Wheat Germ in Breadmaking. I. Composition of Germ Lipids and Germ Protein Fractions[1]

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ABSTRACT

Untreated and defatted granular wheat germ was pin-milled and air-classified. The protein shift was much higher in the defatted than in the untreated germ. Sodium chloride solutions extracted up to 86.3% of germ proteins. The extract contained, on dry matter basis, about 83% protein (N X 5.45). Calcium chloride solutions extracted less proteins, and dilute acetic acid was least effective. Proteins in germ, NaCl extracts, and in low-protein and high-protein air-classified fractions were characterized by starch-gel electrophoresis and amino acid composition. Germ proteins contained a whole spectrum of fast-moving salt-soluble proteins, and gluten proteins were virtually absent. Proteins in defatted germ and in a salt extract of defatted germ were rich in most of the essential amino acids. The high-protein fraction from air classification contained less than the low-protein fraction of lysine, serine, proline, sulfur-containing amino acids, alanine, leucine, and tyrosine. Free and bound germ lipids were characterized by thin-layer chromatography. Free lipids contained practically no polar components; bound germ lipids contained small amounts.

Germ is not an intentional component of white flour, though some germ invariably finds its way into the flour. The amount of germ in a flour usually increases with increase in extraction rate. Wheat flour contains germ oil either from germ fragments or from endosperm particles to which oil has been transferred from the germ particles on reduction rolls (1). Because some germ is present even in the most refined patent flour, the effects of germ in breadmaking are of interest to cereal chemists. Furthermore, the high-protein content and excellent amino acid balance of germ make it attractive for enriching wheat flour.

Rand and Collins (2) reported that the protein quality of wheat germ was comparable to that of high-quality defatted fish meal. Supplementing wheat flour, rice, barley, oats, and a mixture of those cereals with 10 to 15% defatted wheat germ flour strikingly improved nutritive value. Similar improvement from wheat germ was reported by others (3,4).

Processed whole germ is preferred to raw germ for feeding, since raw germ depresses growth and fat utilization (5). The depression was eliminated when germ was steamed (6) or autoclaved (5). Raw germ contains deleterious hemagglutination and antitrypsin factors.

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Moran et al. (7) recently reviewed the effects of toasting and autoclaving wheat germ meal (fed as a sole source of dietary protein) on utilization and protein quality for the growing chick. Both toasting and autoclaving destroyed hemagglutination and antitrypsin activities. Whereas toasting significantly improved net protein utilization (NPU) values, autoclaving had no apparent effect. Prolonged autoclaving destroyed lysine, cystine, and arginine; reduced NPU; and seriously impaired chick growth and feed utilization.

Rehfeld (8) and Olsen (9) found no toxic components in raw wheat germ but reported that the high nutritional value was reduced by heat-treatment. Little is known about the effects on germ of oven heat during bread-baking. Storage of wheat germ reduces proteolytic and amylolytic activity but also enhances fat deterioration and tocopherol destruction (10). Freshly prepared germ develops a rancid taste within several days. Wheat lipases, producing oxidizable free fatty acids, increase deterioration of germ during storage (11). Storage at low temperatures (around 4°C.) prolongs shelf-life. The germ is commonly stabilized by wet or dry heat-treatment to inactivate the enzymes responsible for rancidity. Drying at low temperatures to 5% moisture and storing the dried product in waterproof containers brings similar results (12). Such drying maintains consumer acceptance, with regard to taste and flavor, with little damage to tocopherols and thiamine. Samples stabilized by the above method were acceptable after 2.5 years of storage. Such stabilization does not imply inactivation of factors or components deleterious in breakmaking. Such drying actually may render glutathione inactivation (important in breadmaking) more difficult. Consequently, in selecting drying or heating conditions, to improve nutritional value and stability in storage, the effects on utilization in breadmaking must be considered.

This investigation was made to devise methods for concentrating germ proteins and characterizing the concentrates. Use of physical treatment and chemical additives in baking white bread, nutritionally enriched with high levels of germ or germ concentrates, is discussed in Part II of this investigation (13).

MATERIALS AND METHODS

Germ

Fresh granular wheat germ from a commercial mill was stored in moistureproof containers at +4°C. The germ contained 14.9% moisture, 26.0% protein (N × 5.45) (14), 4.3% ash, 12.0% petroleum-ether extract, 2.0% bound lipids (water-saturated butanol extract, following petroleum-ether extract), and 1.8% crude fiber. Germ dried at 45°C. to about 4% moisture was twice defatted, in a large Soxhlet with petroleum ether, for protein extraction and air classification.

Flour and Gluten

The flour, Regional Baking Standard (RBS), and gluten prepared from that flour were as described by Shogren et al. (15).

