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Wheat dough syruping in cold storage is related to structural changes of starch and non-starch polysaccharides

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ABSTRACT

Even though the refrigerated dough industry is growing quickly due to the convenience and freshness of refrigerated dough over a prolonged storage period, dough syruping, which is a brownish liquid that leaches out from dough during the storage, is a quality-diminishing factor that needs to be resolved. The objectives of this study were to understand dough syruping and how it is related to structural changes in water-soluble arabinoxylan (WS-AX) and starch in wheat flours during refrigeration as well as to prevent syruping by applying exogenous cell wall polysaccharides. Dough syruping increased to 6.5, 6.9, and 17.2% in weak, strong, and *jopoom* wheat flours, respectively, after a 35-day storage period. The endoxylanase activity of *jopoom* wheat flour was substantially greater compared to other commercial flours, but the activity of this flour did not change over the whole cold storage period. The molecular size reduction of WS-AX was inversely related to the degree of dough syruping. The addition of β-glucan, carboxymethylcellulose, and xylan effectively reduced syrup formation in *jopoom* wheat flour dough.

1. Introduction

Wheat flour consists mainly of starch (70–75%), water (14%), and proteins (10–12%). Additionally, 2–3% non-starch polysaccharides (NSPs) are important minor flour constituents related to bread production and quality. Starch is the major storage polysaccharide and the most abundant constituent of many plants, including cereals. Starch granules are composed of two constituent polymers: a basic linear polysaccharide (α-1,4 linked glucose) named amylose and a highly branched polysaccharide named amylopectin (α-1,4 linked glucose and α-1,6 linked glucose) (Whistler & BeMiller, 1997). Starch has some unique properties that determines its functionality in many food applications, especially breadmaking (Romano et al., 2016). Its structure and physicochemical properties have been the subject of many extensive reviews (Hizukuri, 1996). The NSPs from the cell wall of the aleurone and endosperm of the wheat kernel are polysaccharides that contain pentose and/or hexose sugars. The pentose polymers are called pentosans, and arabinoxylan (AX) is the most important backbone. Approximately 1.5–2.5% AX in wheat flour consists of 25–30% water extractable AX (WEAX) and 70–75% water unextractable AX (WUAX). The WEAX and WUAX differ in physicochemical and functional properties, such as viscosity-forming potential, water-binding capacity, solubility, and gel-forming capacity (Courtin & Delcour, 2002). Furthermore, the arabinose to xylose (A/X) ratio plays a crucial role in the physicochemical properties of AX (Izydorczyk & Biliaderis, 1995). AX, even though it is present in minor amounts, is an extremely important functional ingredient because it can bind to water almost ten times as much as its own weight, which accounts for almost 30% of the water binding capacity of wheat flour and exerts a significant effect on the dough and bread quality (Courtin & Delcour, 2002).

Amylases and endoxylanases of microbial origin are often used to improve starch and AX functionalities in breadmaking by changing the properties and the molecular mass of these polysaccharides. Numerous reports are available that have investigated the effects of these added microbial enzymes on both polysaccharide properties and dough and bread quality. The effects of enzyme supplementation on bread volume and quality largely depend on the dosage, the substrate selectivity, the inhibition sensitivity, and other biochemical characteristics of the enzymes (Dornez, Cuyvers, Gebruers, Delcour, & Courtin, 2008; Goesaert, Leman, Bijttebier, & Delcour, 2009; Steffolani, Ribotta, Pérez, & León, 2012). However, little is known about the impact of wheat flour-associated endogenous amylase and endoxylanases on structure and the

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properties of starch and AX in dough. Wheat flour contains several important enzymes such as amylases, xylanase, and lipoxygenase. In the wheat grain, amylase is located mainly in the pericarp with small quantities present in the aleurone layer and the seed coat (Kruger & Tipples, 1980). Variations have been reported in the distribution of amylase in various mill streams of soft winter wheat (Finney, Natsuaki, Bolte, Mathewson, & Pomeranz, 1981). In general, the amylases from cereals (wheat and malted barley) were less sensitive to the presence of some ingredients, additives, and metabolites (Rosell, Haros, Escrivá, & Benedito de Barber, 2001). Endoxylanases associated with wheat kernels originate from the wheat plant itself and from microorganisms populating the outer layers of the wheat kernel as well. This corresponds with the previous observations that endoxylanases in wheat grains are mainly concentrated in the outer kernel layers (Bonnin, Le Goff, Saulnier, Chaurand, & Thibault, 1998; Dornez, Joye, Gebruers, Delcour, & Courtin, 2006; Gys, Courtin, & Delcour, 2004). Endoxylanases have been shown to have an impact on wheat flour dough after the dough is mixed and rested (Dornez, Gebruers, Cuyvers, Delcour, & Courtin, 2007). Wheat flour-associated endoxylanases not only solubilize WUAX but also seriously degrade WEAX to lower MM fragments during the resting phase. It is possible that high wheat flour-associated endoxylanase activity levels nullify the beneficial effects of added microbial endoxylanases. In addition, the wheat flour-associated endoxylanase level is a major determinant of refrigerated dough syruping (Gys et al., 2004).

