

# A Fast Colorimetric Method to Estimate Corn Quality Loss

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## ABSTRACT

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A fast method to estimate the loss of corn quality for wet milling as a result of excessive thermal treatment during drying is proposed. Proteins that remain soluble after drying were quantitatively extracted with a solution of phosphoric acid and ethanol. The soluble proteins were colorime-

trically quantified using Coomassie Brilliant Blue G dye and measuring at 595 nm. A decrease in absorbance values indicates a reduction in commercial quality of the corn. The application time of the proposed technique is  $\approx$ 30 min.

Artificial drying is currently used on crops to adjust moisture content to maintain the quality of the stored grain. Corn drying can cause problems of commercial quality loss in both dry and wet milling (Thompson and Foster 1963). At higher drying rates, the increase in grain breakage susceptibility and stress cracks is more feasible, although reduced final moisture contents produce the same effects. Both viability and test weight are also changed (Freeman 1973; Westerman et al 1973; Hall 1974; Brown et al 1979; Paulsen and Hill 1985). Corn chemical composition is slightly affected by drying temperatures up to 149°C (300°F), (Peplinski et al 1975). Air temperatures at  $\approx$ 116 or 120°C in corn drying have negative effects on quality (Gustafson and Morey 1981; De Dios and Puig 1984). Thermal treatments such as drying negatively influence the nutritious grain value (Hathaway et al 1952). Other changes subsequent to drying and storage include a decreased solubility of proteins, changes in nutritive value, changes in sensory properties (Abramson et al 1980), and decreased *in vitro* digestibility (Onigbinde and Akinyele 1989). High drying temperatures may also destroy certain amino acids, notably lysine (Stefanov 1971), which is already deficient in corn proteins. Several corn wet-milling processes can be adversely affected by artificial drying, including starch and protein separation, starch quality, and recovery percentage of starch (MacMasters et al 1954, 1959; Watson and Hirata 1962; Lambert et al 1967; Vojnovich et al 1975). Solubility of corn proteins in the steep solution is reduced in grain that has been overheated (McGuire and Earle 1958). Many tests have been proposed to determine heat damage in corn. The difficulty in separating starch from proteins caused by drying has the greatest effect on the commercial quality of corn. Watson et al (1951), Watson and Sanders (1961), Neyring and Reilly (1984), and Mazzoni and Robutti (1990), among others, have proposed laboratory methods to accurately reproduce the wet-milling industrial process stages to measure commercial losses. These methods are cumbersome and time-consuming. Other methods have been proposed and are used to assess whether corn was submitted to adverse thermal treatments. These methods include flotation test (Wichser 1961), stress cracks and breakage susceptibility (Thompson and Foster 1963; Szaniel et al 1984; Eckhoff et al 1985; Paulsen and Hill 1985), the tetrazolium method to determine germinative ability (Lakon 1949), test weight (CGC 1975), available lysine (Stefanov 1971), glutamic acid decarboxylase (Batista and Linko 1962), millability test (Freeman and Watson 1969), spectrophotometric system (Christenbury and Buchele 1977), changes in protein solubility (McGuire and Earle 1958), and the inflation coefficient, water reabsorption test, and turbidity test (Le Bras 1982).

Most of the damage produced by heating is due to the modification of the protein structure. Changes in protein solubility and effects on corn wet milling have been established by McGuire and Earle (1958). Proteins that remain soluble after a thermal treatment may be quantitatively extracted using a suitable solution and can be spectrophotometrically quantified using Coomassie Brilliant Blue G (CBBG) dye (Bradford 1976; Hook 1979, 1980). The maximum absorption of the dye shifts from 465 to 595 nm when the dye binds to the protein. Absorbance values of the dyed solution of extracted soluble proteins at 595 nm may be related to heating effects. A fast method that quantifies the thermally modified protein by a spectrophotometric determination of the remaining soluble protein, previously extracted and dyed with CBBG, has been proposed by Tosi et al (2000). The method is used to estimate wheat breadmaking quality modifications as a consequence of thermal drying.

