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Flour Sodium Dodecyl Sulfate (SDS)-Extractable Protein Level as a Cookie Flour Quality Indicator

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Flour characteristics of laboratory-milled flour fractions of two wheat cultivars were related to their cookie-baking performance. Cultivar (cv.) Albatros wheat milling yielded fractions with lower damaged starch (DS) and arabinoxylan levels and higher sodium dodecyl sulfate-extractable protein (SDSEP) levels than did cv. Meunier wheat milling. During baking, cv. Albatros flour doughs spread faster and set later than their cv. Meunier counterparts and, hence, resulted in larger cookie diameters. DS levels negatively affected spread rate during both cv. Albatros (R^2 = 0.68) and cv. Meunier (R^2 = 0.51) cookie baking. SDSEP levels also influenced cookie quality. The use of flour heat-treated to reduce its SDSEP levels to different degrees led to reduction of the set time (R^2 = 0.90). It was deduced that larger gluten polymer sizes limit dough spread time during baking and that, apart from DS level, the SDSEP level is an indicator for cookie flour quality.

KEYWORDS: Sugar-snap cookie; SDS-extractable protein level; set time; gluten polymer size

INTRODUCTION

During baking, sugar-snap cookie dough spreads on the baking surface (*1*). Miller and Hoseney (*2*) noticed that, during baking, the dough piece diameter of such cookie dough linearly increases and then suddenly sets. The final spread depends not only on the spread rate but also on the time at which the cookie dough stops expanding or spreading, that is, the set time (*2*,*3*). Published work relates higher spread rate to lower levels of dough water binding components, that is, damaged starch (DS) (*1*,*3*−*5*), proteins (*2*,*6*), and nonstarch polysaccharides (*7*, *8*). However, in the literature, there is no consensus on the phenomena that determine the time and temperature at which the cookie dough sets (*9*).

According to Doescher et al. (*10*) and Miller et al. (*11*), proteins have an important role in the cookie dough setting mechanism. In their view, during baking, proteins undergo an "apparent" glass transition, which gives them the mobility that, in turn, allows them to swell and entangle. The resulting viscosity increase would then be sufficient to stop dough spreading (*11*). However, according to Slade et al. (*12*), cookie dough setting is not determined by a glass transition. In their view, cookies made from variable quality flours show different spread behavior, but they do not set. Slade et al. (*13*) and Chevallier et al. (*14*) even speculated that a continuous protein network would be absent in sugar-snap cookies. Ram and Singh (*15*) related cookie dough set time to the level of free dough water and to dough strength, which, in their opinion, is largely influenced by the quality and quantity of gluten protein. Recently, Pareyt et al. (*16*), using a model approach based on gluten−starch blends, observed that proteins aggregate during cookie baking and that the aggregation goes hand in hand with reduced spread. This made us wonder about the importance of the molecular size of the gluten in flour and, hence, presumably also at the onset of the baking phase. Indeed, as proteins aggregate during cookie baking, it seems logical that their size before baking codetermines their aggregation in the oven and, hence, that, apart from DS, the protein characteristics may codetermine final cookie diameter.

The present study was performed to understand the role of flour protein in determining cookie dough set time during baking. In a first set of experiments the differences in cookie making behavior between (milling fractions of) two European wheat cultivars (cv.), with similar protein content but different kernel hardness, were studied, focusing on the flour constituents, such as DS level, arabinoxylan (AX) level. and sodium dodecyl sulfate (SDS)-extractable protein (EP) level. In a second experimental setup, the importance of the SDSEP level was further substantiated. As heat treatment of dry flour changes the SDS extractability of its proteins (*17*, *18*) while leaving its other constituents largely unaffected, this provided a way to selectively manipulate cookie flour protein characteristics and to subsequently investigate the impact of gluten molecular size on cookie dough setting, which was monitored with time-lapse photography.

MATERIALS AND METHODS

Materials. All chemicals and reagents were of the highest purity available and from Sigma-Aldrich (Steinheim, Germany), unless specified otherwise. Commercial cookie flour [moisture content, 13.7%; protein content (N × 5.7), 10.7% (dry base)] was obtained from Meneba (Hasselt,

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Belgium). Wheat cv. Albatros and Meunier (protein contents of 11.6 and 11.7%, respectively) were kindly provided by Clovis Matton (Avelgem-Kerkhove, Belgium). Margarine [moisture content, 18.9%; solid fat contents at 20 and 25 °C, 31 and 20%, respectively] was donated by Vandemoortele (Izegem, Belgium). Commercial sugar (average crystal size, 470 μm) was from Iscal Sugar (Moerbeke-Waas, Belgium) and sodium bicarbonate (BICAR) from Solvay Chemicals International (Brussels, Belgium).

Determination of Wheat Kernel Hardness. Wheat kernels were compressed (50%) with a Texture Analyzer (TAXT2i, Stable Microsystems Ltd., Surrey, U.K.) equipped with a metal blade (70 mm wide and 3 mm thick) until they were crushed. Pretest, test, and posttest speeds were 1.0 mm/s. At least 15 kernels were crushed, and the maximum force (in N) was taken as a measure for kernel hardness.

