduction of endosperm is inversely related to the protein content. 2) Air classification makes, to some extent, a separation of endosperm particles derived from different regions of the endosperm.

Flour Fractions Separated by Sedimentation. Table III shows the yields of fractions separated by sedimentation from the coarse portion of the HRW pin-milled flour blends A–D, together with their protein and subaleurone endosperm contents after correction for nonendosperm constituents. Values for protein content ranged from 8 to 53%. The microscopical appearance of two of the fractions, i.e., those having protein content 8 and 53%, respectively, is shown in Fig. 4.

The weighted averages for protein and subaleurone endosperm contents are in reasonable agreement with the values obtained by direct determination on the entire coarse fractions.

The protein and subaleurone endosperm contents given in Table III are graphed in Fig. 5. The curve may be considered in three sections:

Section 1. Fractions with 0–3% of subaleurone endosperm; protein content 8–16%. The protein values refer to endosperm alone and indicate that, as subaleurone endosperm was practically absent, the protein content of the *inner* endosperm varied at least over the range of 8–15% in this sample of HRW wheat.

Section 2. Fractions with 13–50% of subaleurone endosperm; protein content 19–25%. There was a linear relationship between protein con-

TABLE III

CHARACTERISTICS OF FRACTIONS SEPARATED BY SEDIMENTATION FROM COARSE[a] AIR-CLASSIFIED PORTIONS OF PIN-MILLED FLOURS FROM HRW WHEAT

Flour Blend	Density of Fraction	Yield of Fraction	Protein Content of Endosperm	Subaleurone Endosperm Content of the Endosperm
	g./ml.	%	%	%
A	<1.39	20.6	53.1	97
	1.39–1.41	9.3	37.2	85
	1.41–1.42	14.3	28.3	62
	1.42–1.44	19.1	21.3	20
	1.42–1.47	29.9	13.5	2
	>1.47	6.8	9.2	2
	Weighted average		28.2	44
	Direct determination		26.6	44
B	<1.39	4.5	53.3	98
	1.39–1.41	5.8	35.3	78
	1.41–1.42	10.5	25.2	49
	1.42–1.44	18.4	19.0	13
	1.44–1.47	52.0	11.3	1.6
	>1.47	8.8	8.6	1.4
	Weighted average		17.3	18
	Direct determination		17.2	20
C	<1.39	3.9	51.4	95
	1.39–1.42	3.4	34.1	71
	1.42–1.44	4.4	22.4	33
	1.44–1.47	36.5	15.6	3
	>1.47	51.8	8.0	1
	Weighted average		13.9	9
	Direct determination		14.2	7
D	<1.39	2.5	43.4	82
	1.39–1.42	2.9	31.6	66
	1.42–1.44	3.8	19.2	13
	1.44–1.47	37.0	13.6	2
	>1.47	53.8	8.2	0.3
	Weighted average		12.2	5
	Direct determination		12.4	5

[a] Over 35 μ.

tent (y) and subaleurone endosperm content (x) according to the regression: $y = 17.2 + 0.163\ x$. Such a relationship would result if the endosperm consisted of blends in various proportions of two components: inner endosperm of 17% protein content, and subaleurone endosperm of 33.5% protein content.

Section 3. Fractions with 62–98% subaleurone endosperm content; protein content 28–53%. In this section the curve rises steeply, indicating that the subaleurone endosperm had protein content increasing from 34 to about 54% in the fractions consisting of 97–98% subaleurone endosperm.

If it be assumed that the inner endosperm in the section 3 fractions had 17% protein content (as found from the regression relationship

