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The effects of temperature on the crystalline properties and resistant starch during storage of white bread

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ABSTRACT

Resistant starch (RS) can form during storage of foods, thereby bestowing a variety of potential health benefits. The purpose of the current study has been to determine the influence of storage temperature and time on the crystallinity and RS content of bread. Loaves of white bread were baked and stored at refrigeration, frozen and room temperatures with analysis over a period of zero to seven days. RS determination and X-ray diffraction (XRD) were used to evaluate the influence of storage temperature and time on total crystallinity and RS content. The rate of starch recrystallisation was affected by storage temperature and time, where refrigeration temperatures accelerated RS formation and total crystallinity more than storage time at both frozen and room temperature. A strong statistical model has been established between RS formation in bread and XRD patterns, having a 96.7% fit indicating the potential of XRD to measure RS concentrations.

1. Introduction

Starch is one of the most abundant polymers on earth and is the primary energy reserve in plants (Copeland, Blazek, Salman, & Tang, 2009). Starch exists in two forms, known as amylose and amylopectin, in which both α(1→4) and α(1→6) bonds are present to link the monomer units, D-glucose. Amylopectin has a much higher average degree of polymerisation with a larger proportion of the α(1→6) bonds so it is more highly branched than amylose (Wrolstad, 2012). When individuals consume starch-based foods, enzymes particularly α-amylase hydrolyse the glycosidic bonds during digestion in both the mouth and small intestine. Eventually, glucose is produced and transported across the small intestinal wall into the bloodstream where it can either serve directly as an energy source or be converted into fat for storage (Jackson & McLaughlin, 2009).

In some cases, a portion of the starch is not enzymatically degraded into glucose and this is referred to as resistant starch (RS) (Wrolstad, 2012). The occurrence of this starch fraction was first reported by Englyst, Wiggins, and Cummings (1982). To date, five forms of RS have been identified in the scientific literature (RS1–RS5) and these differ in mechanism of resistance (Homayouni et al., 2014). RS1, often found in seeds, milled grains and legumes, is characterised by a physical barrier enveloping the starch preventing enzymatic access (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). RS2 is raw or native starch, found in certain foods particularly green bananas and high amylose corn starches (Dupuis, Liu, & Yada, 2014). RS3 occurs in foods which have been subjected to one or more heating and cooling cycles during which amylose has recrystallised and cannot be enzymatically degraded by human enzymes. It is found in pasta and potato salads and is of significant interest to the food industry as it offers the potential for manipulation of processing to enhance the RS content of foods (Sajilata, Singhal, & Kulkarni, 2006). RS4 refers to starch that has been chemically modified by the addition of functional groups, including ethers and esters, thereby limiting enzymatic access (Ashwar, Gani, Shah, Wani, & Masoodi, 2016). RS5 has been recognised relatively recently and arises when amylose complexes with free fatty acids thereby reducing digestibility (Fuentes-Zaragoza et al., 2010).

RS has been shown to act as a carbon source for fermentation by naturally occurring beneficial bacteria in the large intestine (Zaman & Sarbini, 2016; Zhang, Xu, & Jin, 2012). A number of important health benefits are associated with this fermentation which has led to increased research for methods to enable the industry to enhance RS concentrations in various starchy foods. Such benefits include the production of short chain fatty acids

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which lower the pH of the colon aiding in preventing the growth of pathogenic bacteria and encouraging the proliferation of friendly bacteria (Sharma, Yadav, & Ritika, 2008). One in particular, butanoic acid, is not only a preferred energy source for the colonic cells, but also has been linked with inducing apoptosis of tumour cells in the colon (Clarke et al., 2012). In addition to its potential contribution to bowel health, RS also has strong applications in the management of weight loss and diabetes through its low glycaemic index functionality (Srikaeo & Sangkhiaw, 2014) and hormone regulation (Zhou et al., 2006).