Milling of Wheat Cultivars. Cv. Albatros (16.0% moisture level) and cv. Meunier (16.5% moisture level) grains were milled with a Bühler MLU-202 laboratory roller mill (Bühler AG, Uzwil, Switzerland) and yielded six milling fractions each. For the milling flow sheet, the interested reader is referred to Delcour et al. (*19*). The yields of straight grade (SG) flour (i.e., the sum of break and reduction milling streams) were 74.0 and 72.7% for cv. Albatros and Meunier, respectively.

Analytical Procedures. Wheat and flour moisture contents were determined with AACC Approved Method 44-19 (*20*) and protein contents with the Dumas method, an adaptation of the AOAC Official Method (*21*) to an automated Dumas protein analysis system (EAS vario Max N/CN, Elt, Gouda, The Netherlands), using 5.7 as the conversion factor. DS and total AX levels of different flour fractions were determined according to the Megazyme (Bray, Ireland) procedure (AACC Method 76-31) and by gas−liquid chromatography with corrections for water-extractable arabinogalactan levels as outlined by Courtin et al. (*22*) respectively.

Heat Treatment of Commercial Cookie Flour. Separate batches of commercial cookie flour (250 g) were weighed in bottles (1000 mL). The bottles were closed to avoid moisture loss and then stored at 80 or 100 °C for 2, 5, and 8 h. To avoid lumping, the bottles were shaken every hour and at the end of the heat treatment.

Differential Scanning Calorimetry (DSC). DSC of heat-treated flours was conducted as described by Pareyt et al. (*16*) with a DSC Q1000 (TA Instruments, New Castle, DE). Onset (T_o, °C), peak (T_p, °C), and conclusion (T_c, °C) temperatures and gelatinization enthalpies [ΔH, J/g dry matter (dm) starch] were determined with TA Q Series Advantage Universal Analysis software. Results are averages of at least triplicate measurements and the coefficients of variation were < 3.0%.

Rapid Visco Analysis (RVA). RVA measurements were performed with flour suspensions (12.0%, w/v, 25.0 g) in deionized water or in deionized water containing dithiothreitol (DTT; 0.15%; Acros Organics, Geel, Belgium) (*23*). The temperature profile included a holding step (1 min at 50 °C), a linear temperature increase to 95 °C at 7.5 °C/min, a holding step (6 min at 95 °C), a linear temperature decrease to 50 °C at 7.5 °C/min, and a final isothermal step at 50 °C (10 min). Each analysis lasted 29 min and was performed at least in duplicate.

Cookie Making and Time-Lapse Photography. Cookie making with constant dough moisture level (15.0%) and time-lapse photography were carried out as described by Pareyt et al. (*16*). Margarine (90 g) and sucrose (144 g) were mixed in a KitchenAid Professional KPM5 mixer (Kitchen Aid, St. Joseph, MI) for 3 min with intermediate scraping every minute. Then, deionized water was added and mixing continued for 2 min with intermediate scraping every minute. Finally, flour (200 g, 14.0% moisture) and sodium bicarbonate (4.0 g) were added before mixing for another 2 min with scraping every 30 s. Cookie doughs were sheeted to 6.35 mm thickness with an electrical sheeter (National Manufacturing, Lincoln, NE) and cut with a circular cookie dough cutter (inner diameter, 63.5 mm). At least three pans of five cookies of each individual recipe were baked in an electrically heated rotary oven (National Manufacturing) at 185 °C for 14 min. Coefficients of variation on cookie diameter were < 3%. Time-lapse photography was conducted on at least two dough pieces of separate dough batches.

Defatting of Dough and Cookie Samples. Prior to defatting, dough was divided into small pieces while cookies were ground. Samples (1.0 g) were defatted for 60 min with 10.0 mL of hexane in a 30 mL test tube. Next, the hexane was removed, and the procedure was repeated. The samples were finally dried under a stream of nitrogen.

Determination of SDS-Extractable Protein. Size exclusion (SE) high-performance liquid chromatography (HPLC) was conducted as described by Lagrain et al. (*24*) with a Shimadzu LC-2010 system (Shimadzu, Kyoto, Japan) with automatic injection. Samples of flour, defatted dough, or cookies [1.0 mg dm protein] were extracted with 1.0 mL of 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v) SDS (Acros Organics) (here often referred to as SDS buffer). Extracts were filtered through a 0.45 μm Millex HP filter (Millipore Corp., Billerica, MA) and loaded (60 μL) on a Biosep-SEC-S4000 column (Phenomenex, Torrance, CA). The elution solvent was acetonitrile/water [1:1 (v/v), containing 0.05% trifluoroacetic acid]. The flow rate was 1.0 mL/min, and the column temperature was 30 °C. Eluted protein was detected at 214 nm. SDSEP levels were calculated from the peak areas and expressed as percentage of the peak area of an extract obtained from unheated flour with the SDS buffer in the presence of 1.0% (w/v) DTT and 2.0 M urea (coefficients of variation < 2%) and analyzed under the same conditions. The profiles were divided into two fractions using the lowest absorbance reading between the two peaks as the cutoff point. The first fraction then corresponded to the SDS-extractable glutenin, whereas the second was assigned to the SDS-extractable gliadin (*24*).