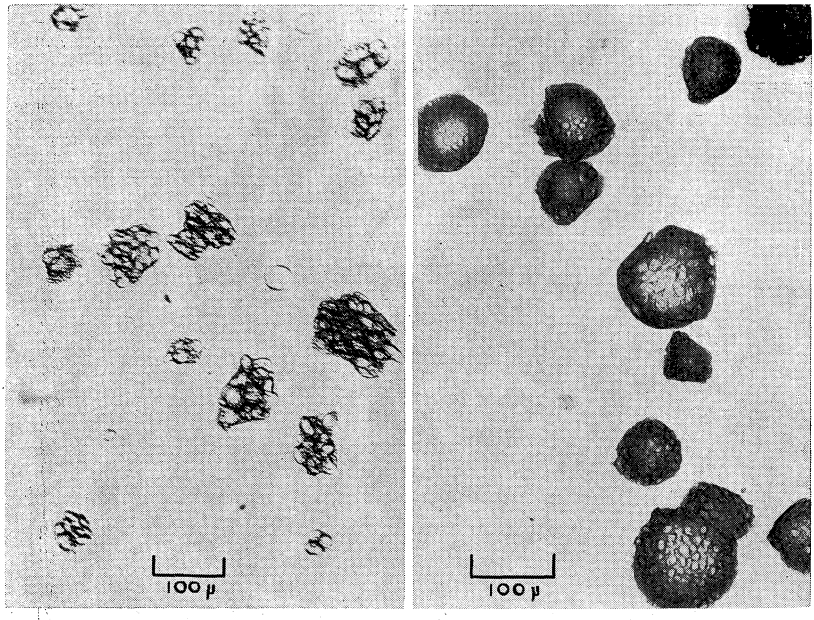

Fig. 4. Fractions separated by sedimentation from the coarse air-classified portions of pin-milled HRW flour. Left: fraction of density >1.47, protein content 8%, consisting of inner endosperm particles. Right: fraction of density <1.39, protein content 53%, consisting of subaleurone endosperm particles.

applying to section 2), the protein content of the subaleurone endosperm moiety of these fractions can be calculated. Similarly, the protein content of the inner endosperm moiety of the section 1 fractions can be calculated if it be assumed that the small amount of subaleurone endosperm in these fractions had 33.5% protein content. The values thus calculated are shown in Table IV. The weighted average protein contents of the subaleurone endosperm were calculated as 45.8, 40.3, 43.3, and 41.6% in the coarse fraction of the four flour blends, and 42.9% in the coarse fraction of the pin-milled straight-run flour from HRW wheat. This figure may be compared with the value 45% for the protein content of subaleurone endosperm in the coarse fraction of the roller-milled HRW flour, calculated independently from the regression curve of Fig. 3.

Subaleurone Endosperm Cells in the Wheat Grain. Figure 6 shows part of a transverse section of HRW wheat. Many of the subaleurone endosperm cells are only sparsely filled with starch granules. The remainder of the contents of these cells stained a deep orange color with Orange G, indicating a high protein content. The inner endosperm, in contrast, took on only a feeble color.

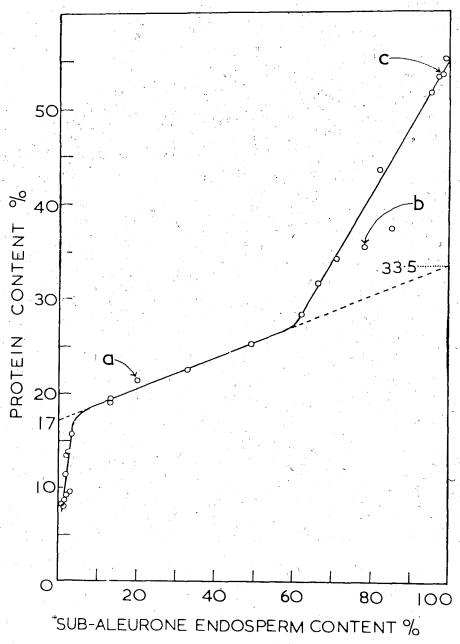

Fig. 5. Relation between protein and subaleurone endosperm contents of the endosperm in fractions separated by sedimentation from the coarse portion of pin-milled HRW flour.

