A SEPARATION OF ANATOMICAL PARTS OF BARLEY FROM THE BY-PRODUCTS OF BARLEY PEARLING[1]

E. J. NOVACEK,[2] C. F. PETERSEN,[2] AND A. E. SLINKARD[3]

ABSTRACT

Preparatory to chick feeding trials for energy determinations, five anatomical parts of the barley kernel were obtained by separating the by-products from the three passes of barley pearling. The separations were based on particle size and/or density and were made on a rebolt sifter and/or gravity separator. Data on all weights of barley, recorded during pearling, and on the subsequent separations of the three by-products were used to calculate each disappearance as a percentage of the barley prior to pearling. A staining-microscopy study was used to partition each of the separations of three by-products into one or more of three anatomical classifications as a percentage of each respective pass. The pericarp and germ were partitioned on the basis of yield from select mixtures. The barley contained 7.74% husk, 0.41% pericarp, 1.29% germ, 7.19% aleurone, and 83.43% endosperm. Accuracy of separation was tested by determining protein on all anatomical parts, and on the whole and pearled barley and by-products. Protein values and percent yields were used to compare calculated and actual protein content of the barley or by-products; agreement was satisfactory.

Studies involving chemical reactions in cereal grains are often best carried out when the kernel is divided into several parts. In the past, anatomical parts have been obtained by hand-dissection in a pin-vise (1). Obviously this is a tedious task and only a small amount of product is obtained. Other methods have been employed such as fractionation with a small pearling machine (2,3), but the heterogeneous products are often unsuitable for analytical studies.

The study reported here is part 1 of an investigation into the effects of water-treating on the metabolizable energy of barley for chicks. The objective of this study was to obtain the five principal anatomical parts of barley in quantities large enough for metabolizable-energy trials with chicks. The parts obtained were the husk (lemma and palea), pericarp, germ, aleurone, and endosperm. This paper presents the techniques employed in the separation of these parts from the by-products of barley pearling.

Materials

The anatomical parts were separated from the by-products of commercially pearled Compana barley. The by-products separated were

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[2]Department of Poultry Science, University of Idaho, Moscow.
[3]Department of Plant Science, University of Idaho, Moscow.

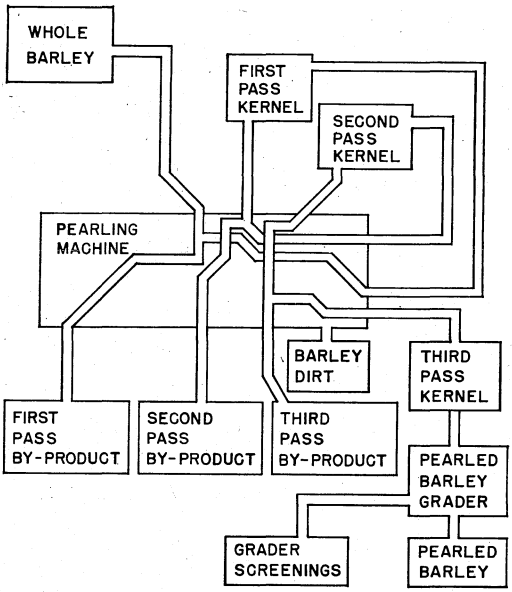

Fig. 1. Flow diagram of the process of pearling barley.

from the first, second, and third pass of barley pearling. A flow diagram of the process of barley pearling, Fig. 1, shows the relative position of each by-product with respect to the kernel. Information regarding the barley is given below.

Variety	Compana
Location of harvest	Northeastern Montana
Harvest year	1963
Weight per bu.	51.5 lb.
Kernel size:	
plump on 6/64 screen	90.74%
medium on 5.5/64 sieve	2.83%
thin through 5.5/64 sieve	6.43%
Protein content	13.75%
Moisture content	9.81%

A total of 500 kg. of the by-products of barley pearling was necessary to obtain 12.3 kg. of pericarp and at least 16.9 kg. of each of the other four anatomical parts. Of the 500 kg., 318 was first-pass by-product and 91 was from each of the second- and third-pass by-products.

All material from the pearling plant was recovered near the midpoint of each pass of pearling, in an effort to obtain a more representative sample.

