Evaluation of Starch Damage Values Determined Enzymatically or Amperometrically

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ABSTRACT

Cereal Chem. 71(6):578–581

Damaged starch values were determined in six commercially produced flours (three each from hard winter and soft wheats) using two enzymatic methods and a modified rapid iodometric method. All flours were intentionally damaged with a ball mill for varying lengths of time, or with additional passes through reduction rolls. For the hard and soft wheat flours, respectively, the damaged starch values averaged 11.7 and 7.4% by the reducing sugar determination (AACC method 76-30A); 7.2 and 4.7% by the spectrophotometric method (AACC method 76-31); and 9.6 and 7.4% by the Chopin Rapid Flour Test method. High degrees of correlation were observed between the Chopin electric current (E_c) and the reducing sugars determination ($r = 0.94$) or the spectrophotometric method ($r = 0.95$). Those correlations improved slightly when hard and soft wheat flours were analyzed separately, or when the data were analyzed with quadratic equations. Eliminating the ball-milled samples from consideration also improved most of the correlations between E_c and the enzymatic methods. Therefore, the Chopin electric current value can be used as an appropriate indicator of flour starch damage.

Starch damage is an important, well-recognized criterion of flour quality (Farrand 1964, Tipples 1969, Evers et al 1984, Hoseney 1986, Salmon et al 1990). Historically, the preferred methods for measuring starch damage have been enzymatic methods (Farrand 1964, Audidier et al 1966). The standard AACC method 76-30A (AACC 1983) has generally been used in the United States. This method depends upon starch hydrolysis by fungal-amylase, with quantitation of reducing sugars. Such methods, however, are time-consuming and require a high degree of operator skill. An enzymatic assay kit has recently been developed to overcome some of those problems (Gibson et al 1992, 1993). This is used in standard method 76-31 (AACC 1983). Fungal α-amylase hydrolyzes the starch, which is then further hydrolyzed by amyloglucosidase, then treated with glucose oxidase-peroxidase and measured spectrophotometrically. However, variations in enzyme activity and stability may still cause difficulties in consistency over time.

Nonenzymatic methods are also available, and generally require less time and operator skill than do the enzymatic procedures (Medcalf and Gilles 1965, McDermott 1980, Douglas et al 1981, Finney et al 1988). One such method, the Chopin Rapid Flour Test (RFT) system (Seedburo Equipment Co., Chicago, IL), depends upon the fact that, under acidic conditions, the standard iodate solution reacts with potassium iodide to form free iodine, which is absorbed by the starch and, in particular, by the damaged starch granules. The unit automatically measures the current flowing between two electrodes attached to a platinum sensor. Internal equations within the system convert the electrical current value to more commonly recognized units of starch damage. Previous investigators have used this system to report damaged starch values expressed in units of Audidier (Berger 1987), Farrand (Salmon et al 1990), AACC 76-30A (Ranhotra et al 1993), or all of the above (Blyth et al 1992). Although such reports suggest acceptable correlations with the enzymatic methods, discrepancies have been noted. In addition to the internally calculated units (such as those in AACC method 76-30A), the new RFT system provides the raw data (electrical current, E_c), which is inversely proportional to the amount of starch damage. This study was undertaken to assess the relationships between E_c values and those obtained from two routinely used enzymatic methods.

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MATERIALS AND METHODS

Flours

Six commercial flours were used, three each from hard winter and soft wheats. All flours were intentionally damaged in a ball mill (U.S. Stoneware, Process Equipment Division, Akron, OH) for 0, 15, 30, or 45 min using a 250-g sample each time. Additionally, all six commercial flours were damaged in a Chopin CD1 lab mill (Seedburo Equipment Co., Chicago, IL) by passing samples through the reduction rolls 5 or 10 times, in series. A 2-kg weight was placed on the roll lever during the grinding process.

Analytical

All flours were analyzed for moisture and protein (N × 5.7) using the standard methods 44-15A and 46-10 (AACC 1983). Damaged starch was determined enzymatically by two different AACC methods. AACC method 76-30A results were quantitated by titrating to determine reducing sugars, while AACC method 76-31 results were quantitated by spectrophotometric determination of glucose. The method reported by Salmon et al (1990) was followed for Chopin RFT starch damage analysis, with the E_c values also being recorded. All samples were run at least in duplicate. The data were analyzed using PC-SAS (SAS 1988) general linear models procedure. No attempt was made to equalize the scale-units between the different methods.