The aim of this work is to determine whether the fast method to estimate breadmaking quality proposed by Tosi et al (2000) can estimate heat damage of corn for wet-milling industries.

## MATERIALS AND METHODS

Samples of various cultivars of four corn hybrids with different protein contents (% protein content, N  $\times$  5.7, dry weight basis), Trihibrido Cargill (6.4, 7.6, 8.2%), Record 160 Cargill (6.5, 7.5, 8.1%), Funk Ambato (11.7%), and Ensenada Semiprecoz (12.2%) were used. Samples were supplied by the Estación Experimental Agropecuaria Pergamino of the Instituto Nacional de Tecnología Alimentaria (INTA), (Pergamino, Argentina). The picker-sheller machine was set for minimal mechanical damage to the kernel. A low drying temperature of the kernels was achieved using a cross-flow of slow current room temperature (25–28°C) air until the moisture content was 12% (wb). Kernels were stored at 25°C until used. CBBG, C.I. 42655 was from Sigma Chemical (St. Louis, MO). Other ACS reagent-grade chemicals used were from Merck (Darmstadt, Germany).

Thermal treatment tests were conducted on kernels previously rewetted at a suitable initial moisture content (IMC) so that the final moisture content was 13.0–14.0% wb. Kernels were brought to the required IMC by spraying them with a specific amount of water and periodically mixing over 48 hr at 25°C. The amount of water added was calculated by the corn drying empirical kinetic equation proposed by Tosi et al (1987). No differences attributable to rewetting were found in the drying curves between kernels with natural moisture and rewetted ones (results not published). Grain moisture content was determined according to ISO method 711 (ISO 1995).

Kernel thermal treatments were performed at different drying times and temperatures using the fluid bed technique so that a nearly homogeneous treatment of the sample was attained. Samples (2 kg) were treated in a pilot-scale fluidized bed dryer built at the Centro de Investigación y Desarrollo en Tecnología de Alimentos (CIDTA), Argentina (Tosi et al 1982). After the thermal treatment, the dried kernels were cooled immediately by changing the

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fluidized medium to fresh air at room temperature. Control kernels were rewetted at the required IMC and dried by room temperature with slow velocity air current in cross-flow arrangement. Three runs for each thermal treatment were performed. The grains of these three runs were mixed to make up a unique sample for both the industrial quality tests and the extraction tests of the remaining soluble protein. The parameters selected for the industrial quality tests were the stress cracks and breakage susceptibility methods of Thompson and Foster (1963), the flotation method of Wichser (1961), and the starch recovery method of Mazzoni and Robutti (1990). The results obtained by these methods were compared with results obtained by extracting the soluble protein quantified by Approved Method 46-11 (AACC 2000) and the proposed colorimetric method. In preliminary tests, samples were extracted with seven extractive solutions described in Tosi et al (2000). The most suitable extractant was selected by comparing the values of the extracted soluble protein determined by Approved Method 46-11 to the starch recovery method of Mazzoni and Robutti (1990).

Soluble protein quantification by the proposed method has four steps. First, the treated corn sample is milled in a cooled IKA A10 laboratory mill (Janke & Kunkel GmbH & Co., Staufen, Germany) to pass through a ASTM 40-mesh screen. Second, the soluble protein or the extractable protein (EP) is extracted. Extractive solution (30 mL) (85% H<sub>3</sub>PO<sub>4</sub> + 96% ethanol, 2:1 v/v) and 150 mL of distilled water was added to treated corn (1.000 g dwb) and shaken in an orbital shaker for 5 min, diluted to 200 mL, and filtered through 589<sup>3</sup> S&S paper or equivalent; the filtrate was

reserved. Third, the soluble EP protein was dyed with CBBG by adding 10 mL of working dye solution to 1 mL of filtered extract, shaking it in an orbital shaker for 5 min, and allowing the solution to rest for 5 min. Fourth, the absorbance of the colored solution was measured at 595 nm against reagent blank using a UV-vis Jasco 7800 spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). As EP control, Kjeldahl extracted proteins (KEP) were determined on 50-mL aliquots of the same filtrate containing EP determined by Approved Method 46-11 (AACC 2000) and expressed as mg of EP per gram of sample. The most suitable extractant and the relationship between absorbance and KEP were established.

