Production of microbial exopolysaccharides in the sourdough and its effects on the rheological properties of dough

Ali Ketabi, Sabihe Soleimanian-Zad, Mahdi Kadivar, Mahmoud Sheikh-Zeinoddin *

Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, P.O. Box 84156, Isfahan, Iran

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Abstract

Exopolysaccharides (EPS) are exogenous microbial metabolites which are secreted mainly by bacteria and microalgae during growth. In addition to natural polysaccharides present in cereal grains flour and dough, microbial flora is usually involved in production of polysaccharide on sourdough fermentation. Total polysaccharides (microbial and flour) were extracted from sourdough and dough samples dehydrated and were added at the rate of 0%, 0.25%, 0.5%, 1%, 1.5%, 2% and 2.5% (w/w flour based) on the dough to investigate its effects on the rheological properties of the dough. Addition of polysaccharides to the dough increased the water absorption and decreased the dough softening after 20 min. Resistance to extension after 45, 90 and 135 min resting time was decreased by increasing the percentage of the added polysaccharides. Longer fermentation time for each level of polysaccharides led to greater stability. No significant differences were observed in the extensibility of dough. The overall effects of different levels of added polysaccharides resulted in a decrease in resistance to extension ratio of the samples. Energy input decreased in all cases. It seems therefore that addition of polysaccharides may be useful when bread is to be made with stronger flour and longer fermentation time is needed.

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1. Introduction

Exopolysaccharides (EPS) are exogenous microbial metabolites which are mainly produced by bacteria and microalgae during their growth. EPS may be assembled as capsular forms that are tightly associated with the cell surface, or secreted into the extracellular environment as slime materials (Sutherland, 1972). Lactic acid bacteria (LAB) are widely used food grade organisms, which are generally recognized as safe and known to produce different metabolites including EPS (Stiles & Holzapfel, 1997). EPS play different roles such as protection of cell against water absorption, phagocytosis and phage invasion (Cerning, 1990 and Cerning, 1995). Plant based polysaccharides and their modified forms as well as microbial polysaccharides are important additives which are commonly used in the food industry (Armero & Collar, 1996; Belitz & Grosch, 1999; Butt, Anjum, Samad, Kausar, & Tauseef Mukhtar, 2001). These polysaccharides improve texture and increase quality and durability of bread (Armero & Collar, 1996 and Armero & Collar, 1998). Plant based polymeric compounds (polysaccharides) can be replaced by microbial polysaccharides produced by LAB during the dough fermentation (De Vuyt & Degeest, 1999). Mentioned microbial polysaccharides can also be considered as prebiotics (Gibson & Roberfroid, 1995; Korakli, Pavlovic, Gaenzle, & Vogel, 2003).

Cereal products are the most important food sources in the world. Cereal grains are predominantly composed of starch. Non-starch polysaccharides, which are classified as minor parts of flour, are naturally present in dough too (Belitz & Grosch, 1999). In addition to natural polysaccharides from cereal grains, microbial flora of dough are usually involved in sourdough fermentation produce different kinds of polysaccharides (Korakli, Rossmann, Gaenzle, & Vogel, 2001). Increasing the amount of polysaccharides to the dough causes many changes in the dough system, which affects the rheological behavior of dough and finally improves technological quality of dough and bread. Addition of hydrocolloids to the dough also affects retrogradation of starch and delays the staling of bread (Collar, Martinez, & Armero, 1999). While application of EPS produced by lactic culture bacteria is very common in dairy industry, the research on the production of EPS in the dough and the impact of those polysaccharides on the quality of bread is very limited. Emphasizing on the native Iranian species of microorganisms, this study examines the application of polysaccharides in bread dough.
2. Materials and methods

2.1. Sourdough and dough preparation

The flour was obtained from Isfahan Joreh Flour milling company. The sourdough was provided by the bakery located in the Isfahan University of Technology campus. Forty grams of the sourdough were added to 100 g of flour, 58–60 mL of water and 1 g of sugar in order to prepare a new sourdough culture. The new sourdough was incubated at 30 °C for fermentation (pH 3.5–4.0). pH and total titrable acidity of dough were measured according to the AACC standard methods (AACC, 2002). A dough sample was prepared as a control exactly as above without using any sourdough. The control was also incubated at 30 °C and was simultaneously taken out from the incubator.