TABLE IV

CALCULATED PROTEIN CONTENTS OF SUBALEURONE AND INNER ENDOSPERM IN FRACTIONS SEPARATED BY SEDIMENTATION FROM COARSE AIR-CLASSIFIED PORTIONS OF PIN-MILLED HRW WHEAT FLOUR

DENSITY RANGE	ENDOSPERM PROTEIN CONTENTS							
	Subaleurone Endosperm				Inner Endosperm			
	Blend A	Blend B	Blend C	Blend D	Blend A	Blend B	Blend C	Blend D
g./ml.	%	%	%	%	%	%	%	%
<1.39	54.2	54.0	53.1	49.2				
1.39–1.41	40.8	40.5	41.1	39.1				
1.41–1.42	35.2	33.7						
1.42–1.44	38.5	32.4	33.4	33.9				
1.44–1.47					13.1	10.9	15.1	13.2
>1.47					8.7	8.2	7.6	8.1
Weighted average	45.8	40.3	43.3	41.6				

Figure 7 shows part of a transverse section of a soft English wheat (variety: Cappelle Desprez). Most of the distinctive subaleurone endosperm cells were well filled with starch granules; cells with high protein content occurred much less frequently than in the hard wheat.

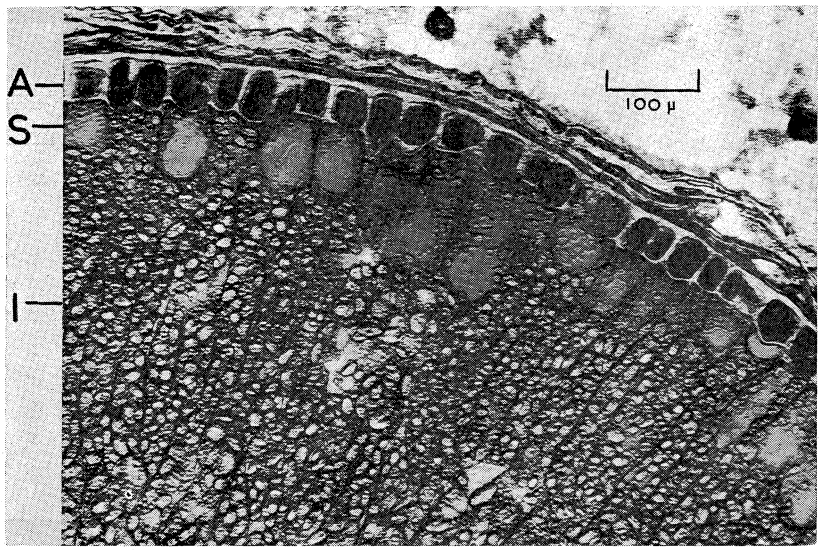

Fig. 6. Part of transverse section of grain of HRW wheat. A, aleurone layer: S, subaleurone endosperm; I, inner endosperm.

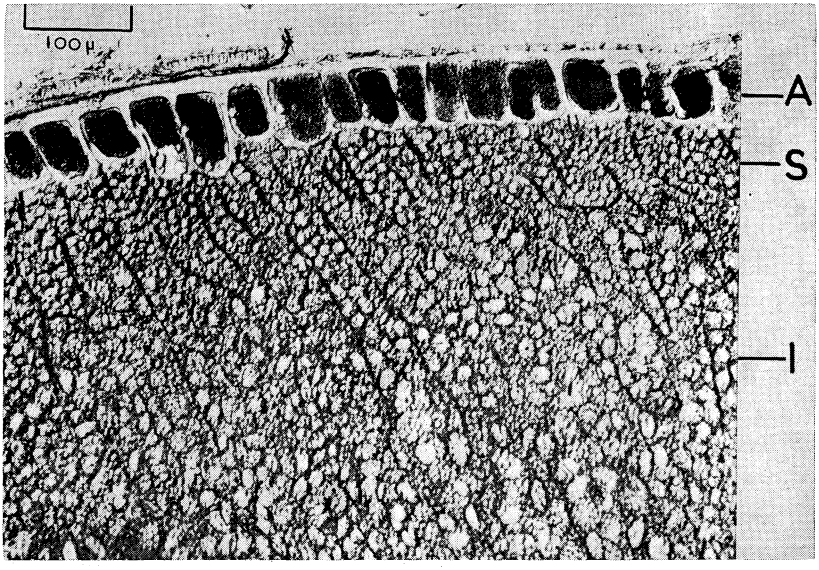

Fig. 7. Part of transverse section of grain of soft English wheat (variety: Cappelle Desprez). A, aleurone layer; S, subaleurone endosperm; I, inner endosperm.

Other types of hard and soft wheats have been similarly examined. The difference in frequency of occurrence of high-protein subaleurone

endosperm cells, exemplified by HRW (Fig. 6) and Cappelle (Fig. 7), seems to be typical of hard and soft wheats in general, and is probably an environmental effect.