RESULTS AND DISCUSSION

Hard wheat flours used in this study were 12.4–13.4% protein; soft wheat flours were 9.1–10.9% protein (Table I). The RFT-AACC values (those calculated by a regression equation programmed internally in the RFT system to predict AACC 76-30A values) were highly correlated with the actual reducing sugar values (AACC 76-30A values; $r = 0.940$). However, the calculated RFT-AACC values slightly underestimated the measured AACC 76-30A values for the soft wheat flours, and substantially underestimated the hard wheat flour values. This was in agreement with Ranhotra et al (1993). Previously, a number of researchers had demonstrated varying correlations between nonenzymatic and enzymatic methods of determining damaged starch (Medcalf and Gilles 1965, Williams and LeSeeleur 1970, Dodds 1971, Belderok 1973, McDermott 1980, and Finney et al 1988).

As shown in Figure 1A and Table I, enzymatic starch damage values determined by titration of reducing sugars (AACC 76-30A) varied between 4 and 16%. Because of the inverse relationship, E_c values varied between 5.4 and 2.0. Although some scatter was present, the correlation between E_c and the reducing sugar method was high ($r = 0.940$). Similar plots could be made between E_c and the spectrophotometric method (AACC 76-31). Because E_c values are the raw data from which the RFT-AACC values are calculated, the remainder of this research concentrated on the relationship between E_c and the enzymatic starch damage values.

Hardness is an important quality characteristic of wheat; it has a major influence on such parameters as milling characteristics, starch damage, and cookie diameter (Rogers et al 1993). Generally, hard wheat flours contain significantly more mechanically damaged starch granules than do soft wheat flours (Moss 1978, Evers and Stevens 1985). Analyzing the data in this study for E_c versus hard and soft wheats separately (Fig. 1B, Table II) improved the correlation coefficients from 0.94 to 0.96 (Table II, equations A and B). Analyzing by protein content, or by protein within hardness, did not significantly improve the analysis (data not shown). However, using quadratic equations instead of linear solutions did improve the fit (Fig. 1C; Table II, equation C). Similar trends were seen when the E_c values were compared to those of the spectrophotometric enzymatic method (AACC 76-31) (Table II, equations D–F).

Ball milling changes the short-range crystalline order (Morrison et al 1994) and the absorption characteristics of starch (Mok and Dick 1991a), with the degree of starch damage dependent upon the wheat class (Mok and Dick 1991b). In excess water, such as is used with the RFT procedure, ball-milled samples would

TABLE I
Protein and Damaged Starch Content of Test Flours

	All Flours		Hard Wheat		Soft Wheat	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Protein (%, dwb)	9.1–13.4	11.4	12.4–13.4	12.8	9.1–10.9	10.0
Damaged starch (%)						
RFT-AACC[a]	3.1–11.7	8.5	6.4–11.7	9.6	3.1–9.8	7.4
Reducing sugars (76-30A)[b]	4.1–15.9	9.4	7.8–15.9	11.7	4.1–11.4	7.4
Spectrophotometric (76-31)[b]	2.7–9.4	6.0	5.1–9.4	7.2	2.7–7.4	4.7
Electrical current (E_c)	5.37–2.00	3.28	4.07–2.00	2.82	5.37–2.76	3.67

[a]Chopin Rapid Flour Test (RFT). Values are calculated by a regression equation programmed internally in the RFT system to predict AACC method 76-30A values.
[b]AACC standard methods (AACC 1983).

