Extraction of Proteins from Wheat Bran: Application of Carbohydrases[1]

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Cereal Chem. 58(4):264–266

ABSTRACT

Proteins (N × 5.7) were extracted from commercial full-fat wheat bran, using a wet alkaline extraction procedure at pH 8.5. The extract thus obtained contained 30% of the total nitrogen originally present in the bran. However, the yield of total nitrogen in the extract could be increased to 38.5% with a pretreatment containing carbohydrases (cellulase, hemicellulase, and pectinase) followed by the normal extraction procedure.

Wheat bran, which represents approximately 20% of the grain, is composed of 16–17% protein (dry basis) and is nutritionally superior to endosperm proteins (D'Appolonia 1979). Fellers et al (1966) used a wet alkaline extraction procedure to isolate bran protein in the form of concentrate/isolate, for protein enrichment of human diets. Subsequently, Woerman and Satterlee (1974), Saunders et al (1975a, 1975b), Hansmeyer et al (1976), Saunders and Betschart (1977), and Lindsay et al (1977) have refined the technique for extraction and recovery of protein, in addition to characterizing functional and nutritional properties of isolated proteins for inclusion in specific food systems.

Isolation of proteins from bran is an example of a solid-liquid extraction process, but, in this case, the underlying mechanism and rate-limiting factors are yet unknown. From the literature cited above, the most important factor affecting the recovery of protein from wheat bran appears to be the pH of the extracting solvent. However, because of the risks involved when operating at higher pH and temperature, risks such as damage to the nutritional quality of isolated proteins (Fellers 1966), alterations in the functional properties (Woerman and Satterlee 1974), and formation of lysinoalanine (Finley and Kohler 1979), obtaining the protein in the "native state" as much as possible by utilizing other approaches instead of pH rise to increase the yield is desirable.

One such alternative is the application of enzymes. Because cell walls in plant materials are composed of cellulose microfibrils embedded in a continuous matrix of lignin, pectin, and hemicelluloses (Kulp 1975), and because these membranes provide certain transport selectivity and thereby limit mass transfer (King 1977), disruption of these membranes by respective enzymes may augment the extraction rate. The feasibility of this approach is shown in the early work of Toyama (1969) on the maceration of vegetable plant cells with cellulases and in more recent work with extraction of alfalfa leaf proteins (Mudgett et al 1978), wherein maximum yield was obtained by using cellulase and pectinase.

Our objective was to study the extraction of proteins from wheat bran by coupling a pretreatment step incorporating cellulase, hemicellulase, and pectinase (applied individually or in combination) to the regular extraction procedure.

MATERIALS AND METHODS

Bran

The composition of the wheat bran utilized in this work was, on a dry basis, 19.2% protein (N × 5.7), 6.0% fat, 6.8% ash, 7.4% fiber, and 60.6% nitrogen-free extract. The bran was obtained from a commercial source (Moinho Carlos Guth, Curitiba, Brazil), ground to pass a 50-mesh USS screen, and stored at 0–5°C before use.

Enzymes

All enzymes utilized in this work were commercial preparations obtained through Sigma Chemical Co. Cellulase (E.C. 3.2.1.4.) was a lyophilized crude powder from *T. viridae*; hemicellulase was a crude preparation from *A. niger*; and pectinase (E.C. 3.2.1.15) was

[1] Presented at the AACC 64th Annual Meeting, Washington, DC, 1979. Taken in part from a thesis submitted by the senior author to the State University of Londrina in partial fulfillment of requirements for the Masters degree in food science.
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0009-0352/81/04026403/$03.00/0
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a purified preparation from *A. niger*. The enzymes had specific activities of 0.18, 0.0034, and 1.1 units per milligram of solid, respectively.

Extraction of Nitrogen

In the pretreatment step, 50 ml of acetate buffer solution at a selected pH and containing a given quantity of each of the enzymes was added to 10 g of bran material. The mixture was agitated at 120 rpm in a constant-temperature (37° C) chamber during a specified period of time. The details of pretreatment are given in Table I. At the end of pretreatment, the volume was adjusted to 100 ml and the pH to 8.5 with 2*N* NaOH, and the agitation of the mixture was continued for 1 hr at 120 rpm in the constant-temperature (25° C) chamber. The mixture was then centrifuged at $1,400 \times g$ for 15 min. The supernatant obtained was filtered through glass wool to obtain the final extract. An aliquot of the extract was analyzed for total N, using the Kjeldahl method described in AOAC method 2.049 (1975). The yield of N in the process was calculated as: (total N in the extract − N content of enzymes used)/ total N in 10 g of wheat bran. The resulting value was multiplied by 100 and expressed as percent N yield.