Determination of Flour Protein Fractions. Flour albumin plus globulin, gliadin, and glutenin fractions were quantified with reversed phase (RP)-HPLC. Samples (100.0 mg dm protein) were extracted (10 min, 150 rpm, 20 °C) twice with 3.0 mL of 0.05 M sodium phosphate buffer (pH 7.6) containing 0.4 M sodium chloride and once with deionized water (albumin plus globulin extract), three times with 3.0 mL of 60% (v/v) ethanol (gliadin extract), and three times (20 min, 150 rpm, 60 °C) with 3.0 mL of 0.05 M Tris-HCl buffer (pH 7.5) containing 50% propan-1-ol, 2.0 M urea, and 1.0% (w/v) DTT and kept under nitrogen (reduced glutenin extract). The extracts were diluted to 10.0 mL and loaded (80 μL) on a Nucleosil 300-5 C8 column (Machery-Nagel, Düren, Germany). The elution solvents were deionized water (A) and ACN (B), each containing 0.1% (v/v) TFA. Proteins were eluted with a linear gradient from 24.0 to 56.0% B in 55 min and detected at 214 nm. The percentage of each protein fraction was calculated from the total area under the curve and expressed as the percentage of the sum of the areas of the different fractions. Experimental errors, expressed as the ratio of the difference between duplicate values and their average, did not exceed 5.0%

Free Sulfhydryl (SH) Determination. Free SH-groups were determined colorimetrically after reaction with 5,5-dithiobis(2-nitrobenzoic acid) (DTNB). Samples (1.0 mg dm protein/mL) were shaken for 60 min in 0.05 M sodium phosphate buffer (pH 6.5) containing 2.0% (v/v) SDS, 3.0 M urea, and 1.0 mM tetrasodium ethylenediaminetetraacetate (EDTA). DTNB reagent [0.1% w/v in the 2.0% SDS, 3.0 M urea, and 1.0 mM EDTA containing 0.05 M sodium phosphate buffer (pH 6.5), 100 μL] was mixed with the sample, and the absorbance at 412 nm was determined after centrifugation (3 min, 11000*g*) after 45 min. Absorbance values were converted to levels of free SH (μmol/g dm protein) using a calibration curve with reduced glutathione (*24*).

Statistical Analysis. Significant differences ($\alpha < 0.05$), determined by the ANOVA procedure, and Pearson's correlation coefficients ($P < 0.05$) were calculated with Statistical Analysis System software 8.1 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Cookie Making with Milling Fractions of Different Wheat Cultivars. Cv. Albatros grains (22.5 N) were softer than those of cv. Meunier (36.6 N). Cv. Meunier grains were tempered to a slightly higher moisture level before milling.

Table 1 lists the milling yields, (SDS-extractable) protein levels, DS levels, and AX levels of the cv. Albatros and Meunier flour milling fractions and the dough spread rates and diameters of the resulting cookies when using these milling fractions and straight grade flours. DS levels varied from 2.6% (dm base) (B1) to 5.2% (C3) for cv. Albatros milling fractions. Cv. Meunier milling fractions had higher DS levels, which ranged from 5.3 to 10.5% (dm base) from fractions B1 to C3, respectively. Total AX levels

Table 1. Milling Yields, Protein, SDS (2.0%, w/v) Extractable Protein (EP, Percent of Protein), and Damaged Starch (DS) and Arabinoxylan (AX) Levels of Different Milling Fractions of Cultivars Albatros and Meunier Milling Fractions and Straight Grade (SG) Flours along with Spread Rates of Doughs and Final Diameters of Cookies Prepared from the Same Milling Fractions and SG Flours[a]