2.2. Dough rheological characteristics

Rheological characteristics of dough including water absorption, dough development time, dough resistance and softening after 20 min were determined by using Brabender Farinograph according to the AACC 54–10 standard methods (AACC, 2002). Resistance (R), extensibility (E) and resistance to extension ratio (R/E) were determined by Brabender Extensigraph based on AACC 54–21 standard methods (AACC 2002)

2.3. Polysaccharides extraction

In order to extract polysaccharides from sourdough and dough, samples were diluted with water in a ratio of 1:2. This suspension was then centrifuged at 11000g for 10 min (Heraeus, Germany). Two volumes of chilled 96% ethanol were added to the supernatant. This mixture was incubated overnight at 4 °C. The precipitate was collected by centrifugation at 2500g for 20 min. at 5 °C (Kokusan OSK1735 Japan). The precipitated EPS were dissolved in deionized water. Two volumes of ethanol were added to the mixture and centrifuged again (2500g for 20 min. at 5 °C). Precipitated polysaccharides were dehydrated by freeze drier (Kokusan OSK 2139, Japan) for further experiments (Van Geel-Schutten et al., 1999). The amount of polysaccharides in sourdough was considered as total microbial and flour polysaccharides. All determinations were made in duplicate.

2.4. HPLC analysis

Both polysaccharides produced by microorganisms and native flour polysaccharides were hydrolyzed by 10.5% (v/v) perchloric acid and incubated at 80 °C for 2 h. Monosaccharide composition and their contents were determined using external standards including xylose, arabinose, glucose and fructose. Carbohydrates were separated by HPLC (Shimadzu, Japan). C18 column (250 mm × 4.6 mm, shim-pack Shimadzu, Japan) was used with a refractive index detector RID-6A (Shimadzu, Japan) and a flow rate of 0.7 ml min⁻¹. The maximum pressure of 150 kg f cm⁻² and the mobile phase of deionized water were employed. Column temperature was 60 °C (Korakli, Gaenzle, & Vogel, 2002).

2.5. Statistical analysis

All determinations were carried out in three replications. Mean values and standard deviation were then calculated. Results were analyzed using the SAS software (version 8). Least significant differences (LSD) test was used to describe means at the 5% significance level.

3. Result and discussion

3.1. Polysaccharides determination in the sourdough and control samples

The polysaccharides and their hydrolyzed sugar contents in the sourdough and control samples were shown in Table 1. Total polysaccharides along with its major constituents, xylose and arabinose, significantly increased during fermentation therefore a 63.3% greater EPS amount was found in sourdough in comparison with the control. Korakli et al. (2001) reported an increased proportion of soluble microbial polysaccharides upon sucrose addition in the dough. HPLC chromatograms showed two divided peaks. Based on the peaks obtained by the injection of external standards, xylose and arabinose were detected in both sourdough and control samples (Table 1). In comparison with control sample, there was 142.3% and 155.6% increase in xylose and arabinose content of sourdough sample, respectively. Comparing with control sample, this phenomenon indicated that polysaccharides were produced in the sourdough sample. Majority of reported EPS produced by LAB are heteropolysaccharides composed of two or more monosaccharides, mainly galactose, glucose, rhamnose and fructose (De Vuyst & Degeest, 1999). Furthermore, production of homopolysaccharides by LAB has been previously described (Van Geel-Schutten, Flesch, Ten Brick, Smith, & Dijkstra, 1998; Van Geel-Schutten et al., 1999). Tieking, Korakli, Ehrmann, and Gaenzle (2003) reported the ability of several lactobacilli of sourdough of intestinal origin to produce EPS of the fructan and glucan types. Detection of xylose and arabinose in the sourdough sample could be an indication of the activity of some native LAB in the sourdough sample which produced polysaccharides of different monosaccharide origin comparing with monomers previously reported. Comparison between xylose and arabinose also indicated that the amount of xylose was greater than arabinose. This result was very similar to values reported by Li, Lu, Gu, Shi, and Mao (2005).