TABLE II
Regression Equations and Correlation Coefficients for the Electrical Current Values Compared with Standard Method Values, Including All Data

Method		Equation[a]	*r* Value
Reducing sugars (76-30A)[b]	A	$DS = 21.16 - 3.64 \times E_c$	0.940
	B	Hard: $DS = 21.02 - 3.47 \times E_c$	0.959
		Soft: $DS = 16.74 - 2.57 \times E_c$	
	C	Hard: $DS = 36.16 - 14.01 \times E_c + 1.77 \times E_c^2$	0.980
		Soft: $DS = 30.97 - 10.06 \times E_c + 0.95 \times E_c^2$	
Spectrophotometric (76-31)[b]	D	$DS = 12.98 - 2.16 \times E_c$	0.948
	E	Hard: $DS = 12.85 - 2.01 \times E_c$	0.966
		Soft: $DS = 10.86 - 1.65 \times E_c$	
	F	Hard: $DS = 18.76 - 6.18 \times E_c + 0.71 \times E_c^2$	0.979
		Soft: $DS = 18.80 - 5.83 \times E_c + 0.53 \times E_c^2$	

[a]DS = damaged starch. E_c = electrical current.
[b](AACC 1983).

TABLE III
Regression Equations and Correlation Coefficients for the Electrical Current Values Compared with Standard Method Values, Using Only Ball-Milled Samples

Method		Equation[a]	*r* Value
Reducing sugars (76-30A)[b]	A	$DS = 24.43 - 4.76 \times E_c$	0.971
Spectrophotometric (76-31)[b]	B	$DS = 14.13 - 2.53 \times E_c$	0.964

[a]DS = damaged starch. E_c = electrical current.
[b](AACC 1983).

TABLE IV
Regression Equations and Correlation Coefficients for the Electrical Current Values Compared with Standard Method Values, Using Only Roller-Milled Samples

Method		Equation[a]	*r* Value
Reducing sugars (76-30A)[b]	A	$DS = 18.29 - 2.84 \times E_c$	0.929
	B	Hard: $DS = 16.87 - 2.22 \times E_c$	0.980
		Soft: $DS = 14.52 - 2.04 \times E_c$	
	C	Hard: $DS = 22.22 - 5.70 \times E_c + 0.55 \times E_c^2$	0.985
		Soft: $DS = 23.62 - 6.58 \times E_c + 0.55 \times E_c^2$	
Spectrophotometric (76-31)[b]	D	$DS = 11.82 - 1.84 \times E_c$	0.924
	E	Hard: $DS = 11.13 - 1.50 \times E_c$	0.982
		Soft: $DS = 9.34 - 1.31 \times E_c$	
	F	Hard: $DS = 15.00 - 4.01 \times E_c + 0.40 \times E_c^2$	0.988
		Soft: $DS = 15.93 - 4.60 \times E_c + 0.40 \times E_c^2$	

[a]DS = damaged starch. E_c = electrical current.
[b](AACC 1983).