	cv. Albatros fraction							cv. Meunier fraction						
	B1	B2	B3	C1	C2	C3	SG	B1	B2	B3	C1	C2	C3	SG
milling yield (%)	17.9	12.7	3.4	32.6	6.2	1.2	74.0	14.6	11.3	2.1	36.1	8.0	0.6	72.7
protein[b] (%)	9.0 (0.1)	11.1 (0.1)	13.4 (0.0)	11.1 (0.0)	13.8 (0.1)	11.8 (0.2)	11.2 (0.1)	9.0 (0.1)	12.1 (0.1)	13.8 (0.2)	10.5 (0.1)	12.3 (0.1)	11.5 (0.1)	10.9 (0.1)
SDSEP (%)	74.9 (0.1)	68.5 (0.2)	74.6 (0.1)	73.3 (1.4)	78.5 (0.1)	79.8 (0.3)	72.2 (1.3)	72.1 (1.0)	73.3 (0.2)	73.5 (1.0)	71.0 (1.9)	79.4 (0.8)	73.5 (2.1)	71.0 (2.2)
DS[b] (%)	2.6 (0.2)	2.6 (0.1)	3.2 (0.2)	3.3 (0.1)	4.6 (0.1)	5.2 (0.1)	3.1 (0.1)	5.3 (0.1)	6.6 (0.1)	6.9 (0.1)	5.9 (0.1)	7.6 (0.1)	10.5 (0.1)	6.1 (0.1)
AX[b] (%)	1.08 (0.02)	1.07 (0.02)	1.30 (0.06)	1.16 (0.01)	1.80 (0.03)	1.41 (0.04)	1.19 (0.02)	1.69 (0.02)	1.85 (0.05)	2.08 (0.02)	2.02 (0.02)	2.90 (0.13)	2.30 (0.09)	2.03 (0.04)
spread rate (cm/min)	0.470 (0.025)	0.445 (0.005)	0.425 (0.020)	0.450 (0.050)	0.435 (0.035)	0.365 (0.005)	0.455 (−)	0.420 (0.010)	0.430 (0.005)	0.385 (0.005)	0.480 (0.010)	0.430 (0.010)	0.360 (0.015)	0.470 (−)
cookie diameter (mm)	92.6 (0.9)	91.7 (1.1)	89.7 (0.5)	90.3 (0.7)	86.8 (0.8)	87.7 (0.6)	88.7 (1.1)	87.0 (0.7)	85.4 (0.5)	84.7 (0.8)	86.5 (0.7)	81.7 (0.5)	83.8 (0.6)	86.1 (0.9)

[a] Sample codes are of the format B*x* and C*x*, where B stands for break flour, C for reduction flour, and *x* for the fraction order. Standard deviations are given in parentheses. [b] Dry matter basis.

Figure 1. Set time of cookies prepared with different flour milling fractions or straight grade flour (TOT) prepared from wheat cultivars Albatros (black bars) and Meunier (white bars). Sample codes are of the format B*x* and C*x*, where B stands for break flour, C for reduction flour, and *x* for the fraction order.

were 1.19% (dm) for cv. Albatros and 2.03% (dm) for cv. Meunier SG flours. **Table 1** shows that cookies made from cv. Albatros SG flour had larger diameters than those made with the corresponding cv. Meunier flour, and, that, for flour of either cultivar, cookies made with different reduction flour streams generally had smaller diameters than those made with break flour streams. The spread rates (**Table 1**) related negatively with DS levels for both cv. Albatros ($R^2 = 0.68$) and cv. Meunier ($R^2 = 0.51$) milling fractions, as also reported earlier by a number of authors (*1*, *3*−*5*). Interestingly, no such relationship was found between DS levels and set time. Although some authors (*7*,*8*) also noted an impact of AX level on cookie spread rate, the present data do not confirm this finding. Notwithstanding the above, negative linear relationships were found between final cookie diameter and flour DS ($R^2 = 0.79$) as well as total AX ($R^2 = 0.92$) levels, which shows that both DS and AX levels are of primary importance in predicting final cookie diameter. Taken together, the present results indicate that cookie flour with relatively more break stream flour probably yields cookies with larger diameter than does flour enriched with reduction streams.

Hoseney et al. (*3*) and Miller et al. (*2*), for sugar-snap cookies, stated that the final cookie diameter not only depends on the spread rate but also on the time at which the cookie dough stops spreading. **Figure 1** shows that cookie doughs made from cv. Albatros flour fractions generally set later than those made with the corresponding cv. Meunier flour fractions. In line with the present results, Doescher et al. (*25*) have already stated that cookies baked with hard wheat flour set earlier than cookies made with soft wheat flour. According to Doescher et al. (*10*) and Miller et al. (*11*), the cookie dough setting mechanism is determined by a viscosity increase due to protein entanglement rather than that it is influenced by DS or AX level. In their view, the latter constituent types, both of which have high water binding capacities, rather influence the spread rate, which also results in a smaller diameter, as indicated in the previous paragraph.

One can logically argue that the protein entanglement and/or subsequent cross-linking that determines the set time during cookie baking could depend on both protein level and protein properties. Because the corresponding cv. Albatros and Meunier flour milling fractions have comparable protein levels (**Table 1**) and cookie making with their fractions results in different set

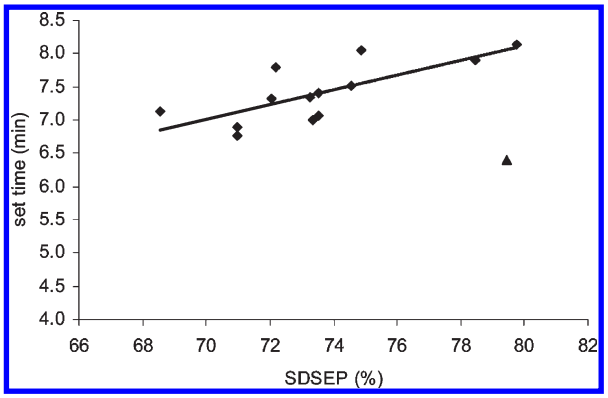

Figure 2. Set time of doughs made with different milling fractions (◆) as a function of the SDSEP level, with data of cultivar Meunier C2 milling fraction (▲) excluded from the correlation.