The refrigerated dough industry is growing quickly due to the convenience and extended freshness of refrigerated dough over a prolonged storage period. Thus, various types of refrigerated dough are widely consumed to save time and costs. However, a brownish liquid, which is called 'dough syrup', migrates to the surface of the dough during refrigerated storage and leads to defects in product quality. The main cause of dough syruping during dough refrigeration has been recognized to be arabinoxylan (AX) degradation by endogenous endoxylanase (Courtin, Gys, & Delcour, 2006; Courtin, Gys, Gebruers, & Delcour, 2005).

The purpose of this study was to gain insight into the impact of endogenous endoxylanase and amylase activity levels on structural changes of AX and starch in wheat flour dough during refrigerated storage and to estimate their potential influence on dough syruping. Furthermore, exogenous cell wall polysaccharides were reinforced in a wheat dough formulation to evaluate their effects on the quality of refrigerated dough.

2. Materials and methods

2.1. Materials

Commercially strong and weak wheat flours were kindly provided from Samyang Co. (Seoul, Korea). The strong wheat flour was Dark Northern Spring (DNS), which is one of the most commonly grown varieties in North Dakota. This wheat flour is commonly used to make bread in Korea. The weak wheat flour was produced with the Soft White (SW) cultivar that is grown in the Pacific Northwest of the United States. SW is used mainly for bakery products other than bread, including pastries, cakes, and cookies. A Korean wheat cultivar, *jopoom*, was a gift from SPC Co. (Seoul, Korea). *Jopoom* wheat is one of the most commonly grown varieties in Korea, and the flour is often used to make bread. The Xylazyme-AX and oat β-glucan were purchased from Megazyme International, Inc. (Wicklow, Ireland). Sodium azide, salt, carboxymethylcellulose, xylan from birch wood, protease, amyloglucosidase, and DMSO were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.2. Dough preparation and storage

The moisture contents of each flour samples were analyzed by Moisture Analyzer (Sartorius MA30, Goettingen, Germany). The sample doughs were prepared using a lean dough formula consisting of 1.8 g of salt, and 65.5, 51.9, and 60.1 mL of water (based on the moisture contents in flour sample) with sodium azide (0.06% w/v) along with 100 g of strong, weak, and jopoom wheat flours, respectively. The moisture content required for each dough was determined based on the results of the Mixolab analysis (Chopin Technologies, Villeneuvela Garenne, France). To test the effects of various non-starch polysaccharides (NSPs) on the bread dough properties, β-glucan, carboxymethylcellulose, and xylan were blended at the 1.0% (w/w) level individually in the dough formulation. The ingredients were mixed in a 100-g pin mixer (National Manufacturing, Lincoln, NE, USA) at 25 °C for 3.5 min, sheeted, molded, and stored in plastic containers at 5 °C for 0, 3, 7, 14, 21, and 35 days. During the storage period, the degree of dough syruping was analyzed, and the dough sample was lyophilized and ground by mortar and pestle to a fine powder.

2.3. Gluten content determination

Dry gluten contents in different flour samples were determined with a hand washing method as described in AACC (2000) method No.38-10.

2.4. Quantification of dough syruping

The degree (%) of dough syruping was defined as the weight fraction of liquid released from the dough and was calculated as (initial mass of dough − mass of centrifuged dough) / initial mass of dough × 100. The stored doughs were divided into four pieces that were approximately 3.0–4.0 g. Accurately weighed dough pieces were centrifuged at 11000 × *g* for 30 min and at 4 °C using a Centrifuge 5804R (Eppendorf Co., Germany). After centrifugation, the tubes were inverted to allow the liquid to drain. The amount of the expelled syrup was quantified according to the difference in weights measured before and after syrup removal and was expressed as the percentage of the initial dough weight.

2.5. Measurement of xylanase and total amylase activities

2.5.1. Effects of temperature and pH on enzyme activity

For the optimal reaction temperature for endoxylanase and total amylase activities in 3 types of wheat flour, the enzyme extract was incubated in sodium phosphate buffer (50 mM, pH 6.0) and sodium acetate buffer (50 mM, pH 5.0), respectively, at temperatures ranging from 30 to 60 °C. The enzyme activities were determined with the AZCL-AX tablet and DNS methods, respectively, described above. To determine the optimum pH of these enzyme activities in wheat flours, the enzyme solution was incubated in a wide range of pH values prepared with sodium acetate (pH 4–6), sodium phosphate (pH 6–7.5), Tris-HCl (pH 7–8), and glycine-NaOH (pH 8–10) buffers.

2.5.2. Effects of cold storage on enzymes

Endoxylanase activity in lyophilized dough samples was measured using the Xylazyme-AX method with an assay kit containing AZCL-AX tablets (Megazyme, Ireland). Lyophilized and ground dough samples (1:5, w/v) were suspended in sodium phosphate buffer (50 mM, pH 6.0), and the enzyme was extracted at 6 °C for 1 h. After centrifugation (5000 × *g*, 4 °C, 10 min), the extract (1.0 mL) was pre-incubated at 40 °C for 10 min. The AZCL-AX tablet was added and incubated at 40 °C for 17 h. The reaction was stopped with a 2.0% Trizma base solution (10 mL). After filtration with Whatman filter paper No.1, the absorbance was read at 590 nm using a UV/Vis spectrophotometer (Beckman coulter, Inc., CA, USA). Xylanase activity was calculated based on a standard curve. Control xylanase from *A. niger* was diluted 1000, 5000, 10000, 20000, and 40,000 times and the dilutions were incubated with AZCL-AX tablets that were used to determine the standard curve. Control xylanase activity was standardized using wheat