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Effects of starch damage and yeast fermentation on acrylamide formation in bread

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ABSTRACT

The effects of starch damage and yeast fermentation on the formation of acrylamide in wheat bread were studied. Four wheat cultivars were milled separately by three laboratory mills to obtain wheat flours with damaged starch content ranging from 1.7 to 6.6%. Reducing sugar contents increased with increasing damaged starch content in flour. Yeast fermentation decreased greatly the content of asparagine by 40–60% in the dough, but increased substantially the content of reducing sugar. Compared with the unleavened bread, dough fermentation significantly decreased the content of acrylamide in leavened bread. The content of acrylamide in bread increased with increasing damaged starch content in wheat flours from the same cultivar. This study clearly showed that damaged starch content in wheat flour and dough fermentation are two major determinants of the formation of acrylamide in bread. The mitigation of acrylamide formation in bread can be achieved by reducing damaged starch in flour and by fermentation of the dough.

1. Introduction

Bread has been a basic part of the diet all over the world for a long time, especially in Western countries. The baking process during bread making can improve greatly the color, flavor, taste and texture of breads, thereby increasing its attractiveness to the consumer (Birch, Petersen, & Hansen, 2014). However, some hazardous substances, such as acrylamide, can be formed during baking (Lineback, Coughlin, & Stadler, 2012). Although the epidemiological associations have not demonstrated acrylamide to be a human carcinogen, the margins of exposure (MOEs) indicate a concern for neoplastic effects based on animal evidence (EFSA, 2015), such that some regulatory authorities consider it prudent to reduce exposure to acrylamide in foods (www.foodstandards.gov.au).

After dietary consumption, acrylamide is rapidly absorbed from the gastrointestinal tract and widely distributed in tissues. In the liver, acrylamide is metabolized to glycidamide (GA), which is more reactive towards DNA and proteins (Pedreschi, Mariotti, & Granby, 2014). To mitigate the risk of possible detrimental effects on human health, a thorough understanding of mechanism of acrylamide formation is needed. The main pathway leading to acrylamide in foods is the Maillard reaction, which occurs mostly between the amino acid asparagine and reducing sugars, the main precursors of acrylamide formation in bread (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). However, acrylamide can also be formed in other ways, including decarboxylation and deamination of asparagine (Granvogl & Schieberle, 2006), from acrylic acid formed in a Maillard reaction between aspartic acid and reducing sugars (Yaylayan & Stadler, 2005), from lipid degradation, dehydration or decarboxylation of organic acids (Yasuhara, Tanaka, Hengel, & Shibamoto, 2003), and from ammonia released from thermal degradation of amino acids and proteins (Keramat, LeBail, Prost, & Soltanizadeh, 2011).

There are many factors that affect the formation of acrylamide in foods, including the abundance of acrylamide precursors in the foodstuff, the processing methods (for example, baking, frying, toasting), the processing conditions, such as the temperature, heating time, pH, water activity, additives, and matrix and so on (Ahrné, Andersson, Floberg, Rosén, & Lingnert, 2007; Fredriksson, Tallving, Rosén, & Åman, 2004; Gökmen, Açar, Köksel, & Acar,

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2007). Based on previous studies, decreasing the content of reducing sugars or of asparagine inhibits acrylamide formation; a positive correlation was identified between the level of asparagine in cereal products and the amount of acrylamide in bread (Capuano et al., 2009). Several other measures were proposed to reduce acrylamide content in cereal products (Konings, Ashby, Hamlet, & Thompson, 2007). Of these, yeast fermentation was shown to be effective for thermally processed food products (Huang, Yu, Zou, & Tilley, 2008; Zhou, Wang, Chen, & Zhang, 2015).

Many types of bread made from wheat flour dough are potential sources of dietary acrylamide. However, the amount of acrylamide they may contain is likely to differ according to the method of preparation. During milling, starch in wheat grains is subjected to compressive and shear forces, resulting in different degrees of damage to starch granules, which can play an important role in the quality of wheat-based food products. The damaged starch granules can take up water readily and be susceptible to enzymatic hydrolysis. An appropriate level of damaged starch is important in the process of dough mixing, and to facilitate yeast fermentation of the dough. However, an excessive amount of damaged starch in flour can lead to a sticky crumb structure in the finished bread (Ghodke, Ananthanarayan, & Rodrigues, 2009; Mulla, Bharadwaj, Annapure, & Singhal, 2010). Grain hardness, mill types, and flour coarseness are the major factors that determine the content of damaged starch in flour. In general, the amount of damaged starch in hard wheat flour is greater than that in soft wheat flour under the same milling condition (Barrera et al., 2013). The effect of milling on damaged starch content in wheat flour, and in turn on the properties of the dough and the quality of final food products, has attracted much attention. However, the role of damaged starch in wheat flour on the formation of acrylamide in thermally processed foods is little understood. The main objectives of this study were to investigate (i) whether damaged starch in wheat flour can influence the formation of acrylamide in breads, and (ii) to examine the effect of dough fermentation on acrylamide formation.