Table 2. Levels of Extractable Albumin plus Globulin (A+G), Gliadin (GLIA), and Glutenin (GLUT) as a Percentage of the Sum of Extractable A+G, GLIA, and GLUT[a]

cultivar	fraction	A+G (%)	GLIA (%)	GLUT (%)
Albatros	B1	18.4	50.2	31.3
	B2	16.6	50.3	33.0
	B3	16.8	48.5	34.7
	C1	18.3	50.7	31.0
	C2	22.7	47.2	30.1
	C3	nd	nd	nd
Meunier	B1	18.9	48.2	32.9
	B2	17.0	51.2	31.8
	B3	17.2	50.1	32.6
	C1	19.6	47.2	33.3
	C2	25.1	43.4	31.5
	C3	nd	nd	nd

[a] Sample codes are of the format B*x* and C*x*, where B stands for break flour, C for reduction flour, and *x* for the fraction order. nd, not determined.

times, the idea comes to mind that protein quality, in addition to its quantity, also influences the dough set time. **Figure 2** shows a positive linear relationship (R^2 = 0.55) between flour SDSEP level and set time for cookies made with different flour fractions and straight grade flours. Data from MC2, that is, the second reduction stream of cv. Meunier, were excluded from this correlation because this fraction contained significant bran contamination, which was visually observed as specks and translated into a high total AX level (**Table 1**). Furthermore, determination of the levels of the different protein fractions (**Table 2**) showed that this mill stream had a higher level of albumin plus globulin and lower levels of gliadin and glutenin than all other mill streams. Probably, in this case, although higher levels of non-gluten proteins resulted in higher SDSEP levels, they did not impact the dough setting as much as did gluten proteins. However, it may be that not only the SDSEP level but also other protein properties are different for the two wheat cultivars. This led us to use an approach based on heat-treated flours as it would allow us to study the impact of gluten proteins on cookie dough setting and final cookie diameter. Under the conditions applied, we expected that heat treatment of dry flour mainly influences protein properties. We used this approach as it allows examining the impact of cookie flours with different gluten properties, but with equal DS, AX, and protein levels.

Impact of Heat Treatment on Flour Properties. Weegels et al. (*17*, *18*) showed that heating changes gluten extractability in 1.5% (w/v) SDS. To investigate whether differences in flour SDSEP level indeed influence the set time during baking, as postulated above, a commercial cookie flour was heated for different times (2, 5, and 8 h) at different temperatures (80 and 100 °C).

Table 3. DSC Onset (T_o), Peak (T_p), and Conclusion (T_c) Temperatures and Enthalpy Values (ΔH) of Heat-Treated Flours[a]

heat treatment	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g dm starch)
none	55.7 cd	62.5c	71.4b	15.7b	9.4a
80 °C, 2 h	56.0bc	62.9b	73.1ab	17.1ab	9.7a
80 °C, 5 h	56.1bc	62.9b	73.3a	17.2ab	10.0a
80 °C, 8 h	56.3ab	62.8bc	73.5a	17.3ab	10.3a
100 °C, 2 h	55.4d	62.7bc	73.7a	18.3a	9.6a
100 °C, 5 h	55.9bcd	63.0b	74.9a	19.1a	10.3a
100 °C, 8 h	56.7a	63.5a	74.8a	18.1a	9.7a

[a] Values with the same letter are not significantly different (α < 0.05). dm, dry matter.

Table 3 lists the DSC characteristics of the resulting flours. The data show only slight changes in either T_o, T_p, or T_c. However, the gelatinization range generally increased with more severe heat treatment. The observed results could not be attributed to annealing of the starch, because of the low water content (*26*). Therefore, in the following sections, the limited effect noted on the starch is not taken into account, because starch does not gelatinize during sugar-snap cookie baking (*1*, *27*).

