Journal of Cereal Science 79 (2018) 86–92

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

# Investigation of the influence of bakery enzymes on non-yeasted dough properties during mixing

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ARTICLE INFO

*Article history:*
Received 10 July 2017
Received in revised form
3 October 2017
Accepted 5 October 2017
Available online 5 October 2017

*Keywords:*
Bakery enzymes
Mixing
Aeration
Dough
Ultrasound

ABSTRACT

Understanding the influence of bakery enzymes on dough properties during the early stages of the breadmaking process can help optimize the design of enzymes for the bakery industry. The objectives of this study were to determine whether bakery enzymes affected dough aeration during mixing and whether outcomes differed according to flour strength. Doughs were prepared from a strong breadmaking flour and a cookie flour to which various bakery enzymes were added. Dough density was measured, and the ultrasonic phase velocity and attenuation coefficient in the resonance frequency region for bubbles in dough were evaluated. Dough properties differed according to the enzyme and a significant interaction between enzyme type and flour strength was observed. For strong breadmaking wheat flour doughs, the greatest changes were observed for glucose oxidase, followed by xylanase and then cellulose. For the weak flour doughs, the largest changes were observed for doughs containing lipase and xylanase, with the effect of glucose oxidase being much less pronounced. The enzyme-dependent changes in acoustic signatures and in dough density demonstrate that some bakery enzymes influence bread crumb structure as early as at the mixing stage.



## 1. Introduction

The enzyme sector associated with food and beverages is a rapidly growing industry, globally estimated to reach more than 2.5 billion dollars by 2020, with bakery enzymes contributing to more than 35% of this expected demand (Melim Miguel et al., 2013). A variety of bakery enzymes are commonly used during breadmaking to manipulate dough handling properties and machinability, soften bread crumb, increase loaf volume, extend shelf life (Harada et al., 2005), and thus enhance the quality of the final product. Even though the use of these enzymes as processing aids during breadmaking is well established, many of the mechanisms governing the interactions between enzymes, other ingredients and various processing operations are yet to be fully elucidated.

Mixing, the first unit process operation of breadmaking, has a substantial influence on bread quality. During mixing, air is incorporated as small gas pockets into the dough from the headspace of the mixer and these gas pockets are subdivided into smaller bubbles as mixing progresses (Chin et al., 2004). By mixing bread doughs under ambient or reduced pressure, the lack of entrainment of these gas pockets during mixing causes a pronounced deterioration in bread crumb structure (Baker and Mize, 1941). In addition, by mixing ingredients at the right ratios and for the optimal period of time, mixing is the first step in a definition of the dough's mechanical (rheological) properties.

Given the significance of the mixing process in relation to the quality of the loaf, evaluating whether bakery enzymes interact with molecules in the dough's gas phase to alter the mechanical properties of the dough during mixing is a valuable undertaking. However, to the authors' knowledge, the effects of bakery enzymes on dough aeration, as measured by changes in the dough's mechanical properties following mixing, have not been investigated. In this study, our objective was to use dough density measurements (Campbell et al., 2001) and low-intensity ultrasound [a technique sensitive to the bubbles present in dough (Koksel et al., 2016; Leroy et al., 2008)] to investigate how various bakery enzymes affect the properties of doughs made from flours of contrasting dough strength.

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https://doi.org/10.1016/j.jcs.2017.10.002
0733-5210/

## 2. Experimental

### 2.1. Sample preparation

Bread dough samples were prepared without yeast from either a strong or a weak flour, distilled water, and sodium chloride (reagent grade, Fisher Scientific, ON, Canada). The strong flour (12.4% protein content on a 14% m.b.) was milled from Canada Western Red Spring (CWRS) wheat, whereas the weak flour (9.1% protein content on a 14% m.b.) was milled from Canada Western Soft White Spring (CWSWS) wheat. Various enzymes (glucose oxidase, xylanase (bacterial origin), maltogenic amylase, lipase and cellulase) were used at concentrations (20, 50, 100, 50 and 50 ppm, respectively) advised by the manufacturer (Zeelandia International, Netherlands). The moisture contents (10.7% for CWRS and 11.4% for CWSWS) of the flour samples were determined in triplicate, according to the AACCI Approved Method 44–15.02 (AACC International, 2010).

Duplicates of Farinograph (FA-R/2, Germany) curves for each dough formulation were obtained using AACCI Approved Method 54–21.01 (AACC International, 2010). Water absorptions (63.1% for CWRS and 55.3% for CWSWS on f.w.b.) and dough development times (3.5 min for CWRS and 1.2 min for CWSWS) of flours were determined. To attain an even distribution of the enzymes in flour, each flour-enzyme mixture was blended for 1 min in the mixer bowl before the addition of other ingredients. Sodium chloride at 1.6% (f.w.b.) was dissolved in the required amount of distilled water (optimum water absorption as determined by Farinograph) and added to the dry ingredients. Duplicates of doughs at each formulation were prepared using a GRL-200 mixer (operating at 225 rpm). Water set at 26 °C was circulated (Haake C3 heating/cooling unit, Germany) around the mixer bowl so that a dough temperature of 30 ± 1 °C was achieved at the end of mixing.