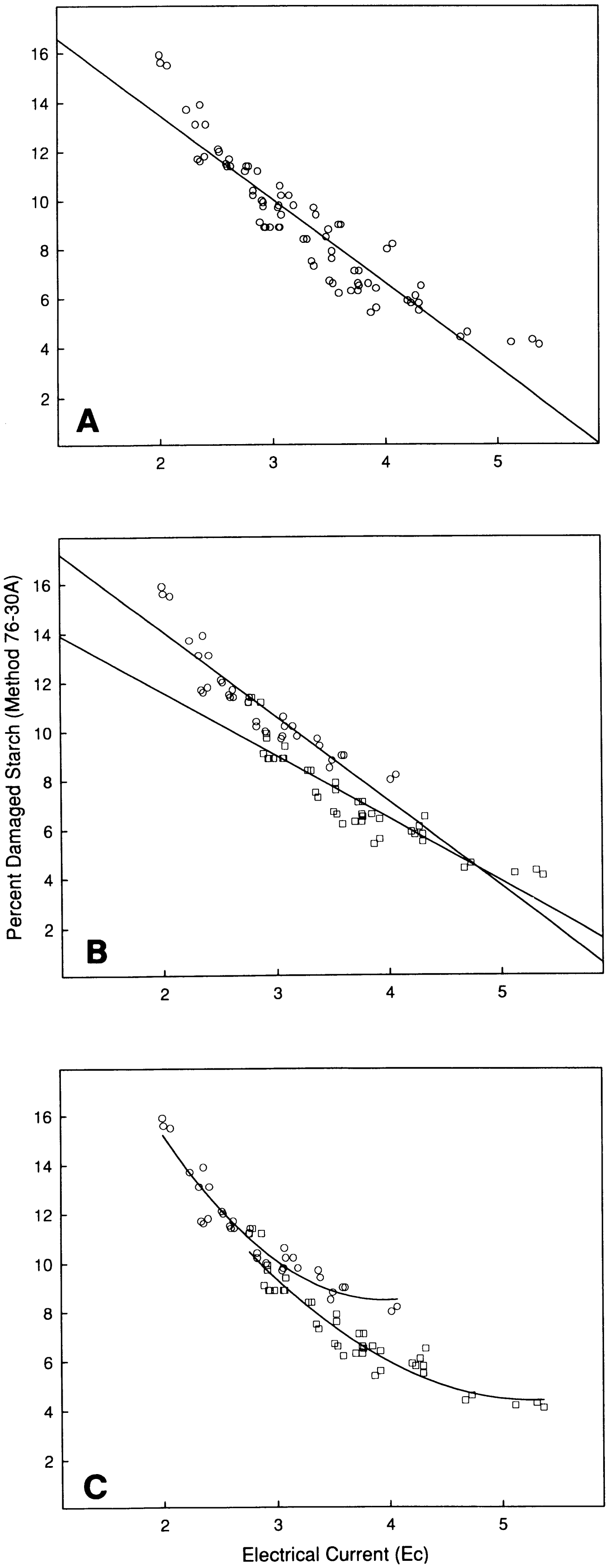

Fig. 1. Relationship between electrical current (E_c) and percent damaged starch, as determined by the reducing sugar method (AACC method 76-30A). **A,** Linear relationship for all flours (r = 0.940). **B,** Linear relationship for hard wheat flours (○) and soft wheat flours (□) (r = 0.959). **C,** Quadratic relationship for hard wheat flour (○) and soft wheat flours (□) (r = 0.980).

be expected to absorb more water than less severely damaged starch granules. Therefore, the iodine absorption characteristics might be different than in the non-ball-milled samples. Table III presents the linear equations for the ball-milled flour samples, which are more highly correlated with either enzymatic method than were the ball-milled samples plus the roller milled samples (Table II, equations A and D; Table III, equations A and B). Based on such information, interpretation of any starch data from ball-milled samples needs to be approached with caution. Analyzing the ball-milled samples by wheat class or with quadric equations resulted in only marginal improvements of the correlation coefficients (data not shown).

Samples with additional damage produced on the roller mill are more representative of commercially available flour and starch than are ball-milled samples. Upon removing the ball-milled data, new regressions were calculated for the roller-milled samples (Table IV, equations A and D). The correlations were greatly improved when the data were grouped by wheat class (Table IV, equations B and E). Further improvement was seen when curvilinear equations were used (Table IV, equations C and F). The effect of blended wheat flours was not investigated in this study. However, the data just presented demonstrate that E_c values can be used to monitor starch damage in both hard and soft wheat flours.

The RFT system generally requires less time and operator skill than enzymatic methods, and it eliminates the concern of enzyme shelf life. The four most commonly used methods for starch determination each present results in different scale-units. RFT values (E_c) correlate well with two of those common methods, although in yet another scale-unit. Therefore, the E_c value is a valid tool for monitoring starch damage in wheat flours.

CONCLUSION

Monitoring the electrical current (E_c) of the Chopin RFT system is a useful means of assessing starch damage in flours. Grouping flours according to wheat class (hard or soft) improved the predicted enzymatic value developed from the E_c (r = 0.94 or 0.95 to r = 0.96 or 0.97, depending upon the enzymatic method used). Applying quadratic equations further improved the correlations (r = 0.98) for either enzymatic method.

Flours were intentionally damaged for this study by two very different milling methods. Regression lines varied between the milling methods. When only the roller-milled samples, which are more representative of commercial flours, were examined, separating the hard and soft wheat flours greatly improved the correlations (r = 0.93 or 0.92 to r = 0.98 or r=0.98). Quadratic equations further increased the correlations slightly (r = 0.99) for either enzymatic method.

Due to the limited scope of this study, optimum regression equations were not developed. Rather, the probability of doing so was evaluated. Although predetermined linear equations are internally available in the instrument to convert the E_c values to enzymatic values, improved regressions could be developed. Mathematical conversion of the E_c data to the units of one of the standard enzymatic methods is optional.

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[Received January 12, 1994. Accepted August 25, 1994.]