AN IMPROVED METHOD FOR THE PREPARATION OF WHEAT GLIADIN[1]

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ABSTRACT

Good yields of gliadin free of lipid, carbohydrate, and protein impurities are obtained in less time and with less cumbersome operations, by a new alcoholic method. Protein impurities present in 35% isopropyl or 30% *tert*-butyl alcohol extracts of gluten are precipitated by pH adjustment and cooling. Lipid impurities are adsorbed on decolorizing carbon. Gliadin is then precipitated by adjusting the pH, diluting with water, and cooling the extract. Carbohydrate impurities remain behind in solution. The precipitate is washed with water and lyophilized. The final product is high in total and amide nitrogen, readily soluble in 60% ethyl alcohol or dilute acids, and bland in flavor.

Methods for preparing high-quality gliadin in substantial quantities are cumbersome and time-consuming because of long extraction periods, the necessity of concentrating dilute extracts, and repeated precipitations to remove impurities (2,3,5,12,13). Freedom from impurities is necessary in food applications where foam-producing capacity, foam stability, and a bland flavor are important (10). Simple and rapid methods for producing high-purity gliadin would increase its potential as a commercial product. The present paper describes an improved laboratory procedure for preparing this product.

Gliadin is the portion of wheat gluten protein soluble in 60 to 70% ethyl alcohol. Recently the material was shown to be a mixture of at least four major electrophoretic components (9,15) which are all present in our product in about the same proportions as reported.

Materials and Methods

Starting material for all preparations of gliadin was commercially available native dried gluten. Both analytical and technical grade isopropyl alcohol were used with no apparent difference in results; only the analytical grade of *tert*-butyl alcohol was used. Distilled water was used for preparing alcohol solutions and making dilutions.

Protein contents of dry preparations were calculated ($N \times 5.7$) from Kjeldahl nitrogen values (1), and amide nitrogen contents were deter-

[1]Manuscript received November 22, 1961. Contribution from the Western Regional Research Laboratory, Albany, California. This is a laboratory of the Western Utilization Research and Development Division, U. S. Department of Agriculture, Albany 10, California.

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mined by the method of Mecham and Olcott (11). For rapid estimations of protein contents a biuret reagent was used (14). Turbid test solutions with this reagent were clarified by filtering in a Hirsch funnel through ¼ in. of dry Celite filter aid supported on Whatman No. 1 filter paper.

Carbohydrate impurities were estimated colorimetrically with an orcinol reagent (7) which was used directly on 0.5- to 6-mg. samples of the gliadin preparations. Results were compared to a standard curve obtained with xylose and are expressed as micromol. of xylose.

Lipid impurities were estimated colorimetrically by a hydroxamic acid method (8). It was necessary to add *tert*-butyl alcohol to prevent the development of turbid test solutions and low color values. The following procedure was used: Gliadin (10–25 mg.) dissolved in 4 ml. of 50% (v/v) *tert*-butyl alcohol was rapidly mixed with 4 ml. of fresh pH 11.7 hydroxylamine (prepared by mixing 7 ml. of approximately 3.5*N* sodium hydroxide with 10 ml. of 2*M* $NH_2OH \cdot HCl$). After exactly 5 minutes 1 ml. of approximately 5*N* hydrochloric acid was added. Then, after addition of 1 ml. of *tert*-butyl alcohol, 1 ml. of 1.1*M* ferric chloride (in 0.1*N* hydrochloric acid) was added, and the light absorption at 540 mμ was determined within 3 to 5 minutes against a water blank. The pH of the final solution was 1.2 ± 0.2, as recommended in the original method. Absorption differences due to reagents were corrected by controls containing everything but the sample. Values were determined from a standard curve of glycerol mono-oleate and were expressed as micromol. of this substance.

Estimates of alcohol remaining in dried samples were made as follows: 1 g. of gliadin was hydrated with 2 ml. of water in a 250-ml. ground-joint, round-bottomed flask. The flask was fitted to one leg of a U-tube assembly and a ground-joint receiver tube on the other leg. The joints were greased with silicone stopcock grease. The wet gliadin was frozen in a dry ice-acetone bath, and then the system was evacuated through a stopcock. The liquid was distilled from the flask to the receiver tube by heating the flask with a water bath and cooling the receiver with dry ice and acetone. The distillate was warmed to room temperature and diluted to 5 ml. The alcohol was estimated colorimetrically at 495 mμ after addition of 1 ml. of ceric ammonium nitrate reagent (6). The blank contained only reagent and water.

Apparent pH of alcohol-containing solutions was measured with a Model G Beckman pH meter. Changes in pH of the extracts were made by careful addition of either 2*N* hydrochloric acid or 2*N* sodium hydroxide.

Electrophoresis runs were made in a Perkin-Elmer model 38 electrophoresis apparatus.

Experimental Results

Extraction of Gliadin. Isolation procedures were developed using both aqueous *tert*-butyl and isopropyl alcohols as extraction solvent. Because of the much lower cost and commercial applicability of isopropyl alcohol, the latter was selected as being the more suitable.