3.2. Polysaccharides and Farinograph results

The effect of polysaccharides on the water absorption capacity of the dough is shown in Table 2. Addition of polysaccharides...
significantly increased the water absorption when polysaccharides were added at concentration higher than 0.25%, and mainly at 2.5% of polysaccharide concentration. Beside the important and basic role of gluten and starch in preparation of bakery products, it is very important to consider the roles that minor parts of the flour play on the quality of such products. Fat, ash, endogenous enzymes and non-starch polysaccharides are those, which should be considered in bread production. Arabinoxylans, which categorized as non-starch polysaccharides can affect both the dough characteristics and final bread products. Different parameters such as chain length, cell wall pattern, degree of substitution or arabinose to xylose ratio determine the arabinoxylan characteristics (Courtin & Delcour, 2002).

The characteristics of dough containing different amount of polysaccharides is shown in Table 2. Increase in polysaccharides proportions from 0% to 2% did not significantly modified dough development time, an indicator of primary grain hardness, neither dough stability. Since the amount of polysaccharides added to the dough was limited and they were also partly insoluble, the effect of polysaccharides may not contribute to influence the mentioned parameters. Comparison of different levels of polysaccharides with the control sample showed that dough stability did not change greatly and there was no significant difference between samples containing 0.25–2.5% polysaccharides concentration. Rosell, Rojas, and Benediti de Barber (2001) reported that dough resistance was very different for various hydrocolloids. For example, dough resistance was increased by addition of xanthan or alginate, whereas it was decreased by using kappa carragenan or hydroxypropil methyl cellulose (HPMC). In our work, changes in dough softening after 20 min showed that addition of polysaccharides to the dough significantly increased the dough toughness at polysaccharide concentration higher than 0.25% (Table 2). It is well documented that polysaccharides in grains such as wheat and rye have lower concentration higher than 0.25% (Table 2). Increase in polysaccharides concentration higher than 1.5% produced dough with lower extensibility after 90 min of resting. No clear tendency in extensibility was observed as resting time increased to 135 min. Rosell et al. (2001) and Courtin and Delcour (2002) reported the similar behavior after addition of different hydrocolloids to the dough. They indicated that extensibility could be decreased by addition of soluble and non-soluble polysaccharides to the dough. Mettler and Seibel (1993) also reported a slight decrease in dough elasticity by adding hydrocolloids such as guar gum and carboxy methyl cellulose (CMC) to the dough.

The resistance to extension ratio (R/E) of the dough containing different levels of polysaccharides is also shown in Table 3. The overall effect of increase in polysaccharide content of the dough resulted in a lower resistance to extension ratio (R/E), which was mainly due to the influence of polysaccharide addition on the resistance parameter (R). Increasing fermentation time from 45 to 135 min showed better stability of dough. Rosell et al. (2001) also showed that most of hydrocolloids decrease the resistance to extension ratio, but analysis through the time indicated better stability of the dough containing hydrocolloids.

Statistical analysis in this study showed that energy (area, Table 3) was also affected by polysaccharides concentration, fermentation time and their combination effect. Energy input decreased at resting times higher than 45 min, as well with polysaccharide addition. These events may indicate that a lower resistance to reach the tearing point is achieved after polysaccharides were sufficiently hydrated. This coincided with earlier findings (Rosell et al., 2001).

4. Conclusion

Application of oligosaccharides producing species of prebiotic LAB in the sourdough of cereal products may help to improve rheological properties of the dough as well as consumer’s health.

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