Breadmaking Quality Estimation By Fast Spectrophotometric Method

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ABSTRACT

A fast method to estimate wheat breadmaking quality diminution as a result of excessive thermal treatment during drying is proposed. Proteins that remain in soluble after drying were extracted with a solution of phosphoric acid plus ethanol, and colorimetrically quantified after dyeing with Coomassie Brilliant Blue G and measuring at 595 nm. A decrease in absorbance values indicates a decrease in loaf volume and breadmaking quality. The application of the proposed technique required ≈30 min.

Hot-air-drying of wheat can affect the breadmaking quality of the flour. The main factors influencing quality are previous history, moisture content, time of exposure, drier design, and wheat cultivar (Nellist 1978). Damage may be quantified by biological, biochemical, chemical, and physicochemical tests. According to Nellist (1978), the most important tests include large-scale baking tests that give results in accordance with flour behavior in the industrial process; microbaking tests; extensimeter measurements; farinograms; Chopin's alveogram; gluten agglutination test (Hutchinson and Booth 1946); glutamine decarboxylase and ribonuclease activities (Linko 1961, Slusanschi et al 1973); turbidity test (Greer et al 1964, McDermott 1971); and germination test (Ghaly et al 1974). Thermal treatments modify protein solubility (McGuire and Earle 1958), thus the measure of quantitative extracted protein in soluble form may be useful in estimating damage. Soluble proteins may be quantified by colorimetric assay using dye. The dye binds to the protein and causes a shift in the absorption maximum of the dye from 465 to 595 nm (Bradford 1976; Hook 1979, 1980).

Hot-air-drying is a common practice in Argentina where wheat price is set according to protein content. But protein content does not assure good breadmaking quality because the functional properties of proteins, and not the quantity, were modified first by the drying effects. When wheat is received, a rapid determination of breadmaking quality is required to facilitate grain storage according to quality. The aim of this work was to develop a fast method for estimating diminution of wheat breadmaking quality as a consequence of excessive thermal treatment during drying. The method quantifies the thermally modified protein by extracting the remaining soluble protein with a suitable extractive solution and then making a colorimetric determination of the extracted protein.

MATERIALS AND METHODS

Four wheat cultivars with different protein contents (N × 5.7) were used: PROINTA Super (12.4, 13.4, 14.7, 15.5, 17.0, 17.6%), Las Rosas INTA (9.4, 15.6%), Klein Chamaco (9.6, 14.5, 16.6%), and Klein Dorado (13.2%) supplied by EEA INTA Marcos Juárez (Marcos Juárez, Argentina). Grains were air-dried to 11% (wb) moisture content and stored at 25°C. Air-dried samples were used as controls. Coomassie Brilliant Blue G dye, was obtained from Sigma Chemical Co. (St. Louis, MO). Other ACS reagent-grade chemicals were obtained from Merck (Darmstadt, Germany). Water was ASTM 1 grade.

Drying treatments were conducted at different temperatures and times (Table I) on rewetted grains at an initial moisture content (IMC) adequate for a final moisture content (FMC) of 13.5–14.0% (wb). The tests were performed on a pilot-scale fluidized bed dryer built at the CIDTA (Tosi et al 1982). After the thermal treatment, the dried grains were cooled immediately by changing the fluidizing medium from hot to fresh air at ambient temperature. Grains were brought to the required IMC by spraying a quantity of water calculated by a drying kinetic empirical equation (Tosi et al 1984) and mixing periodically over 48 hr at 25°C. No differences were found between the drying curves of wheat grains with natural moisture and those of rewetted grains. Grain moisture content was determined in triplicate according to Method 711 (ISO 1995).

Three runs for each drying condition were performed on the PROINTA Super cultivar (protein content, 13.4% dwb). Grains were mixed to make up a unique sample for both the breadmaking quality tests and the extraction tests of the remaining soluble protein. The parameters selected for breadmaking quality tests were loaf volume, dry and wet gluten, and Chopin's alveograph indexes (W = dough strength [$\times 10^{-4}$ J]; L = extensibility [mm]; P = tenacity [mm]; P/L = tenacity-to-extensibility ratio). The extraction of the remaining soluble protein was accomplished on 1,000 g (dwb) of treated wheat previously milled in a cooled laboratory mill to pass through a ASTM 40 mesh. Extractive solution (30 mL) (Table II) and 150 mL of distilled water were added, shaken for 5 min, diluted to 200 mL, and filtered through paper (589[3] S&S or equivalent). Aliquots (50 mL) of the filtrate were analyzed for extracted protein (KEP) using Approved Method 46-11 (AACC 2000). Aliquots (1 mL) of the same filtrate were used in the colorimetric assay. KEP was correlated with loaf volume for selection of an optimum extractive solution.