The working dye solution was prepared by dissolving 0.01 g of CBBG in a mixture of 5 mL of ethanol and 10 mL of H<sub>3</sub>PO<sub>4</sub>, diluting to 100 mL with water, and filtering through 589<sup>3</sup> S&S paper or equivalent. The dye solution remains stable for three weeks.

The influence of treatment time and temperature on stress cracks, breakage susceptibility, floating test, starch recovery, and EP determined by both KEP and absorbance was evaluated on Trihibrido Cargill (6.4%) cultivars (Table I). Comparing KEP values to drying tests at selected temperatures and times (Table II) shows influences of corn cultivar and protein content on the extractive method. This comparison was performed between pairs of corn samples with similar protein contents, such as Trihibrido Cargill (6.4, 7.6, 8.2%) and Record 160 Cargill (6.5, 7.5, 8.1%) for low protein content, and Funk Ambato (11.7%) and Ensenada

**TABLE I**  
Drying Time and Temperature Effects on Stress Cracks, Breakage Susceptibility, Flotability, Starch Recovery, and Protein Extracted Using Phosphoric Acid and Ethanol Solution (PAE) Determined by Kjeldahl Extracted Proteins (KEP) and Absorbance (A) on Trihibrido Cargill Corn Cultivar<sup>a,b</sup>

Drying Conditions		Quality Test (%)					PAE Extracted Protein	
Temp. (°C)	Time (min)	Stress Crack Single	Stress Crack Multiple	Breakage	Starch Recovery	Flotability	KEP <sup>c</sup> (mg/g)	A <sup>d</sup>
Control	...	3	1	4.0	83	5	5.64	0.726
50	5	4	7	12.3	83	5	5.60	0.725
50	10	3	11	16.5	84	4	5.72	0.731
50	20	6	18	22.3	82	5	5.56	0.722
50	30	12	41	36.0	83	5	5.60	0.723
50	40	18	38	47.5	84	5	5.68	0.732
50	60	15	43	51.2	83	6	5.60	0.722
60	5	5	16	28.9	84	5	5.36	0.703
60	10	4	23	33.7	83	6	5.20	0.684
60	20	8	26	40.1	84	8	5.28	0.688
60	30	29	29	50.1	82	8	5.32	0.690
60	40	20	33	53.4	81	8	5.40	0.697
60	60	17	38	58.8	82	10	5.16	0.666
70	5	4	19	22.2	82	11	5.12	0.688
70	10	8	24	25.6	80	15	5.20	0.671
70	20	11	30	30.3	81	19	4.60	0.589
70	30	19	38	59.7	80	21	4.08	0.523
80	5	4	28	28.8	81	10	3.92	0.504
80	10	5	34	41.5	80	16	3.52	0.447
80	20	12	53	71.6	78	29	2.68	0.339
90	5	5	48	65.8	80	30	2.44	0.312
90	10	7	53	73.8	77	36	1.88	0.253
90	20	7	62	81.2	78	52	1.76	0.233
100	5	4	62	70.7	77	35	1.52	0.194
100	10	5	72	90.7	74	76	1.36	0.177
110	5	4	64	78.4	77	71	1.28	0.162
110	10	5	85	96.7	74	94	0.60	0.070
120	5	5	77	80.5	75	82	0.40	0.045
120	10	5	86	93.0	75	100	0.17	0.018
Correlation coefficient <sup>e</sup>		0.79 <sup>f</sup>		0.86	0.94	0.93	0.99	

<sup>a</sup> Protein Content 6.4% (w/w, db).