Analytical Methods

Particle size (average diameter in μ) was determined in a Fisher Sub-Sieve Sizer (No. 14-311) as described by Croteau (16).

Moisture, ash, protein, crude lipids, and crude fiber were determined as

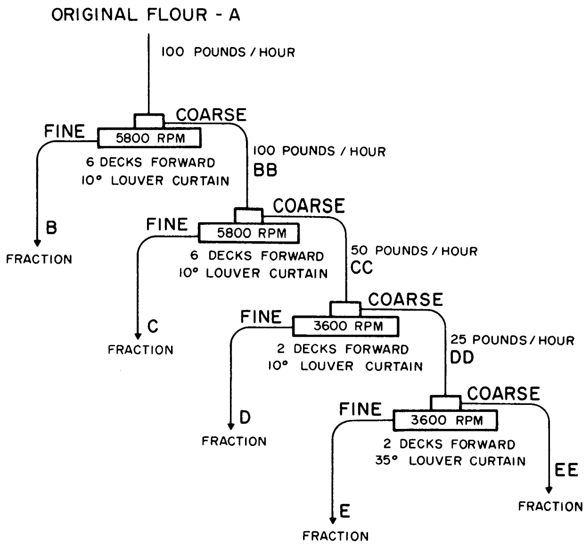

Fig. 1. Flow diagram of the germ air-classification procedure.

described in AACC Approved Methods (17). Bound lipids were extracted according to a procedure described previously (18).

Protein Extracts

Proteins were extracted from wheat germ with 0.05N acetic acid, various concentrations of NaCl, and various concentrations of $CaCl_2$. Five grams of germ was extracted with 50 ml. of liquid for 5 min. in a Waring Blendor. The suspension was cleared by centrifugation at 27,000 $\times$ g. The solids were resuspended with additional 50 ml. of liquid and extracted for another 5-min. period. The two supernatants were combined, dialyzed, frozen, and lyophilized.

Air Fractionation

The germ was air-fractionated into high- and low-protein streams according to the scheme given in Fig. 1. Fractionations were made in a Pillsbury Laboratory Model No. 1 classifier, employing the indicated feed rates, speeds, and internal set-up. Fine fractions B, C, D, and E were removed from the original flour A. Residual coarse germ was designated EE; small amounts were collected in the bag of the air classifier.

Protein and Lipid Composition

Starch-gel electrophoresis was performed on dialyzed samples in a vertical position with pH 3.2 aluminum lactate and lactic acid buffer containing 3.0M urea, at 30 ma. and approximately 325 v. for 4 hr. at room temperature. The gel was sliced and stained with 0.1% Amido Black 10 B. Details of the procedure have been given previously (19).

Acid hydrolysis was used in the preparation of samples for amino acid analyses. Twenty-five milligrams of sample was hydrolyzed with 6 ml. of 6.0N HCl in an

evacuated, sealed test tube for 20 hr. at 110°C. The hydrolysate was filtered, evaporated to dryness over NaOH, and diluted to volume with 0.2N sodium citrate buffer, pH 2.2. The samples were stored at −20°C. until required for analysis. The hydrolysates were practically clear, indicating little, if any, formation of humin. Amino acids were determined on a Beckman Model 120C amino acid analyzer.

Thin-layer chromatography was performed on 150 γ of lipids. Plates were developed with chloroform (for nonpolar lipids) or chloroform-methanol-water (65:25:4) (for polar lipids), then sprayed with a saturated solution of $K_2Cr_2O_7$ in 70% of aqueous sulfuric acid, and charred at 150°C. for 30 min. Details of the procedure were described (18).

RESULTS AND DISCUSSION

Petroleum ether (b.p. 35° to 60°C.) extracted from raw germ 11.7 to 12.2% (average 12.0%) crude fat. Drying at temperatures up to 100°C. for 12 hr. had little effect on fat extractability. Acetone extracted from raw germ 13.4 to 14.8% crude lipid (average 1.9% more crude fat than extraction with petroleum ether).

High drying temperatures drastically affected extractability of germ proteins with 0.05N acetic acid. In germ dried for 12 hr. at 70°C., 32.6% of the total protein was extracted. Heating the germ at 100°C. lowered the yield to 30.0%, and heating at 130°C. drastically reduced the yield to 1.1% of the germ proteins.

Salt solutions (NaCl or $CaCl_2$) gave much higher yields of extractable proteins from wheat germ. When the germ was extracted three times with NaCl solution, consecutive extracts contained 76.5, 8.2, and 1.6% of the total germ protein. The effects of NaCl and $CaCl_2$ concentration on protein extraction (yields of dialyzed and lyophilized material from two-step extractions) are summarized in Fig. 2. Highest yields of protein were extracted with about 3.0% salt solutions. Sodium chloride was a better protein extractant than $CaCl_2$. The dialyzed and lyophilized salt extracts contained about 83% protein on dry matter basis.