Figure 3 (black bars) shows that heating decreases flour SDSEP levels and that the degree to which it occurred depended on the applied temperature and incubation time. Heat treatment at 100 °C decreased the flour SDSEP levels more than did treatment at 80 °C. Also, 8 h of treatment had more effect than either 5 or 2 h. In addition, a decrease in levels of free SH groups was observed (**Figure 4**, black bars). Singh and MacRitchie (*28*) related this to the formation of disulfide bonds. **Figure 5** shows that heat treatment at 80 °C of flour increased the RVA peak viscosity, which was more pronounced for longer treatments. Similar results were obtained for heat treatment at 100 °C (results not shown). Addition of DTT, which cleaves disulfide bonds in gluten, completely or partially (for flours treated at 100 °C for 5 and 8 h) restored the profile (profiles not shown), demonstrating that the effect was related to protein cross-linking, as already demonstrated for rice flour (*29*) and dried pasta (*23*). **Figure 6** shows that heat treatment at 80 °C mainly influenced the extractability of glutenin polymers (**Figure 6a**) and that this effect was more pronounced with longer treatment times. Heating at 100 °C (**Figure 6b**) decreased the levels of both extractable glutenin and gliadin fractions, the second being more pronounced when heating for at least 5 h. In line with the present observations, Singh and MacRitchie (*28*), when heating dispersions of gluten (1.6 mg of protein/mL, pH 5.0) at 120 °C, noted a decrease in gliadin and a corresponding increase in apparent glutenin extractability, which they related to the incorporation of gliadin by sulfhydryl−disulfide interchange into the glutenin structure.

Use of Heat-Treated Flours in Cookie Making. Figure 3 shows that cookie dough making with the respective heat-treated flours did not significantly alter the SDSEP levels. Cookie dough mixing with the recipe used evenly disperses dough ingredients, resulting in a nonextensible dough with minimal if any gluten development.

During cookie baking, proteins aggregated, which decreased SDSEP levels (**Figure 3**). For the flour samples heated at 100 °C during 5 or 8 h, no further significant changes in SDSEP levels

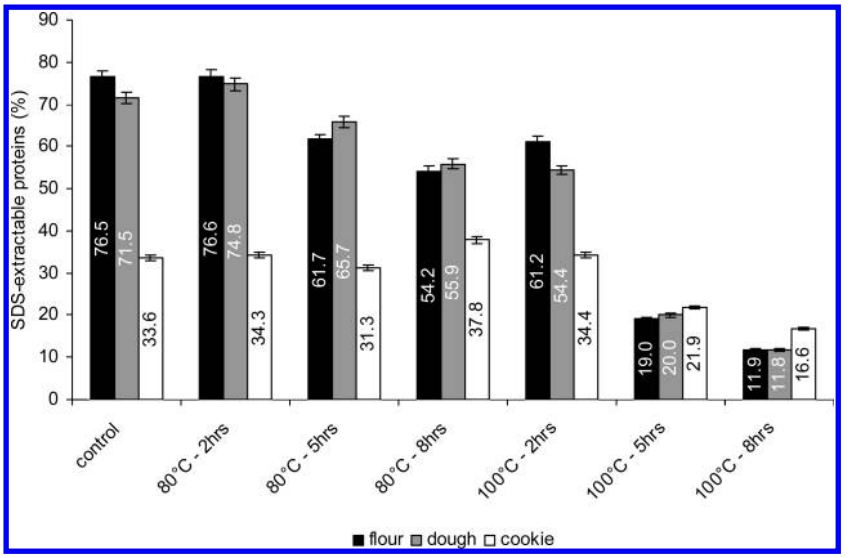

Figure 3. 2.0% (w/v) SDSEP levels of control and different heat-treated flour samples (black bars) and their corresponding doughs (gray bars) and cookies (white bars).

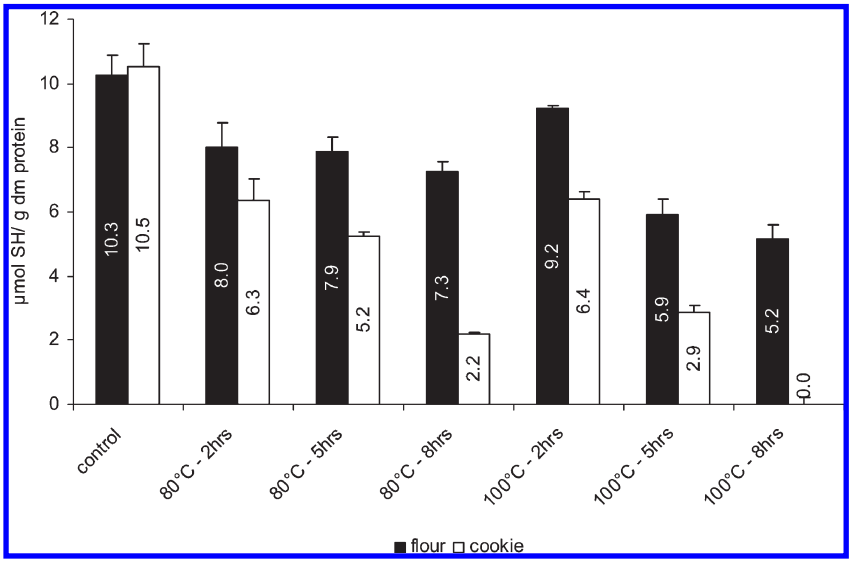

Figure 4. Free sulfhydryl (SH, μmol/g dry matter protein) of control and different heat-treated flours (black bars) and corresponding cookies (white bars).