Protein extraction rates at room temperature from gluten sifted as a dry powder into well-stirred isopropyl and *tert*-butyl alcohol solutions are shown in Table I. Soluble protein was estimated on cloudy

TABLE I

RATES OF PROTEIN EXTRACTION BY AQUEOUS ISOPROPYL AND *tert*-BUTYL ALCOHOL SOLUTION[a]

EXTRACTION PERIOD	ALCOHOL	PERCENT ALCOHOL (v/v)				
		20	30	40	50	60
minutes		*mg/protein/ml*	*mg/protein/ml*	*mg/protein/ml*	*mg/protein/ml*	*mg/protein/ml*
15	Isopropyl	..	36	37	36	..
30		..	37	40	37	..
45		24	35	36	36	34
15	*tert*-Butyl	..	25	28	28	..
30		..	28	32	32	..
45		..	32	37	37	..

[a] Gluten protein to solvent was 48 mg. per ml.

supernatants after centrifugation. About 77% of the total protein dissolved within 15 to 30 minutes in 30 to 60% (v/v) isopropyl alcohol. Within the same extraction period 30 to 50% (v/v) *tert*-butyl alcohol dissolved over 59% of the total gluten protein. Upon standing, a slight amount of gliadin protein appeared to precipitate from the 30% isopropyl alcohol extract but not from a 35% isopropyl alcohol solution tested later or from the 30% *tert*-butyl alcohol extract. The latter isopropyl alcohol concentration and the 30% *tert*-butyl alcohol were selected for extraction of gliadin from gluten with the minimum amount of alcohol. The materials extracted by these solvents contain 16% total nitrogen of which 26% was amide nitrogen (Table II), and therefore should be high in gliadin. Highly purified gliadin is reported to contain 17.5% total nitrogen of which 26% is amide nitrogen (2,3,5). The glutenin portion of gluten, on the other hand, is reported to contain 14 to 22% amide nitrogen (4).

Precipitation of Protein Impurities. The material causing turbidity of the alcoholic extracts appears to be a lipoprotein, possibly glutenin.

It precipitated within several hours after the pH of the extract was raised from 5.8 to neutrality and the solution was cooled. About 10 to 20% of the total protein precipitated from solution. The precipitated

TABLE II

DRY WEIGHT COMPOSITION OF GLIADIN EXTRACTED AND PURIFIED IN 35 PERCENT ISOPROPYL AND 30 PERCENT *tert*-BUTYL ALCOHOL SOLUTIONS

GLIADIN	NITROGEN	NITROGEN AS AMIDE NITROGEN	CARBOHYDRATES PER G. OF PROTEIN	LIPIDS PER G. OF PROTEIN
	%	%	*μM as xylose*	*μM as mono-oleate*
Isopropyl alcohol				
Original extract	16.6	24.7	28	100
Turbid impurities removed	16.8	24.5	25	60
Carbon-treated	16.9	27.5	19	26[a]
Precipitated and washed	17.8[b]	25.8[b]	1.9	24[a]
tert-Butyl alcohol				
Original extract	15.5	25.6	57	150
Turbid impurities removed	15.9	25.4	62	83
Carbon-treated	16.7	25.0	54	25
Precipitated	17.2	25.5	3.6	21

[a] Lipid-free gliadin prepared from flour previously extracted exhaustively with water-saturated n-butyl alcohol to remove all lipids gave a value equivalent to 25 micromol. of mono-oleate per g.
[b] Pure gliadin is reported to contain 17.5% total nitrogen and 26% of the total as amide nitrogen (2,3,5).

material contains 14% total nitrogen of which 22% is amide N, and thus in respect to amide nitrogen it is similar to glutenin (4). Also, like glutenin, it apparently absorbs lipids, for a large part of the lipid impurities are removed in the step (Table II). At 3 to 4% total protein concentration in the isopropyl or *tert*-butyl alcohol extract, adjustment of the pH to 6.8–6.9 for the precipitation gave satisfactory clarification upon centrifugation; but on working with an isopropyl alcohol extract at 5 to 6% protein concentration, dilution of the isopropyl alcohol to 34% and adjustment of the pH to 7.0 were required to obtain reasonably clear supernatants.

Adsorption of Lipid Impurities. Remaining lipid impurities adsorb on decolorizing carbon with little loss of protein. For this procedure the pH of the extract was lowered to 5.9 to prevent precipitation of gliadin and to facilitate subsequent filtration. The carbon was kept suspended in the extract by mechanical stirring for an appropriate period. Celite filter aid was then added, and the mixture was filtered through a Celite mat in a Büchner funnel. Filtration was facilitated by keeping the Celite-carbon particles suspended by stirring and adding

the mixture slowly so as to maintain a thin layer of liquid in the funnel. Clogging of the filter indicates incomplete precipitation of turbid material in the preceding step. The filtrate was water-clear, with a yellow-green fluorescence occurring at 5 to 6% protein concentration. Lipid analysis (Table II) indicates the solids in the filtrate are essentially lipid-free. About 6% of the total protein was lost in this step.