2. Materials and methods

2.1. Materials

The grains of four wheat cultivars (Beijing 0045, Zhongmai 175, 07CA255, 13CA64) were provided by the Institute of Crop Science of Chinese Academy of Agricultural Science, China. Grain hardness of the four wheat cultivars was 3.5, 53.7, 64.1 and 80.6 for Zhongmai 175, Beijing 0045, 07CA255 and 13CA64, respectively, based on the test of Single Kernel Characterization System (SKCS 4100, Perten Instruments, Sweden). Yeast was purchased from Angel Yeast Co. Ltd. (Yichang, Hubei, China). Reducing sugar and starch damage assay kits were purchased from Megazyme International Ireland Ltd., (Wicklow, Ireland). Acrylamide (>99%) and Carrez Reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other chemical reagents were all of analytical grade.

2.2. Preparation of wheat flours

A Buhler Laboratory Mill, Brabender Senior Mill and Brabender Junior Mill (subsequently referred to as Senior and Junior, respectively), which are commonly used mills in wheat quality testing laboratories worldwide, were used to mill the grains according to Approved Method 26-21A (AACC International, 2000). The yields of straight run flour of 07CA255, Beijing0045, Zhongmai 175 and 13CA64 by Buhler Laboratory milling were 76.1%, 72.8%, 71.8% and 72.6%, respectively. The flour yields of 07CA255, Beijing0045, Zhongmai 175 and 13CA64 obtained from the Senior Mill were 73.1%, 66.8%, 66.4% and 67.6%, respectively, and the flour yields of 07CA255, Beijing0045, Zhongmai 175 and 13CA64 obtained from the Junior Mill were 64.4%, 56.2%, 58.9% and 56.5%, respectively.

2.3. Determination of starch damage, asparagine and reducing sugar content of wheat flours

Damaged starch content of the wheat flours was determined using the Megazyme Damaged Starch Kit and expressed as percentage of flour weight on a dry basis. Reducing sugar content was assayed on a water-soluble extract of wheat flour according to the procedure described by Fink, Andersson, Rosén, and Åman (2006) as follows. Wheat flour (3 g) was mixed with 100 mL of distilled water for 20 min in a water bath (50 °C). The samples were centrifuged at 4000 *g* for 5 min, and the supernatant was diluted with water to 100 mL in a volumetric flask. Reducing sugar content was determined using the Megazyme Reducing Sugar Assay Kit and presented as glucose equivalents in g/100 g flour. The content of free asparagine was analyzed according to a method described by Huang et al. (2008).

2.4. Dough preparation and baking procedure

The recipes for baking yeast-leavened and unleavened breads were modified from Mustafa et al. (2009). Ingredients and their weight proportions were wheat flour (100 g), dry yeast (0 or 1 g for unleavened or leavened bread, respectively), sodium chloride (1 g) and warm water (60 g). Wheat flour (100 g) (and yeast for leavened bread) was mixed thoroughly with 60 mL water containing 1% sodium chloride on a flour weight basis. The mixture was kneaded for 5 min to form a dough. The dough was covered with a wet cloth, allowed to leaven for 120 min, kneaded again for 1 min before baking in an electric oven at 220 °C for 25 min. Breads were cut into slices and the outer crust was removed manually. The crumb and the crust were freeze-dried, ground to pass through a 100 μm sieve, and stored at −20 °C until analysis.

2.5. Determination of reducing sugar and asparagine content of the dough

A sample of doughs before baking was freeze-dried, ground, and passed through a 100 μm sieve and used for the determination of reducing sugar and asparagine content as described in section 2.3.

2.6. Determination of acrylamide content of breads

Freeze-dried bread samples (1.00 g) were weighed into 50 mL plastic centrifuge tubes and mixed with 5 mL of methanol at room temperature for 2 min. After centrifuging at 10,000*g* for 10 min at 4 °C, the supernatant was transferred to a 15 mL plastic centrifuge tube and 0.1 mL Carrez I and 0.1 mL Carrez II solutions added to precipitate proteins. After centrifuging at 5000 *g* for 10 min at 10 °C, the supernatant was collected in a 5 mL volumetric flask and made to volume with methanol. A 1 mL aliquot of the solution was carefully evaporated to dryness under a gentle stream of nitrogen at 40 °C and the residue was dissolved in1 mL of distilled water.

For solid phase extraction (SPE) clean-up, a Thermo Carbon column was conditioned sequentially with 5 mL of methanol and 5 mL of water. Then, 1 mL of the sample extract was applied to the SPE column and allowed to pass completely through the sorbent material. The acrylamide was eluted from the SPE column with 5 mL of methanol. The eluate was collected in a 10 mL test tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was dissolved in 1 mL of ethanol and filtered through a 0.22 μm Poly vinylidene fluoride (PVDF) filter before analysis.

Acrylamide content in breads was determined using a high

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