The separation of the anatomical tissues from the by-products of barley pearling was based on physical differences of size and density

between the particles. Apparently these physical differences are due to the prolonged abrasive action of the pearling disks. Perhaps lines of weakness are related to different cell types and fiber content. The cell-type and fiber-content characteristics are probably different for each anatomical part, and thus the separations are possible.

A Rebolt sifter was used to separate each by-product into six different sizes.

An Oliver Model 50B gravity separator was used to separate the germ, pericarp, and husk.

A Sutton, Steele & Steele gravity separator V135A, class demonstrator, was effective in separating the germ only.

Procedure

The procedure for the separation began with a rebolt sifter fitted with size Nos. 34W, 54GG, 70GG, 120SS, and 200SS sieves (see Fig. 2). The top or largest screen size was 34W. The "W" refers to wire, GG to grits gauze (usually silk), and SS to stainless steel. One-half of a 1,000-g. sample from the first pass was placed on the 34W sieve and the machine operated for 3 min. It was then stopped, and the coarse husk remaining on the size 34W sieve was removed and weighed. The remaining 500 g. was then placed on the sieve and the rebolt separator again put in motion for another 3 min. The top sieve was then removed and this material weighed. The machine was continued in operation for 2 min. additional to a total of 8 min. from the time the first sample was introduced. The material remaining on each sieve was then weighed and separately packaged, and the procedure was repeated. This complete procedure was repeated for the second-pass by-product but modified to replace 34W with 38W and 200SS with 135SS, and allowing only 5 min. for sifting each sample. The procedure was again modified for the third-pass by-product in that sieve 38 was removed and 200SS was inserted after 135SS; the time interval was 8 min. These changes were made to further separate aleurone from the smaller particles of endosperm. The material separated was pooled according to sieve size and pass of pearling.

Samples from each of the six separations of the three by-products (18 samples) were then differentially stained to characterize the anatomical parts. An I_2KI differential-stain microscopy (100×) technique was used to estimate the relative concentration of each anatomical part in all separations from the rebolt sifter. Several samples from each separation were stained with the I_2KI stain (Johansen, 4). This stain reacts with starch to produce a black color, and turns cellulose brown. The endosperm stained dark and was readily recognized. The aleurone,

germ, husk, and pericarp stained brownish but were recognized by different color intensity, cell size, and shape. Visual estimates of each component were made from triplicate slides, on the basis of differences in the staining and structural details. The estimate was then expressed as a percentage of the weight of a given separation. As weight records were kept for all separations, it was possible to relate the anatomical parts to the by-products and to whole barley. The stain technique did not permit differentiation between husk and pericarp.

Weight records were kept for all passes of barley pearling and subsequent separations of each by-product. These records were used to calculate the percent anatomical part in each by-product and in whole barley from the data obtained by the stain technique.

The sieve sizes used and estimates of the relative concentration of anatomical tissue are presented in Fig. 2. This is a flow diagram modified in such a way that the yield of each anatomical part is shown as a percentage of the material retained by each sieve used in separating the by-products. For instance, the first sieve used on the rebolt sifter for separation of the first-pass by-product was 34W. The material retained by the 34W sieve and identified by the stain technique was