Precipitation and Recovery of Gliadin. In the final steps gliadin was precipitated and washed to remove carbohydrates, inorganic salts, and alcohol. Raising the pH of the extract to 7.1–7.7, warming to 40°C. in a water bath, diluting with one-half volume of 40°C. water, and finally cooling to 5°–10°C. in an ice bath or cold room precipitated over 90% of the gliadin. Diluting at 40°C. instead of room temperature facilitates handling of the mixture prior to cooling, by preventing coagulation of the gliadin into a sticky mass. After cooling, the then coagulated gliadin may be recovered by centrifugation or by decantation after overnight settling at 5°C. The precipitate can be washed with distilled water at 50°C. with little loss of gliadin, to remove the last bit of impurities and the alcohol. Raising the temperature to 50°C. facilitates this step by lowering the viscosity of the stiff gliadin mass. After the gliadin has been stiffened by cooling in an ice bath, the wash liquid may be removed by decantation. Material recovered from freeze-drying the wet gliadin contains 100% protein (Table II), according to classic criteria, and only traces of impurities. No alcohol was detected by the method employed. The protein exhibits solubility characteristics expected of gliadin, in that water-clear solutions of 10% or greater concentrations can be readily obtained with 60% (v/v) ethyl alcohol, dilute hydrochloric acid, or dilute acetic acid. Its electrophoretic pattern (Fig. 1) is similar to that reported by

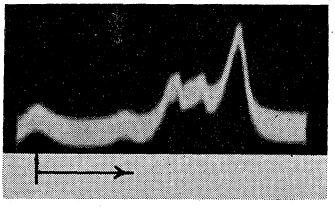

Fig. 1. Ascending limb of electrophoresis pattern for 1% purified gliadin in 0.0083*M* aluminum lactate-lactic acid buffer, pH 3.1, 1°C.; 7.9 v/cm., 45 minutes.

Jones *et al.* (9). The product is bland in flavor.

Complete Preparative Procedure. The complete procedure is described here with 35% (v/v) isopropyl alcohol, but 30% (v/v) *tert*-butyl alcohol may be used with similar results. Gluten is extracted with

stirring, using 9 ml. of alcohol solution per g. of gluten. Up to 350 g. of gluten may be conveniently extracted at one time. After 45 minutes the insoluble material is removed by centrifugation for 10 minutes at $1200 \times g$, and the centrifuge cake is washed with fresh alcohol solution (5.4 ml. per g. of starting material). The wash-solution supernatants may be used for extraction of additional gluten or may be added to the original extract. The cloudy extract is adjusted to pH 7.0 and cooled in a water bath (22°C.). The resulting precipitate ordinarily can be removed by centrifugation within 3 hours and is discarded. Then the supernatant is stirred for 1 hour with 16 g. per liter of Norite A at pH 5.9. The carbon is removed by adding 16 g. per liter of filter aid to the extract and filtering in a Büchner funnel fitted with No. 598 Schleicher and Schuell filter paper prelayered with $\frac{3}{8}$ in. of filter aid. After adjustment of the pH of the water-clear filtrate to 7.0, the protein is precipitated by diluting the extract at 40°C. with one-half volume of distilled water and cooling the mixture to 10°C. The precipitated gliadin is recovered either by centrifugation or by decantation after standing overnight at 5°C. The precipitate is washed several times at 50°C. with distilled water. The precipitate may then be dispersed in 0.1*N* acetic acid and freeze-dried. Yields are about 80% if calculated on the assumption that 50% of the original gluten protein is gliadin.

Discussion

The method for preparing high-purity gliadin developed in the present work has several advantages over previous procedures. First, the initial extraction with isopropyl or *tert*-butyl alcohol and recovery of the extract can be completed in just a few hours. This is much less time than required by the older methods, where overnight extracts and repeated extractions are employed (2,5,12,13).

Nongliadin protein material peptized during the gliadin extraction is simply and quickly removed by centrifugation after appropriate pH adjustment. This avoids the tedious and difficult filtrations used by previous workers (2,5,12,13). Complete removal of the material is absolutely necessary for good filtration during removal of carbon in the subsequent step.

The removal of lipids by adsorption on activated carbon makes unnecessary the repeated precipitations of the gliadin protein previously used by other workers to remove impurities (2,3,5,12,13). In our experiences, lipid impurities are largely carried down with the gliadin precipitate, whereas carbohydrate impurities are not. The latter are

readily removed with one precipitation of gliadin, but repeated precipitations are required for removal of the lipid impurities unless the carbon method is used. The tedious concentrating of solutions which was used by some workers prior to each precipitation (5,12,13) has also been avoided.

The purity of the final product is high, as judged from its high total nitrogen and amide nitrogen contents, low carbohydrate and lipid contents, and high solubility in 60% ethyl alcohol or dilute acids. The yield is as good as, or better than, those few previously reported (2,12). The final advantage of the new procedure is that the product is bland enough in flavor to be used as an ingredient in delicately flavored food products (10).

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