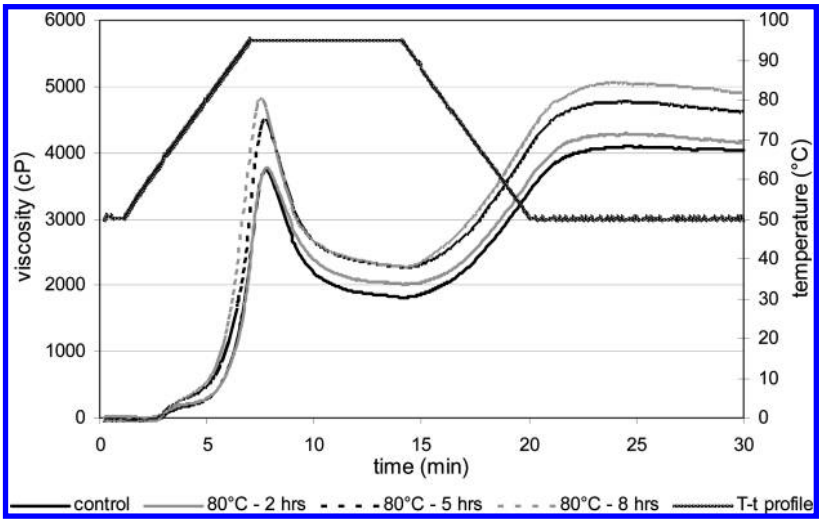

Figure 5. RVA profiles of heat-treated (80 °C) flour dispersions.

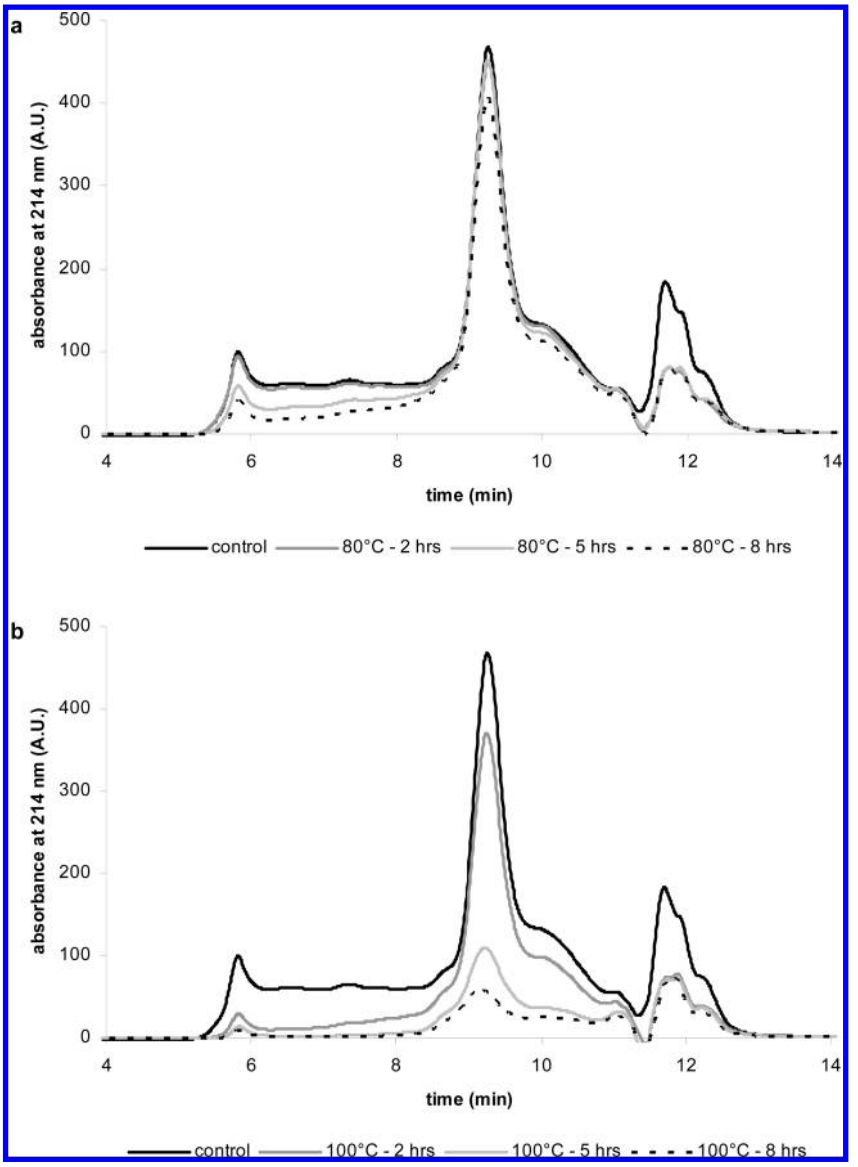

Figure 6. Size exclusion HPLC profiles of 2.0% (w/v) SDSEP of control and heat-treated flour samples at 80 °C (**a**) and 100 °C (**b**). A.U., arbitrary units.

were noted during cookie baking. Apparently, the aggregation due to cross-linking had already largely taken place during the preceding heat treatment.

