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# Impact of mixing time and sodium stearoyl lactylate on gluten polymerization during baking of wheat flour dough

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ABSTRACT

The impact of differences in dough transient gluten network on gluten cross-linking during baking is insufficiently understood. We varied dough mixing times and/or added sodium stearoyl lactylate (SSL; 1.0% on flour dry matter basis) to the recipe and studied the effect on subsequent gluten polymerization during heating. The level of proteins extractable in sodium dodecyl sulfate containing media was fitted using first order kinetics. The extent and rate of gluten polymerization were lower when mixing for 8 min than when mixing for 2 min. This effect was even more outspoken in the presence of SSL. The present observations were explained as resulting from less gliadin incorporation in the polymer gluten network and from interaction of SSL with the gluten proteins. Finally, a higher degree of gluten polymerization during baking increased the firmness of the baked products.

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## 1. Introduction

When wheat (*Triticum aestivum* L.) flour is mixed with an appropriate amount of water, flour particles are increasingly hydrated and viscoelastic dough is obtained. The gluten protein network formed during mixing is transient due to dynamic exchange of disulfide (SS) bonds and entanglements between different protein polymers (Goesaert, Slade, Levine, & Delcour, 2009; Singh & MacRitchie, 2001). This network mainly consists of glutenin proteins. The globular gliadins rather plasticize the glutenin polymeric network (Cornec, Popineau, & Lefebvre, 1994; Khatkar, Bell, & Schofield, 1995). When dough is heated, the transient gluten network is transformed into a permanent, continuous, thermoset network (Goesaert et al., 2009; Levine & Slade, 1990). This is due to gluten protein polymerization and involves the formation of (permanent) SS bonds through oxidation of sulfhydryl (SH) groups (Singh & MacRitchie, 2004) and incorporation of gliadins into the glutenin structure through SH/SS interchange reactions (Lagrain, Thewissen, Brijs, & Delcour, 2008; Singh & MacRitchie, 2004). The formation of a permanent gluten structure as well as its properties are important in cereal processes such as bread, cookie and pasta making (Delcour et al., 2012) as they largely affect, if not determine, the quality of the final products. For bread, gluten protein polymerization in the baking phase contributes to its final structure and quality (Lagrain, Thewissen, Brijs, & Delcour, 2007), whereas, in cookies, such thermoset network determines cookie spread, the internal structure as well as the break strength (Pareyt, Van Steertegem, Brijs, Lagrain, & Delcour, 2010b). Also, during pasta making, gluten polymerization creates a three-dimensional interconnected structure (Wagner, Morel, Bonicel, & Cuq, 2011), of which the degree of protein polymerization during drying and/or subsequent cooking determines pasta quality in terms of firmness and cooking losses (Bruneel, Pareyt, Brijs, & Delcour, 2010). However, the relevance of structure formation of the transient network during dough mixing, and the influence of the latter on the formation and the final permanent gluten network structure in baked products has, to the best of our knowledge, never been investigated.

Surfactants such as the anionic surfactant sodium stearoyl lactylate (SSL) can strengthen dough (Pareyt, Finnie, Putseys, & Delcour, 2011) and affect the gluten network structure (formation) during mixing. They present an interesting tool to change dough's mixing properties and, in the end, the transient gluten network structure. The dough strengthening effect of SSL has been attributed to its interactions with gluten proteins during dough mixing, which may cause gluten aggregation and increase dough strength

*Abbreviations:* DDT, dough development time; SH, sulfhydryl; SS, disulfide; SSL, sodium stearoyl lactylate; SDSEP, sodium dodecyl sulfate extractable proteins; SE-HPLC, size-exclusion high performance liquid chromatography; dm, dry matter; SDSglut, SDS extractable glutenin; SDSglia, SDS extractable gliadin; DSC, differential scanning calorimetry; WSB, water saturated butanol.

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(Gomez, Ferrer, Anon, & Puppo, 2013; Gomez et al., 2004). In contrast, according to Collar, Armero, and Martinez (1998), during dough mixing, SSL preferentially binds to the inside part of the starch granules and only loosely to gluten.

Against this background, we here set out to study the potential relation between dough transient gluten network structure formation by mixing and further gluten cross-linking during baking. To that end, flour and water, to which in some cases SSL was added, were mixed into dough during different mixing times in order to yield dough with different gluten network (formation). The effect of varying mixing times and/or addition of SSL during mixing on gluten polymerization during subsequent heating was studied. The level of proteins extractable in sodium dodecyl sulfate containing media (SDSEP) in dough and at different stages during baking was monitored using size-exclusion (SE) high performance liquid chromatography (HPLC). Finally, the effect on the firmness of the baked model dough was investigated.

## 2. Materials and methods

### 2.1. Materials

Commercial wheat flour [moisture level: 10.9%; protein level: 10.8% on dry matter (dm) basis] was from Moulins de Kleinbettingen (Kleinbettingen, Grand Duchy of Luxembourg). Flour moisture and protein ($N \times 5.70$) contents were determined according to AACC Method 44-15.02 (AACC-I, 1999a) and the Dumas method, an adaptation of the AOAC (1995) to an automated protein analysis system (EAS Vario Max N/CN, Elt, Gouda, The Netherlands), respectively. SSL was from Palsgaard (Juelsminde, Denmark). All chemicals, solvents and reagents used were of analytical grade and from Sigma–Aldrich (Steinheim, Germany) unless specified otherwise.