<sup>b</sup> Standard error (minimum, maximum, mean): stress crack single (0.5, 2.6, 0.8); stress crack multiple (1.4, 4.5, 3.5); breakage (1.5; 5.8; 3.9); starch recovery (2.28, 3.54, 3.1); flotability (2.1, 4.9, 3.4); KEP (0.06, 0.1, 0.8); A (0.0038, 0.0051, 0.0046).

<sup>c</sup> Protein (N × 5.7) AACC Approved Method 46-11.

<sup>d</sup> Absorbance units; 1.96 ± 10 mg of protein/AU.

<sup>e</sup> Correlation coefficient between each of the quality tests or KEP and absorbance.

<sup>f</sup> Sum of single and multiple cracks.

Semiprecoz (12.2%) for high protein content. Determinations of stress cracks, breakage susceptibility, floating test, starch recovery, extraction with PAE, KEP, and absorbances were made in triplicate.

## RESULTS AND DISCUSSION

Of the seven extractants tested, the mixture containing two volumes of PO<sub>4</sub>H<sub>3</sub> (85%) and one volume of ethanol (96%) (PAE) was the one that gave the highest correlation coefficient (0.94) when KEP quantity was correlated with starch recovery. The same was found when the proposed method was developed on wheat. Because of this high correlation, the PAE mixture was used in all subsequent tests. Table I shows the effect of drying time and temperature on the corn quality of Trihibrido Cargill (6.4% protein content). Industrial quality decreases as temperature and drying time increase.

Correlation between absorbance and KEP was linear ( $r = 0.99$ ) and mean KEP/absorbance was  $1.96 \pm 0.10$  mg/absorbance units. Correlation coefficients of stress cracks, breakage susceptibility, floating test, and starch recovery values, and the absorbance values obtained by the proposed method are included in Table I and show significant agreement.

The influence of corn cultivar and protein content is shown in Table II. Proteins extracted with PAE and determined as KEP on Trihibrido Cargill (6.4, 7.6, 8.2%) and Record 160 Cargill (6.5, 7.5, 8.1%) or Funk Ambato (11.7%) and Ensenada Semiprecoz (12.2%) cultivars show significant agreement between pairs of same protein content. Correlation coefficients are included in Table II. The linear correlation coefficients between KEP values for samples submitted to the same heating conditions (rows in Table II) and corresponding cultivar protein content have maximum values at mild heating conditions (0.99) and decreases at the strongest heating conditions (0.70), with a mean value of 0.96.

### Proposed Fast Spectrophotometric Method

The corn sample is split into two portions and moisture is determined on one portion while the other is milled in a cooled laboratory mill to pass through an ASTM 40-mesh screen. An aliquot (0.100 g, dwb) of the milled grain is placed in a 20-mL

volumetric centrifugation tube, PAE solution (3 mL) is added and diluted to 20 mL with distilled water. The sample plus the extractant mixture is mixed in an orbital shaker for 5 min. It is filtered through paper (589<sup>3</sup> S&S or equivalent) or centrifuged at  $850 \times g$  for 10 min. Dyeing is performed by adding 10 mL of working dye solution to 1 mL of filtered extract (or centrifugate supernatant), shaking it in an orbital shaker for 5 min, and allowing the mixture to rest for 5 min. Absorbance is measured on colored mixture at 595 nm against a reagent blank. The values remain constant for 30 min. The industrial quality is determined by comparing the absorbance values of the tested sample to a calibration curve of absorbance values of kernels with the same protein content, dried at 25°C. Lower absorbance values indicate a possible decrease on the industrial wet-milling aptitude.