To prepare dough subsamples for the ultrasonic tests, the procedure of Koksel et al. (2014) was used. One dough subsample was ultrasonically tested from each freshly mixed dough batch, and results were reported as averages of duplicate doughs.

### 2.2. Experimental methods

The experimental ultrasonic setup (in transmission mode) was the same as Koksel et al. (2014). The dough subsample was squashed between acrylic delay lines so that the thickness of the dough subsample between the delay lines was 0.34 mm. The ultrasonic signal was acquired and recorded 30 min after the end of mixing. The longitudinal attenuation coefficient ($\alpha$) and the phase velocity ($v$) were calculated according to Strybulevych et al. (2007) and corrected for acoustic impedance mismatch between dough/delay line interfaces according to Leroy et al. (2011). All ultrasonic experiments were performed inside a temperature (30 ± 0.1 °C) and humidity (85 ± 1.0% relative humidity) controlled cabinet (Caron 6010, Marietta, OH, USA) based on the proofing conditions stated in AACCI Approved Method 10–09.01 (AACC International, 2010).

Dough densities at ambient pressure ($\rho$) were determined according to Koksel and Scanlon (2012). For dough made from the strong flour, dough density at reduced pressure ($\rho_{RP}$) was also measured at 0.06 atm by connecting the mixing bowl to a vacuum pump during the second half of mixing. For dough made from the weak flour, dough density at reduced pressure was calculated using the $\rho_{RP}$ of strong flour and adjusting the density according to the differences in the mass fraction of water (63.1% for CWRS and 55.3% for CWSWS on f.w.b.). The gas-free dough (matrix) density ($\rho_{GF}$) was calculated by linear extrapolation of dough density to zero pressure (Koksel et al., 2014). Gas volume fraction ($\phi$) in the dough was calculated using $\rho$ and $\rho_{GF}$ [$\phi = 1\text{-}(\rho/\rho_{GF})$].

## 3. Results and discussion

### 3.1. Air entrainment effects of enzymes

In Fig. 1, the gas volume fraction of doughs prepared from strong (CWRS) and weak (CWSWS) flours without (control) and with various enzymes is presented. Overall, strong flour doughs had similar or higher gas volume fractions ($\phi$) compared to weak flour doughs, indicating that dough aeration was flour type (strength) dependent. The mixing time for strong flour doughs in this study was almost 3 times as long as that of weak flour doughs (3.5 min vs. 1.2 min). Accordingly, owing to a net increase in dough aeration with mixing time until optimum development is reached (Koksel and Scanlon, 2012), a higher $\phi$ in strong flour doughs was not unexpected. A lower $\phi$ in doughs prepared from strong flour compared to weak flour doughs has been reported in the literature previously (Campbell et al., 2001), but in that study the water content as well as the mixing time were identical for both strong and weak flour doughs.

For strong flour doughs, an increase in $\phi$ was observed in the presence of all enzymes, indicating that enzyme addition altered dough aeration during mixing. With enzyme addition to weak flour doughs, net aeration (gas entrainment minus distrainment) during mixing stayed the same, with the exception of glucose oxidase. With glucose oxidase addition, an increase in $\phi$ compared to the control dough was observed. Glucose oxidase's strengthening effect on dough mechanical properties through cross-linking of gluten proteins has been previously reported (Hilhorst et al., 1999; Bonet et al., 2006). Doughs containing glucose oxidase are therefore expected to become stronger due to this cross-linking. Since the same mixing time was used for a given control and its enzyme-containing dough, doughs containing glucose oxidase developed faster and occluded more air, regardless of dough strength, resulting in higher $\phi$ compared to the control doughs.

In Fig. 2, the attenuation coefficient ($\alpha$) and the phase velocity ($v$) of strong flour doughs without and with various enzymes are presented as a function of frequency. As expected, both $\alpha$ and $v$ exhibited peaks due to the presence of the bubbles entrained into dough during mixing; the peak is due to the bubbles resonating in response to the incident ultrasound waves (Koksel et al., 2017; Leroy et al., 2008). The frequency dependence of both $\alpha$ and $v$ is sensitive to changes in the gas volume fraction and/or the size distribution of bubbles in dough (Koksel et al., 2017; 2014). For instance, an increase in gas volume fraction in dough brought about by modification of dough formulation increased the magnitude of the peak in $\alpha$ (and a decrease in the magnitude of the peak in $v$) (Koksel et al., 2014; Leroy et al., 2008), while an increase in the median bubble size in unyeasted dough due to disproportionation was associated with a shift in the peaks in $\alpha$ and $v$ to lower frequencies (Koksel et al., 2017).

It is evident from Fig. 2 that the magnitudes of the peaks in $\alpha$ and $v$ were enzyme dependent, especially for the attenuation coefficient. From the results of Fig. 1, $\phi$ increased in the presence of enzymes compared to the control for the strong flour doughs. This increase would, in part, explain the difference observed in the frequency dependence of $\alpha$ and $v$ for the enzyme-free versus the enzyme-supplemented doughs (Fig. 2). However, the volume fraction of bubbles alone does not explain the variation in the magnitude of the peak in attenuation coefficient ($\alpha_{max}$). Even though doughs essentially had the same $\phi$ for the different enzymes, enzymatic modification of dough properties (both the rheology of the dough matrix and the specific distribution of its gas phase) is not unexpected. For instance, glucose oxidase stiffened a