Concentration of wheat germ proteins by salt extraction is expensive and time-consuming. Air classification of cereal products is relatively simple and

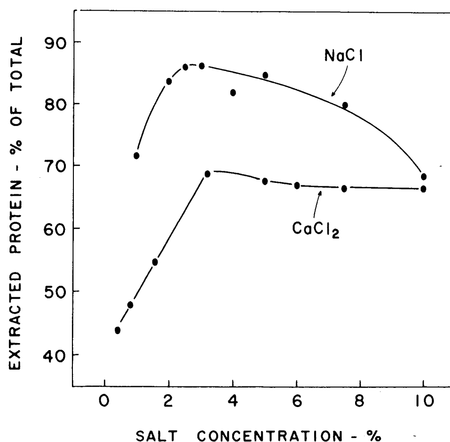

Fig. 2. Effects of NaCl and $CaCl_2$ concentration on extraction of germ proteins.

TABLE I. AIR CLASSIFICATION OF WHEAT GERM[a]

Source and Fraction	Yield %	Moisture %	Average Particle Size	Ash %	Protein (NX5.45) %
Whole germ					
A	100.0	6.2	10.1	4.7	29.1
B	1.8	6.7	3.9	5.0	28.5
C	11.8	6.8	6.1	5.2	30.0
D	10.4	6.6	8.4	5.1	30.7
E	19.4	7.1	10.0	4.9	27.6
EE	56.3	7.1	15.8	4.5	28.1
Bag	0.3	9.6	2.4	6.3	31.4
Petroleum ether-defatted germ					
A	100.0	7.2	5.2	5.4	33.0
B	11.9	7.5	2.6	7.6	35.2
C	22.0	7.2	4.7	6.0	36.8
D	24.7	7.5	8.4	5.5	35.3
E	14.3	7.7	10.9	4.6	30.0
EE	26.1	7.7	19.8	3.7	26.4
Bag	1.0	8.9	1.8	7.3	37.2

[a]Expressed on as-is basis.

inexpensive. However, the expected concentration of protein from a shift during air classification would be considerably smaller than with an efficient and selective wet extraction. The authors know of no published study on air fractionation of wheat germ. The results of air classification of germ that was pin-milled on an Alpine mill at 15,000 r.p.m. are summarized in Table I. Both the original germ and germ that had been dried and defatted were fractionated. Air classification of the original fat-rich germ is fast and economical. Stability of pin-milled and air-classified germ was limited. Defatting the dried germ yielded a more stable product and substantially increased the protein shift. In the original fat-rich germ, the protein increase in the major fractions was 0.9 and 1.6%. In the defatted and dried germ, the protein increased (in a fraction that comprised 22.0% of the total) 3.8%. Reclassification of the protein-enriched fraction (without regrinding) increased protein 1.7% more; however, the yield of that latter fraction was substantially decreased.

Starch-gel electrophoretic patterns of proteins from gluten and germ are compared in Fig. 3. Gluten proteins contained glutenins (mostly retained at the point of origin) and gliadins that moved a short distance into the gel. The gluten proteins were virtually absent in germ. Germ proteins contained a whole spectrum of fast-moving salt-soluble proteins separated into several bands. The most prominent band was somewhat reduced in the NaCl extract and was much smaller in the high-protein than in the low-protein air-separated fractions.

Amino acid composition of proteins in defatted germ differs substantially from that of wheat flour and especially of wheat gluten proteins (Table II). Results are in general agreement with those reported in literature (3,4). The amino acid composition of the NaCl extract differed little from the composition of proteins in

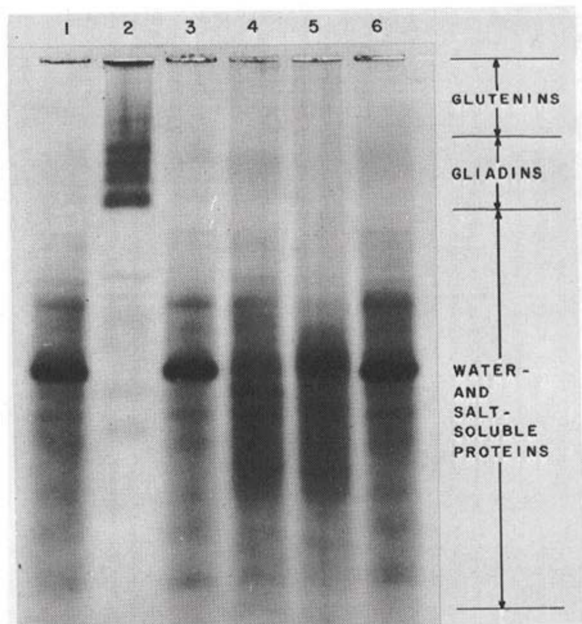

Fig. 3. Starch-gel electrophoretic patterns (migration from slots on top of figure) of proteins in 1) whole germ, 2) wheat flour gluten, 3) germ air-fractionated low-protein fraction, 4) germ air-fractionated high-protein fraction, 5) NaCl extract of germ proteins, and 6) whole germ.