Figure 4 shows that, for control cookie making, no decrease in free SH levels occurred during baking. In contrast, when cookies were made with heat-treated flours that themselves already had lower free SH levels than control flour (**Figure 4**), a further decrease in free SH during cookie baking was observed. It is logical to assume that, during cookie baking, flour protein can physically entangle and that the degree to which sulfhydryl−disulfide exchange and sulfhydryl oxidation can occur depends on the properties of the protein at the onset of the process. When cookies were baked with control wheat flour, no loss of SH-groups occurred during the process (**Figure 4**). When flour was heat-treated, the level of free SH-groups decreased, and further decreases occurred during baking of cookie doughs made with such flour. It seems that, during cookie baking with the heat-treated flours, the SH containing low molecular weight compounds and proteins were subject to a more pronounced formation of disulfide bonds. In the case of the proteins, this may indicate that the preceding heat treatment made the free SH-groups (more) available because of conformational changes. This may well have led to an increased tendency to entangle and/or cross-link.

Table 4. Spread Rate, Set Time, and Diameter of Cookies Made with Control and Heat-Treated Cookie Flours[a]

heat treatment	spread rate (cm/min)	set time (min)	diameter (mm)
none	0.410 (0.015)	7.2 (0.2)	88.3 (0.7)
80 °C, 2 h	0.400 (0.005)	7.2 (0.1)	87.8 (1.6)
80 °C, 5 h	0.335 (0.005)	7.0 (0.0)	85.4 (1.7)
80 °C, 8 h	0.375 (0.010)	6.8 (0.0)	85.0 (0.9)
100 °C, 2 h	0.375 (0.010)	6.9 (0.1)	86.4 (1.8)
100 °C, 5 h	0.400 (0.010)	6.5 (0.0)	84.2 (0.9)
100 °C, 8 h	0.390 (0.005)	6.6 (0.1)	85.7 (1.5)

[a] Standard deviations are given in parentheses.

Table 4 lists dough spread rates, set times, and final diameters of cookies made with the heat-treated flours. The heat treatment had no clear-cut influence on the spread rate during baking. Long ago, Doescher et al. (*10*) and Miller et al. (*11*) found spread rate to depend on protein level and, therefore, on the level of water that it binds, rather than on protein aggregation and/or entanglement. According to the same authors, the latter determines the set time during baking because of the resulting viscosity increase. In the

present work, set time, which correlated well with cookie diameter ($R^2 = 0.73$), decreased linearly ($R^2 = 0.90$) with decreasing SDSEP level. This indicates that larger (less soluble) gluten polymers cause earlier setting during baking, probably because their entanglement and/or cross-linking has more effects on the system mobility than that of smaller ones. This would mean that wheat flour, with relatively smaller gluten polymers, that is, smaller than a critical gluten polymer size, favors cookie spread. Indeed, in that case, relatively more interactions would be required to obtain a similar degree of entanglement/cross-linking. It follows that not only the flour protein level but also its SDSEP level is a good indicator for cookie baking behavior.

In conclusion, the aim of this work was to understand the impact of flour protein characteristics other than their level on the cookie dough setting mechanism. Different milling fractions with comparable protein levels were obtained from wheat cv. Albatros and Meunier. Cv. Albatros SG flour yielded cookies of larger diameter than did cv. Meunier SG flour. Cookie making with the separate cv. Albatros flour fractions also resulted in cookies with larger diameter than from those prepared with the corresponding cv. Meunier fractions. A linear relationship was found between DS and spread rate and between SDSEP and set time. Our results concerning DS confirmed literature data maintaining that cookie diameter is negatively affected by flour DS content and that this can be attributed to the cookie dough spread rate. Heat treatment of commercial cookie flour progressively decreased the SDSEP level with time and temperature. This resulted in earlier setting during baking and, consequently, in smaller cookie diameter. A hypothesis was proposed in which the polymer size of the gluten proteins plays a crucial role in cookie dough setting. Flour with relatively smaller, and thus more SDS-extractable, gluten protein would need relatively more interaction before the aggregation stops the spreading than flour with relatively larger polymers. In addition, the tendency of the gluten to entangle and/or cross-link may be another parameter to take into account. However, further research will be necessary to elucidate the specific contribution of the different protein fractions.

ABBREVIATIONS USED

AX, arabinoxylan; cv., cultivar; dm, dry matter; DS, damaged starch; DSC, differential scanning calorimetry; DTNB, 5,5-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; EDTA, tetrasodium ethylenediaminetetraacetate; RVA, Rapid Visco Analysis; SDS, sodium dodecyl sulfate; SDSEP, SDS-extractable protein; SE-HPLC, size exclusion high-performance liquid chromatography; SH, sulfhydryl; SG, straight grade.

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