### 2.2. Methods

#### 2.2.1. Mixograph study

Mixing properties can be evaluated with the Mixograph which produces a mixing curve (*i.e.* the mixogram) that shows the optimum dough development time (DDT). DDT is the time required to reach a peak in the mixogram. At the DDT, flour particles are completely disrupted and an optimally developed gluten network is formed (Delcour & Hoseney, 2010; Hoseney, 1985).

Mixograms were produced at least in duplicate with a 10-g Mixograph (National Manufacturing, Lincoln, NE, USA) according to AACC Method 54-40.02 (AACC-I, 1999b) using 8.6 g of flour dm and, thus, 9.65 g flour. SSL (1.0% on flour dm basis) was added as such. In the present study, the width of the mixogram tail was considered as an indication of dough strength (Van Steertegem, Pareyt, Brijs, & Delcour, 2013b).

#### 2.2.2. Dough preparation and sampling during baking

Control dough and dough with SSL were mixed in the Mixograph for 2, 4, 6 and 8 min. After mixing, each dough was divided over 7 cylindrical open glass test tubes [8 mm outer diameter $\times$ 45 mm; part 66658, Bruker (Ettlingen, Germany)] which were placed in larger test tubes (10 mm diameter $\times$ 180 mm; part E1405321_03, Bruker) and heated in an oil bath at 110 °C. At specific times (0, 0.5, 1, 2, 4, 6 and 8 min) a single test tube was removed from the oil bath and immediately submerged in liquid nitrogen to stop the heating process. Afterwards, the frozen (partially baked) dough pieces were freeze-dried, ground, and used for further analyses.

#### 2.2.3. Temperature measurements during baking

Dough temperature during heat treatment was evaluated with a MultiPaq21 Data Logger (DataPaq Ltd, Cambridge, UK).

#### 2.2.4. Determination of protein extractability in SDS containing medium

SDSEP levels of flour, freeze-dried dough and baked products were determined with SE-HPLC as described by Pareyt, Wilderjans, Goesaert, Brijs, and Delcour (2008). Samples (1.0 mg dm protein/mL) were extracted with a sodium phosphate buffer (0.05 M, pH 6.8) containing 2.0% (w/v) SDS (Acros Organics, Geel, Belgium). So-called total protein extracts were prepared under reducing conditions: samples (1.0 mg dm protein/mL) were extracted under nitrogen atmosphere with the above described buffer containing 1.0% (w/v) dithiothreitol (Acros Organics, Geel, Belgium) and 2.0 M urea. All samples were centrifuged (10 min, 10,000*g*) and filtered on polyethersulfone (0.45 mm, Millex-HP, Millipore, Carrigtwohill, Ireland). Extracted proteins were separated with SE-HPLC conducted as described by Lagrain, Brijs, Veraverbeke, and Delcour (2005) using a LC-2010HT system (Shimadzu, Kyoto, Japan) with automatic injection. The extracts (60.0 μL) were loaded on a BioSep SECS4000 column (Phenomenex, Torrance, CA, USA) and eluted with acetonitrile/water (1:1, v/v) containing 0.05% (v/v) trifluoroacetic acid. The flow rate was 1.0 mL/min, the column temperature 30 °C and eluted protein was detected at 214 nm. All extractions were performed at least in duplicate. The elution profiles of unreduced samples were divided into two fractions using the lowest extinction value between the two peaks as the cutoff point (Lagrain et al., 2005; Pareyt, Bruneel, Brijs, Goesaert, & Delcour, 2010a). The higher molecular weight proteins referred to SDS extractable glutenin (SDSglut) eluted first, the lower molecular weight proteins corresponding to SDS extractable gliadin (SDSglia) thereafter. SDSEP, SDSglut and SDSglia levels were calculated from the corresponding peak areas and expressed as a percentage of the peak area of flour proteins extracted under reducing conditions. The relative loss of SDSEP between dough and products baked for 8 min was calculated as follows:

$$\text{SDSEP loss } (\%) = \frac{\text{SDSEP dough} - \text{SDSEP baked product}}{\text{SDSEP dough}} * 100$$

#### 2.2.5. Differential scanning calorimetry (DSC) measurements

DSC analyses were performed as in Van Steertegem, Pareyt, Brijs, and Delcour (2013a). Products baked for 8 min were accurately weighed (2.4–2.7 mg) into aluminium pans (Perkin-Elmer, Waltham, MA, USA) and deionized water was added in a ratio 1:3 w/w dm sample/water.

#### 2.2.6. Gluten starch separation

Control dough and dough containing 1.0% SSL (on flour dm basis) were mixed (4 min) in quadruplicate in a Mixograph as outlined above. Two dough pieces were immediately freeze-dried. Starch was washed from the remaining dough pieces with deionized water until the washing water was clear (*ca.* 100 mL). Both the starch and the remaining gluten fraction were freeze-dried. Flour (500 mg or 500 mg + 4.4 mg SSL), freeze-dried dough and freeze-dried isolated starch and gluten fractions (with and without SSL) were extracted with hexane to extract free lipids, while bound lipids were subsequently extracted with water saturated butanol (WSB). Control dough and dough with SSL were weighed on an equal dm basis as that of the flour (without and with SSL). In addition, the amount of starch and gluten weighed corresponded to their respective amounts present in the freeze-dried dough pieces. Hexane (10.0 mL) was added and the samples were shaken (60 min, 150 rpm) and centrifuged (10 min, 1000*g*, 20 °C).

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