The dye solution was prepared by dissolving 0.01 g of Coomassie Brilliant Blue G in 5 mL of ethanol and 10 mL of H_3PO_4 mixture, diluting to 100 mL with distilled water, and filtering through paper (589[3] S&S or equivalent). This dye solution is stable for three weeks.

The spectrophotometric quantification of the extracted proteins was accomplished by adding 10 mL of working dye solution to 1 mL of filtered protein extract, agitating by orbital shaker for 5 min, and allowing the solution to rest for 5 min, then measuring absorbance at 595 nm against a reagent blank. The relationship between the absorbance (A) and the KEP values was established.

The influence of grain protein content on the quantity of extracted protein was established by drying tests at various conditions (Table III) on PROINTA Super samples with different protein contents, extraction with the optimum extractive solution, and KEP determination.

Cultivar influence on quantity of extracted protein was evaluated by KEP and by colorimetric assay by comparing pairs of different cultivars of similar protein content. Klein Chamaco (9.6%) and Las Rosas INTA (9.4%), Klein Dorado (13.2%) and PROINTA Super (13.4%), PROINTA Super (14.7%) and Klein Chamaco (14.5%), Las Rosas INTA (15.6%) and PROINTA Super (15.5%), Klein Chamaco (16.6%) and PROINTA Super (17.0%).

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TABLE I
Drying Temperature and Drying Time on Wet and Dry Gluten (WG and DG), Alveograph Dimensions and Values and Loaf Volume (LV) Influence, and Phosphoric Acid-Ethanol Solution (PAE) Extracted by Both (KEP) and Absorbance (A) on Wheat Samples[a]

Drying Temp. (°C)	Drying Time (min)	WG (%)	DG (%)	Alveograph[b]						LV (mL)	KEP (mg)	A
				P	*G*	*L*	*W*	*P/G*	*P/L*			
Control	Control	27.8	10.1	122	20.5	85	392	5.9	1.43	755	1.54	0.710
50	55	27.6	9.8	122	20.6	84	391	6.0	1.45	760	1.52	0.731
60	55	27.8	10.0	121	20.5	85	389	5.9	1.42	755	1.53	0.732
70	12	25.8	9.4	119	20.3	84	376	5.9	1.42	700	1.54	0.734
70	21	23.2	9.1	120	16.1	53	285	7.4	2.29	690	1.53	0.711
80	5	26.1	9.8	120	17.6	63	312	6.8	1.91	685	1.42	0.681
80	21	14.1	6.3	151	11.0	25	159	13.7	6.14	490	1.32	0.631
90	5	19.3	7.5	171	11.0	25	185	15.5	6.95	450	1.35	0.612
90	21	na	na	110	7.3	11	54	15.1	10.14	295	1.30	0.593
100	5	na	na	98	6.4	8	5	15.3	11.81	265	1.20	0.594
100	12	na	na	nr	nr	nr	nr	. . .	. . .	245	0.92	0.495
110	5	na	na	nr	nr	nr	nr	. . .	. . .	230	1.06	0.345
110	12	na	na	nr	nr	nr	nr	. . .	. . .	220	0.78	0.416
120	5	na	na	nr	nr	nr	nr	. . .	. . .	220	0.92	0.325
120	12	na	na	nr	nr	nr	nr	. . .	. . .	220	0.76	0.359

[a] PROINTA Super wheat cultivar, protein content 13.4% dwb.
[b] Alveograph indexes: *W* = dough strength ($\times 10^{-4}$ J); *L* = extensibility (mm); *G* = inflation index; *P* = tenacity (mm); *P/L* = tenacity-to-extensibility ratio; *P/G* = tenacity to inflation index ratio.
[c] na = nonagglutining; nr = not recorded.