## CONCLUSIONS

Absorbance values of PAE-extracted proteins were well correlated with the values determined by the AACC Approved Method 46-11 (KEP) and with stress cracks, breakage susceptibility, flotation, and starch recovery tests. The remarkable high correlation coefficient between the starch recovery test and the proposed method is probably due to the influence of protein damage in both methods. No difference attributable to kernel protein content or cultivar was significant. For application purposes, it is convenient to determine both sample moisture and protein content by reliable fast methods such as NIR, where the full determination requires  $\approx 30$  min; standardization of user conditions is required. No heating, cooling, or concentration adjustment steps are needed, making application faster and more simplified. Breakage susceptibility and starch recovery methods are time-consuming and they both may require moisture adjustment or steeping steps that take from several hours to two days, respectively. On the other hand, the floating test and the stress cracks methods require 15–20 min and are slightly faster than the proposed method, but both may be considered as indicative methods because they are based on measuring the physical consequences of heating damage. The proposed method allows estimating the actual thermally damaged protein. The method is not as simple as the stress crack, breakage, or flotation methods, but it can be satisfactorily performed by an industrial

TABLE II  
Influence of Cultivar<sup>a</sup> and Protein Content on Quantity of Protein Extracted Using Phosphoric Acid and Ethanol Solution (PAE) on Pairs of Cultivars of Similar Protein Content of Four Corn Hybrids Dried at Different Temperatures and Times

CPC (% dwb)		TC (6.4)	RC (6.5)	RC (7.5)	TC (7.6)	RC (8.1)	TC (8.2)	FA (11.70)	E (12.20)	CPC-KEP
Temp. (°C)	Time (min)	Extracted Protein (KEP) <sup>b</sup>								
(r) <sup>c</sup>										
Control	Control	1.41	1.45	1.60	1.65	1.73	1.81	2.58	2.59	0.997
50	10	1.43	1.43	1.66	1.67	1.78	1.85	2.53	2.62	0.999
50	60	1.40	1.34	1.50	1.65	1.65	1.83	2.40	2.50	0.991
60	10	1.30	1.30	1.53	1.52	1.63	1.68	2.33	2.43	0.999
60	60	1.29	1.28	1.49	1.51	1.61	1.68	2.35	2.45	0.999
70	10	1.30	1.03	1.17	1.53	1.30	1.65	1.88	1.95	0.999
70	30	1.02	0.91	1.03	1.19	1.08	1.32	1.62	1.70	0.998
80	10	0.88	0.65	0.82	1.05	1.01	1.10	1.22	1.29	0.969
80	20	0.67	0.49	0.55	0.80	0.63	0.90	0.88	0.91	0.998
90	10	0.47	0.45	0.55	0.57	0.60	0.61	0.80	0.89	0.989
90	20	0.44	0.40	0.45	0.55	0.53	0.62	0.71	0.74	0.994
100	5	0.38	0.36	0.40	0.47	0.50	0.5	0.69	0.70	0.987
100	10	0.34	0.30	0.38	0.43	0.40	0.5	0.60	0.63	0.995
110	5	0.32	0.16	0.21	0.40	0.21	0.42	0.29	0.30	0.934
110	10	0.15	0.09	0.08	0.23	0.10	0.18	0.19	0.21	0.977
120	5	0.10	0.05	0.06	0.08	0.06	0.11	0.07	0.08	0.707
120	10	0.04	0.05	0.06	0.05	0.06	0.05	0.07	0.08	0.885
Correlation coefficient <sup>d</sup>		0.99		0.99		0.99		0.99		0.96 <sup>e</sup>

<sup>a</sup> Trihibrido Cargill (TC); Record 160 Cargill (RC); Funk Ambato (FA); Ensenada Semiprecoz (E).

<sup>b</sup> PAE-extracted proteins determined by AACC Approved Method 46-11 ( $N \times 5.7$ ).

<sup>c</sup> Correlation coefficient between original cultivar protein content (CPC) and KEP.

<sup>d</sup> Correlation coefficient of PAE-extracted proteins between pairs of cultivars of similar protein content submitted at equal thermal treatment.

<sup>e</sup> Mean value CPC-KEP correlation coefficient.

quality control laboratory operator acquainted with colorimetric and spectrophotometric determinations, and it also can be applied as a routine method. The proposed method aims only to provide a fast estimation of the possible wet-milling quality loss so as to choose a proper grain storage bin and processing conditions and not to estimate the industrial and commercial quality of corn just for commercial purposes.

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