Because of the number of independent variables and the complexity of interactions among them, we employed response surface methodology, a fractional factorial design with five independent variables each at five levels, giving a total of 32 experiments, including six repetitions at the central point (Bender et al 1976, Henika 1972, Henika and Palmer[3]). The details of the experimental design are shown in Tables I and II. From the data obtained a multiple regression equation (Taylor expansion series) was built for yield of N, followed by optimization for selecting the levels of variables needed to maximize the yield of N in the process (Davies and Goldsmith 1972, Draper and Smith 1966). The computer analysis of data was carried out by the Foremost Research Center, Dublin, CA.

RESULTS AND DISCUSSION

The data for multiple regression analysis relating the yield (as percent) of N extracted (Y) to the independent variables (X1–X5) gave the following equation:

$$\begin{aligned} Y = {} & 28.744 + 1.569X_1 + 0.855X_2 + 1.587X_3 + 0.897X_4 - 0.275X_5 \\ & - 0.582X_1^2 + 0.594X_2^2 + 0.237X_3^2 + 0.409X_4^2 - 0.067X_5^2 \\ & + 0.002X_1X_2 + 0.057X_1X_3 + 0.086X_1X_4 + 0.293X_1X_5 \\ & - 0.384X_2X_3 + 0.569X_2X_4 - 0.216X_2X_5 - 0.113X_3X_4 \\ & + 0.342X_3X_5 - 0.099X_4X_5 \end{aligned}$$

where X_1 is cellulase (units); X_2 is hemicellulase (units); X_3 is pectinase (units); X_4 is time (hr); and X_5 is pH (units).

The following estimates were associated with the above polynomial: standard error, 1.30; standard deviation of replicates, 0.21; and multiple correlation coefficient, 0.96. The model explained 91.4% of the variation. The percent of the variation attributable to linear effects was 72.6; to quadratic effects, 13.2; to interaction effects, 5.5; to experimental error, 0.1; and 8.48% of the variation was not explained.

Analysis of variance for the above data is given at the top of Table III.

From the analysis presented thus far, the regression as a whole and the linear as well as second order effects appear to be significant. But the lack of fit was also significant, thus casting doubt on the entire model. However, Henika[4] has suggested that whenever the mean square for experimental error takes on extremely low values, tests of significance for lack of fit should be considered irrelevant. Nevertheless to improve the precision and reliability of our conclusions, we repeated the analysis of variance calculations using "lack of fit" mean squares instead of "total residuals" for calculating F values, giving the results shown at the bottom of Table III.

This analysis confirms the significance of linear effects. Because the terms in the model are independent, based on the significance tests, the second order and interaction terms could be omitted, giving a simplified model consisting only of linear effects.

Therefore, if all three enzymes used in the experiment were held at their maximum level (+2), incubation time at 7 hr, and initial pH at 3.7, we could obtain a yield of 38.5% for N extracted. Our data (Nina 1979) with minimaps and contour maps indicate that a real maximum was not reached within the ranges of the variables. This report confirms the potential for carbohydrases in increasing the yield of intracellular constituents such as proteins from solid substrates like wheat bran during the process of wet extraction. However, under practical conditions, the choice of the enzyme and its level of usage are dictated by the economics of the process. In our case, more research is needed to elucidate the mechanism of action

[3]R. G. Henika and G. M. Palmer, 1976. Response surface methodology revisited. Presented at the AACC 61st Annual Meeting, New Orleans, LA.

[4]R. G. Henika. 1978. Personal communication.