TABLE II
Tested Extractive Solutions and Corresponding Correlation Coefficient Values Determined Between Loaf Volume and Kjeldahl Determination of Extracted Proteins

	Extractive Solution	Correlation Coefficient
a	0.1*M* $Na_2B_4O_7 \bullet 10\ H_2O$ pH = 10	0.64
b	0.1*M* $Na_2B_4O_7 \bullet 10\ H_2O$ + 0.6 % v/v 2-mercaptoethanol pH = 10	0.58
c	0.1*M* $Na_2B_4O_7$ pH = 7	0.52
d	0.1*M* Na_2HPO_4 + 5% K_2SO_4 pH = 7	0.56
e	0.8*M* NaCl	0.63
f	0.1*M* Na_2HPO_4 + 5% K_2SO_4 + 0.8*M* NaCl pH = 7	0.59
g	85% H_3PO_4 + 96% ethanol (2+1 in volume) (PAE)	0.91

TABLE III
Influence of Drying Temperature and Drying Time on Quantity of Extracted Protein with Phosphoric Acid-Ethanol Extractive Solution (PAE) on Various Treated PROINTA Super Wheat Cultivars of Different Protein Content

Drying Temp. (°C)	Drying Time (min)	Quantity (mg) of Extracted Protein (KEP)[a]					
		Protein Content (% dwb)					
		10.7	11.6	13.4	14.7	15.5	17.0
Control	Control	1.12	1.26	1.54	1.64	1.75	1.96
50	55	1.10	1.24	1.52	1.63	1.74	1.94
60	55	1.12	1.24	1.53	1.63	1.76	1.95
70	12	1.10	1.23	1.52	1.62	1.74	1.93
70	21	1.07	1.20	1.49	1.62	1.72	1.95
80	5	1.04	1.16	1.42	1.50	1.60	1.95
80	21	0.88	1.18	1.32	1.40	1.46	1.80
90	5	0.88	1.20	1.35	1.40	1.48	1.83
90	21	0.76	1.02	1.20	1.24	1.32	1.68
100	5	0.84	1.14	1.26	1.30	1.44	1.75
110	5	0.62	0.94	1.06	1.16	1.30	1.68
120	5	0.54	0.72	0.92	1.04	1.18	1.54

[a] Standard deviation range: 0.05–0.07.

RESULTS AND DISCUSSION

As shown in Table I, a negligible modification of breadmaking quality was produced at low drying temperature and time. Quality deterioration was noted with increased drying temperature and time. From all the parameters selected for estimating breadmaking quality, only loaf volume gave measurable values for the entire range of tested conditions. Values of remaining parameters were considered as indicative. At 80°C and 21 min of treatment, loaf volume diminished >35.1% compared with the control. This was the lowest limit of the deteriorating treatments, the increase corresponds with loaf volume diminution.

Phosphoric acid-ethanol (PAE) solution was selected as the optimum extractive solution because it had the highest correlation coefficient (Table II) of KEP values with loaf volume. Detectable modifications in breadmaking quality were associated with a 14% reduction on the amount of PAE — KEP. This correlated well with a decrease in the loaf volume (r = 0.91).

Correlation between absorbance (A) and KEP was linear (r = 0.99) when both are measured on proteins extracted with PAE solution; KEP/A was 2.15 mg with a standard deviation of 0.93. PAE extracted proteins on PROINTA Super wheat cultivars with different protein contents (determined by KEP) were a linear function of grain protein content (r = 0.98, standard deviation 0.02) (Table

III) and were also significantly correlated with drying temperature and time. The differences among the PAE extracted proteins determined by KEP or colorimetrically on cultivars with the same protein content were not statistically significant.

Fast Spectrophotometric Method

The wheat sample was divided into two portions. Moisture was determined on one portion and the other was milled in a cooled laboratory mill to pass through a ASTM 40 mesh. An aliquot (0.100 g, dwb) of milled grain was placed in a 20-mL volumetric centrifugation tube. PAE solution (3 mL) was added and diluted to 20 mL with distilled water. Sample was mixed for 5 min in an orbital shaker and then filtered through paper (589[3] S&S or equivalent) or centrifuged at $2,000 \times g$ for 10 min. Dye solution (10 mL) was added to 1 mL of protein extract and mixed in an orbital shaker for 5 min. The reaction was allowed to rest for 5 min. Absorbance was measured at 595 nm against a reagent blank. The values remained constant for 30 min. The modification of the breadmaking quality is determined by comparing the quantity of the extracted protein in thermally treated samples with the extracted protein values of air-dried wheat grains with the same protein content. Lower values indicate a possible decrease of the loaf volume as consequence of the thermal effect.

CONCLUSIONS

Absorbance values of PAE extracted proteins were well correlated with extracted proteins obtained by the AACC Method 46-11 and with loaf volume. A decrease in absorbance values indicates a decrease in breadmaking quality. For the proposed method application, it is convenient to determine sample moisture and protein contents by reliable fast methods. In such cases, the full determination requires ≈30 min and needs to be standardized for the user's conditions. The proposed method aims only to provide a fast estimation of the possible breadmaking quality lost so as to choose a proper grain storage bin and not to estimate the breadmaking quality for commercial purposes.

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