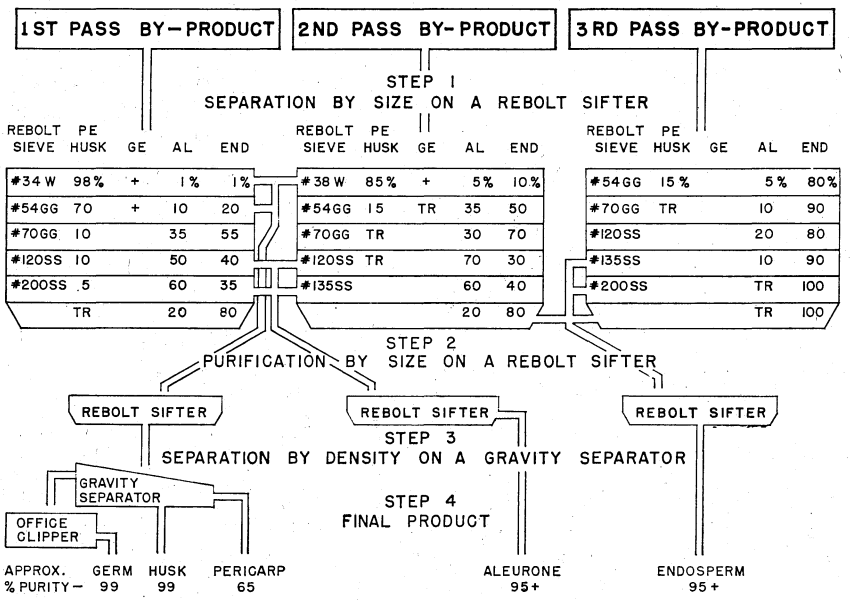

Fig. 2. Flow diagram of separation of husk, pericarp (Pe), germ (Ge), aleurone (Al), and endosperm (End) from three by-products of barley pearling. The separation data are presented as a percentage of the total material retained by each rebolt sieve.

98% pericarp and husk, 1% aleurone, and 1% endosperm. Although germ was present on this sieve, it was not quantitatively identified by stain-microscopy. The stain technique was not applicable for germ because of the relatively large particle size and small percentage in the sample. Quantitative classification was based upon the weight of germ obtained from the gravity separator.

The diagram, Fig. 2, shows which separations were selected for purification and further separation to yield the final products. Samples which were shown to contain a high percentage of the desired part were pooled and purified on the rebolt sifter before they were further separated. As illustrated in the diagram, the material remaining on the 34, 38, and 54 sieves was first pooled, then purified; and finally the husk, germ, and pericarp were recovered separately when subjected to the action of a gravity separator. The germ was then purified on an Office Clipper to remove traces of husk and a few broken kernels.

The pericarp obtained from the gravity separator was run over the separator several times to obtain a more concentrated product.

Discussion

Each of the three by-products of pearling was separated into six classifications based on particle size. The five anatomical parts were obtained from those separations containing the greatest concentration of the desired part, irrespective of the pass of pearling from which they were obtained or the sieve size which contained them. Those separations of greatest concentration were combined and purified to obtain a final product which would respond to the staining procedure characteristic for that anatomical part.

Unfortunately, an overlapping of tissue size was found. The largest fragment of one tissue was the same size as the smallest fragment of another. Thus some sieves contained almost equal amounts of tissue of more than one type but of the same size. These mixtures were not separable by the techniques employed and were discarded.

All of the pooled separations of greatest concentration were further concentrated on the rebolt sifter to obtain greater purity of the desired anatomical part.

The separation for aleurone was analyzed by stain-microscopy and was estimated to be at least 95% free of other anatomical tissues. The foreign material in the aleurone separation was almost entirely endosperm adhering to aleurone particles. Aleurone contributes 7.15% of whole barley; 6.9% was contributed by the by-products and about 0.25% from barley dirt and loss. Most of the aleurone was recovered from the first- and second-pass by-products. Table I shows the amount

of each anatomical part obtained from each by-product expressed as a percentage of whole barley. Table II shows the amount of each part obtained from each by-product but expressed as a percentage of each pass of pearling.

TABLE I

YIELDS OF ANATOMICAL PARTS FROM EACH OF THE THREE BY-PRODUCTS OF BARLEY PEARLING EXPRESSED AS A PERCENT OF WHOLE BARLEY

	HUSK	PERICARP	GERM	ALEURONE	ENDOSPERM
	%		%	%	%
First pass	7.20		1.17	3.67	4.26
Second pass	0.85		0.12	2.25	6.24
Third pass	0.10		0	1.02	8.57
Total	7.74	0.41	1.29	6.94	19.07

The endosperm preparation contained less than 5% foreign material, predominantly aleurone particles as small as the larger particles of endosperm. Endosperm contributes 83.45% of whole barley; 61.5% was contributed directly from pearled barley, 19.1% from the by-products, and 2.85% from barley dirt and grader screenings. Of the endosperm recovered from the by-products, the first pass and the third pass contained markedly different size particles. A much smaller particle size was recovered from the first-pass by-product. The explanation is offered that the cells at the periphery of the kernel were the last to develop from the inward inversion of the aleurone cells (5). The size of the peripheral starch granules may then be smaller at the time of kernel maturation. When the pearling disks disrupt the inward aleurone layer, these smaller granules are released.