TABLE I
Experimental Design for Response Surface Methodology

Variables	Levels[a] −2	−1	0	+1	+2
X_1 Cellulase (units)[b]	0.0	10.0	20.0	30.0	40.0
X_2 Hemicellulase (units)[c]	0.0	0.5	1.0	1.5	2.0
X_3 Pectinase (units)[d]	0.0	30.0	60.0	90.0	120.0
X_4 Incubation time (hr)	3.0	4.0	5.0	6.0	7.0
X_5 Initial pH (units)	3.7	4.2	4.7	5.2	5.7

[a]Coding relationships for variables are as follows: (Variable, X_i − Variable at central point, X_i at "0")/ Interval, ΔX. For example, $X_1 = 2 = (222.2-111.1)/55.55$.
[b]1 unit = 5.5 mg of solid.
[c]1 unit = 294.1 mg of solid.
[d]1 unit = 0.91 mg of solid.

TABLE II
Details of Experimental Set-Up and Observed Values for Nitrogen Extracted (Y) from 10 g of Wheat Bran

Test No.	Experimental Variables: Cellulase (mg)	Hemicellulase (mg)	Pectinase (mg)	Time (hr)	pH	Y_{obs}[a] (%)
1	55.6	147.1	27.3	4.0	5.2	23.93
2	166.7	147.1	27.3	4.0	4.2	27.12
3	55.6	441.2	27.3	4.0	4.2	27.52
4	166.7	441.2	27.3	4.0	4.2	27.38
5	55.6	147.1	81.8	4.0	4.2	29.70
6	166.7	147.1	81.8	4.0	5.2	32.01
7	55.6	441.2	81.8	4.0	5.2	28.61
8	166.7	441.2	81.8	4.0	4.2	31.53
9	55.6	147.1	27.3	6.0	4.2	26.64
10	166.7	147.1	27.3	6.0	5.2	27.30
11	55.6	441.2	27.3	6.0	5.2	27.60
12	166.7	441.2	27.3	6.0	4.2	32.40
13	55.6	147.1	81.8	6.0	5.2	29.28
14	166.7	147.1	81.8	6.0	4.2	32.07
15	55.6	441.2	81.8	6.0	4.2	32.64
16	166.7	441.2	81.8	6.0	5.2	32.04
17	0.0	294.1	54.5	5.0	4.7	21.44
18	222.2	294.1	54.5	5.0	4.7	31.30
19	111.1	0.0	54.5	5.0	4.7	29.36
20	111.1	588.2	54.5	5.0	4.7	32.79
21	111.1	294.1	0.0	5.0	4.7	27.62
22	111.1	294.1	109.1	5.0	4.7	31.67
23	111.1	294.1	54.5	3.0	4.7	28.51
24	111.1	294.1	54.5	7.0	4.7	32.16
25	111.1	294.1	54.5	5.0	3.7	27.71
26	111.1	294.1	54.5	5.0	5.7	29.15
27	111.1	294.1	54.5	5.0	4.7	28.94
28	111.1	294.1	54.5	5.0	4.7	28.76
29	111.1	294.1	54.5	5.0	4.7	28.35
30	111.1	294.1	54.5	5.0	4.7	28.91
31	111.1	294.1	54.5	5.0	4.7	28.78
32	111.1	294.1	54.5	5.0	4.7	28.82

[a]For controls (ie, extraction without pretreatment with enzymes), Y_{obs} was 30.0% (Nina 1979).

TABLE III
Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	F_{calc}	$F_{tab (95\%)}$
Original Analysis					
Regression	198.9	20	9.5	5.85[a]	2.65
First order	158.1	5	31.6	18.60[a]	3.20
Second order	28.7	5	5.8	3.38[a]	3.20
Interaction	12.1	10	1.2	0.71	2.85
Residuals	18.7	11	1.7		
Lack of fit	18.5	6	3.1	61.50[a]	4.95
Experimental error	0.23	5	0.05		
Total	217.2	31	7.0		
Modified Analysis[b]					
Regression	198.9	20	9.5	3.06	3.87
First order	158.1	5	31.6	10.02[a]	4.39
Second order	28.7	5	5.8	1.87	4.39
Interaction	12.1	10	1.2	0.39	4.06
Lack of fit	18.5	6	3.1		

[a] Significant at $P < 0.05$.
[b] F values calculated with "lack of fit" mean squares.

of these enzymes on the recovery of proteins from the extract and on the functional and nutritional properties of the proteins isolated. In future work, the use of purified sources of enzymes would be desirable because impurities (particularly cross-contamination) encountered in commercial preparations of carbohydrases may complicate the model development.

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[Received December 27, 1979. Accepted October 30, 1980]