TABLE II. AMINO ACID COMPOSITION[a] OF WHEAT FLOUR, GLUTEN, AND DEFATTED GERM OR GERM PRODUCTS

					Air-Fractionated Germm	
Amino Acid	Flour g.	Gluten g.	Whole Germ g.	NaCl Extract of Germ g.	High-Protein Fraction g.	Low-Protein Fraction g.
Lysine	1.78	1.53	7.76	8.33	6.41	7.30
Histidine	1.82	1.77	2.65	2.58	3.35	2.40
Ammonia	3.02	3.33	1.71	1.54	2.26	3.15
Arginine	3.23	3.14	8.86	9.85	10.60	7.27
Aspartic acid	3.81	3.05	10.21	8.81	9.67	9.58
Threonine	2.31	2.36	4.82	4.92	4.27	4.40
Serine	4.43	4.18	4.62	4.64	4.10	4.60
Glutamic acid	37.19	39.30	15.45	15.94	16.47	16.76
Proline	11.55	11.49	4.37	4.04	4.22	5.07
Glycine	3.37	3.03	6.54	6.95	6.58	6.34
Alanine	2.87	2.37	7.00	6.89	6.40	6.70
Cystine	1.44	1.61	0.66	0.88	0.75	1.09
Valine	3.99	3.58	5.65	5.51	5.80	5.06
Methionine	1.45	1.45	1.88	2.11	1.77	1.89
Isoleucine	3.80	3.51	3.91	3.59	3.64	3.61
Leucine	6.64	6.31	6.79	6.37	6.53	7.45
Tyrosine	2.15	3.30	3.12	3.26	3.02	3.34
Phenylalanine	5.16	4.68	4.07	3.86	4.20	4.02

[a]Grams per 100 g. amino acids.

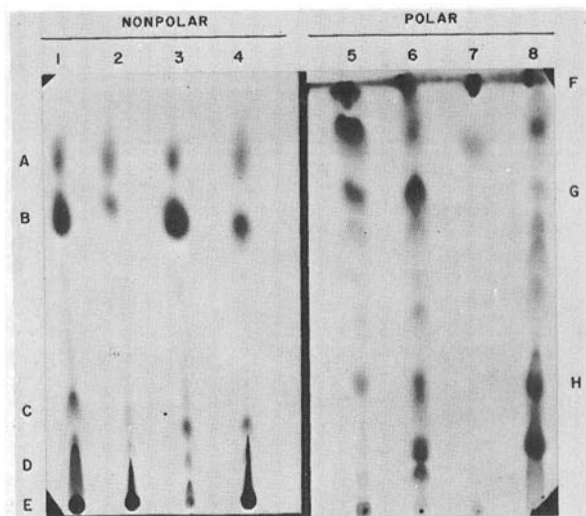

Fig. 4. Thin-layer chromatography of lipids in wheat flour and wheat germ. Samples 1 to 4 fractionated with chloroform, 5 to 8 with chloroform-methanol-water (65:25:4). Samples 1 and 5 free lipids (petroleum-ether extract) of flour; 2 and 6, bound lipids (water-saturated butanol extract following petroleum ether) of flour; 3 and 7, free lipids of germ; 4 and 8, bound lipids of germ. Tentatively identified as A) hydrocarbons and steryl esters, B) triglycerides, C) diglycerides, D) free fatty acids, E) unresolved polar lipids, F) unresolved nonpolar lipids, G) digalactosyldiglycerides, and H) phosphatidyl choline.

defatted germ. The high-protein fraction from air classification of defatted germ contained less than the low-protein fraction, of lysine, serine, proline, sulfur-containing amino acids, alanine, leucine, and tyrosine.

Thin-layer chromatography of lipids (Fig. 4) shows virtual absence of polar lipids in the petroleum-ether extract of germ. The bound germ lipids contain small amounts of polar components (presumably phospholipids and small amounts of glycolipids). Concentration of the polar compounds in total germ lipids is relatively negligible, however, as the ratio of free to bound lipids in wheat germ is approximately 12 to 2. This might explain the fact that whereas wheat flour lipids are essential in producing an optimum loaf of bread, germ lipids are ineffective or only partly effective (20,21).

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