The husk, pericarp, and germ were first obtained as a mixture on sieves 34, 38, and 54. The tissues retained by these sieves from the first- and second-pass by-products were pooled and recleaned on the rebolt sifter, then separated on a gravity separator. The difference in density between the germ and the husk or pericarp was very apparent, and this separation was possible over a wide range of machine adjustments.

Nearly all of the germ was obtained intact from the first-pass by-product. Apparently the germ was broken off *in toto* during the first pass of pearling before it had been subjected to prolonged abrasive action by the pearling disks. The germ was similar in size and density to the rounded broken kernels obtained from the first pass. The separation of these broken kernels from the germ was obtained by size difference on an Office Clipper. The final preparation of germ was estimated to be more than 99% pure. The contribution of germ to whole barley was 1.3% (see Table II).

The pericarp separated from the husk when the mixture was subjected to the action of the gravity separator. A very close adjustment was imperative, to minimize the overlapping effect of the smaller fragments of husk and larger fragments of pericarp. The overlapping was due to the very small difference in density between the husk and pericarp. Staining procedures failed to differentiate between husk and pericarp, so the percentage of pericarp in the final separation for pericarp (65%) was estimated by macro observation based on color, texture, and particle size. This criterion was also used to identify pericarp, as the staining procedures were inapplicable. It is recognized that the criterion used for this identification was inadequate, but the classification is supported by elimination of any other possible anatomical part.

Pericarp was found in the first two by-products and represents 0.41% of whole barley. The sample obtained was estimated to be 65% pericarp and approximately 35% husk. No attempt was made to separate this mixture by means other than a gravity separator.

Husk was found predominantly in the first-pass by-product and contributes 7.74% of whole barley. The product was at least 99% free of foreign material.

The final data on husk and pericarp are presented as combined data in Table I, because those samples containing a large percentage of husk and pericarp had inadvertently been pooled.

The percentage contribution of anatomical parts of whole barley from each by-product is shown in Table I. Table II includes the percentage of each part within each by-product and within whole barley.

TABLE II

SUMMARY OF THE PERCENT CONTRIBUTION OF ANATOMICAL PARTS TO THE BY-PRODUCTS OR WHOLE BARLEY

BARLEY PART	PROTEIN	WHOLE BARLEY	BY-PRODUCT			PEARLED BARLEY
			First-Pass	Second-Pass	Third-Pass	
	%					
Husk	2.81	7.7	44.1	9.0	1.1	
Pericarp	3.89	0.4				
Germ	35.69	1.3	7.2	1.3		
Aleurone	21.46	6.9	22.5	23.8	10.5	
Endosperm	18.76[a]	19.1[b]	26.2	65.9	88.4	100.0
Pearled barley	12.20	61.5				
Other[c]	13.75	3.1				
Whole barley	13.75	100.0	16.3	9.5	9.7	61.5

[a] Sample from composite of second- and third-pass by-products.
[b] Endosperm is presented for the by-products only. The remaining endosperm is listed as pearled barley.
[c] Other materials include barley dirt, grader screenings, and loss. In calculating the protein content for comparison, this material was presumed to have the same protein content as whole barley. It was estimated that of the 3.1%, 0.25% was aleurone and 2.85% endosperm.

The endosperm from the by-products is listed separately from pearled barley because of the difference in protein content. The difference in protein content between endosperm from the by-product and from pearled barley was unexpected; however, it is in agreement with data published on the distribution of protein in the wheat kernel (6).

The accuracy of the separation data was tested by comparing calculated and chemically determined protein values for whole barley and for the by-products. The protein values for whole barley, each by-product, product, and anatomical part were chemically determined. The values so determined for barley and each by-product were then compared with the calculated values obtained by using the protein values and percent distribution of the parts as given in Table II. The calculated value for each product and by-product is the sum of the protein contribution from each constituent anatomical part. The comparative values for testing the accuracy were as follows:

	Whole Barley	*By-Products*		
		1st Pass	*2nd Pass*	*3rd Pass*
Chemically determined	13.75	13.58	18.86	18.15
Calculated from the parts	13.69	13.55	18.19	18.87

The similarity of the values indicates that the accuracy is acceptable and that the percentage figures given in Table II are rational.

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