Discussion

High-Protein Fractions Separated by Sedimentation. The sedimentation method described in this paper is similar to Hess's method of separating wedge protein (20), but whereas the starting material used by Hess was the fine air-classified fraction of flour, in which particles of free wedge protein are abundant, in this study it was the coarse (over 35-μ) fraction from which free wedge protein was absent. There seems no doubt that the high-protein fractions separated by sedimentation in this study consisted of subaleurone endosperm cells which, in most cases, were the complete cellular contents (see Fig. 4). Some of the particles retained their cell wall, as shown by staining with molybdenum oxide blue (6). The identity of the particles, seen in these fractions, with subaleurone endosperm cells in grain sections (Fig. 6) was established by comparison of cell size and shape, and starch granule characteristics (25). The particles in these fractions did not in the least resemble particles of free wedge protein.

No other reports of any endosperm cells of wheat having protein content of the order of 40% (14% moisture basis) have been found, although Watson *et al.* (25) separated outer endosperm of 40–41% protein content (dry basis) from sorghum and corn.

Röhrlich and Niederauer (26) separated wedge protein from air-classified fractions of flour by Hess's sedimentation technique. "Wedge protein" was recovered from fractions of each particle size, even from the coarsest, but protein contents were not reported. As the amount of wedge protein recovered was minimal for fractions of intermediate particle size, and increased for both finer and coarser fractions, it is possible that the high-protein material separated from the coarser fractions consisted of subaleurone endosperm cells of high protein content, rather than "wedge protein." Microscopical examination would have confirmed this possibility.

Protein Gradient in Endosperm of Hard and Soft Wheats. Investigation of the protein gradient in wheat endosperm by analysis of dissected fractions has generally been made on soft wheats. Cobb (27), working with Purple Straw wheat, reported a 2.2-fold gradient in protein content (7.4 to 16.5%) from center to periphery. Hinton (21), working with soft English wheat (variety: Vilmorin 27), also found a 2.2-fold gradient (5.7 to 12.5%). The protein range reported here for HRW wheat (8–43%) is so much greater than those previously re-

ported as to suggest a real difference between hard and soft wheats in this respect.

Comparison of the photographs of grain sections (Figs. 6 and 7) provides further evidence that hard and soft wheats differ as regards the nature of the subaleurone endosperm. Other work, not fully reported here, has shown that subaleurone endosperm cells of high protein content could be separated from the coarse fractions of flour milled from U.S. Soft White wheat, but in much smaller yield than that obtained from the HRW wheat. This finding is consistent with the results for yield and protein content of coarse air-classified fractions of hard and soft wheat flours shown in Fig. 2.

Further confirmation of a difference between hard and soft wheats in the nature of the outer layers of the grain is provided by Normand *et al.* (28) who, by removal of successive peripheral layers, by tangential abrasion, from hard wheat of 17.4% protein content, obtained "flour" with protein content up to 24% (dry basis), whereas the protein content of corresponding "flour" from soft wheat was only about 1% higher than that of the whole kernels.

Variation in Protein Content of Subaleurone Endosperm. The wide range of protein contents (33–54%) of the subaleurone endosperm in the sedimented fractions (see Table IV) was due mainly to variation in structure and composition of the subaleurone cells, and to concentration of the various cell types in different fractions. For example, in the fractions represented by the points marked *a*, *b*, and *c* in Fig. 5, with 21.3, 35.3, and 53.3% protein content, respectively, the subaleurone endosperm cell contents were generally intact, and in *a* were well filled with starch granules, in *b* showed a small central area devoid of starch granules, and in *c* showed merely a peripheral ring of starch granules surrounding a large mass of protein.

It was concluded that in the intact grains of HRW wheat the range of protein contents of the subaleurone endosperm cells is probably similar to that (33–54%) found for this type of cell in the coarse fractions of the flour. The process of sedimentation in liquids of various densities not only separated subaleurone endosperm from inner endosperm, but also classified the subaleurone endosperm into fractions ranging from 33 to 54% protein content and, similarly, classified the inner endosperm into fractions ranging in protein content from 8 to 15%.

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