As RS3 is recrystallised amylose, RS may contribute to total crystallinity and hence measurement of X-ray patterns via X-ray diffraction (XRD) has potential for evaluating relative RS concentrations. XRD has also been used by numerous authors in an attempt to understand the complex mechanisms involved during staling (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014; Ribotta, Cuffini, León, & Añón, 2004). Aguirre, Osella, Carrara, Sánchez, and Buera (2011) investigated the effect of storage temperature on crystallinity using XRD, but RS content was not determined in that study. Further, Yadav (2011) examined the effects of dough formulations and baking conditions on RS content, although storage temperature was not taken into account and XRD measurements were not performed. Therefore XRD could be particularly advantageous as the current enzymatic method is expensive and time consuming. Hence, the overall aim of this investigation was not only to see the effect of storage temperature on RS formation during breadmaking but also to observe if there is a relationship between RS content and X-ray diffractogram patterns.

2. Methods and materials

2.1. Bread preparation, storage and sampling

The experiments involving preparation, storage and analysis of baked loaves were carried out in triplicate.

2.1.1. Loaf preparation

For white bread production, the ingredient proportions were: 100.0% white baker's flour, 63.0% water, 2.0% caster sugar, 2.0% canola oil, 1.7% table salt, 1.5% dried yeast and 1.1% bread improver. Note that all percentages are relative to the total flour weight. All ingredients were weighed and then mixed in a Kitchen Aid heavy duty 10 speed bench mixer (Model 5kPM50, Benton Harbour, USA). The bread flour (Manildra Group, Gladesville, New South Wales), contained 13.1% protein, 66.8% carbohydrates, 1.6% total lipid, 0.61% ash and 13.5% moisture.

Initially, mixing was carried out on a slow speed (setting 1) for 4 min followed by a further 6 min at a higher speed (setting 6), until gluten was well developed. The dough was then covered with a damp tea towel and allowed to prove at room temperature for one hour. The dough was then briefly kneaded and divided into 380 g portions and moulded into greased bread tins. The second proofing occurred in a humidifier at 37 °C for 30 min at a relative humidity of 85%. Finally, the doughs were baked initially for 10 min at 230 °C and then for a further 15 min at 200 °C.

2.1.2. Storage of bread loaves

Once baked and cooled, loaves were packed into airtight sealable bags and placed either at room, refrigeration or frozen temperatures and stored for various periods of up to 7 days. The storage conditions corresponded to temperatures that were measured as 20 °C, 3.5 °C and −17 °C, respectively. The temperatures and storage times were chosen as these conditions reflect how the majority of Australian consumers store bread.

2.1.3. Sample preparation

Samples were prepared by freeze drying, using a procedure similar to that of Buddrick, Jones, Hughes, Kong, and Small (2015) and Mihhalevski et al. (2012), with minor adaptations. Samples were taken from each representative storage sample on days 0 (day of baking), 1, 4 and 7. This was done by taking 1 cm slices from the middle of the loaf and finely dicing. Once diced, they were placed into sealable bags which were immersed into a Dewar of liquid nitrogen, instantly freezing the samples. The frozen bread samples were freeze dried (Opergon Freeze Dryer, Korea) for a period of 4 h at −55 °C. The resultant samples were ground using an IKA Universal Mill 20 fitted with a 1.0 mm sieve and the freeze dried powders were stored at −18 °C until analyses were carried out.

2.2. Sample analysis

2.2.1. X-ray diffraction

X-ray diffractograms of the subsamples were obtained using a Bruker D4 Endeavor under the following conditions: 2θ range of 5–90°, voltage of 40 kV, current of 35 mA, rate of 0.3/s and a step-size of 0.02. From the X-ray diffractograms, the degree of crystallinity (DC) was calculated using the formula used by both Mihhalevski et al. (2012) and Ribotta et al. (2004).

$$\textit{Degree of crystallinity}\ (\%) = \frac{I_c}{I_c + I_a}$$

Where:

I_c = The integrated area of the crystalline phase; and
I_a = The integrated area of the amorphous phase.

2.2.2. Moisture content

The moisture content of the freeze dried, ground bread samples was measured according to the American Association of Cereal Chemists (AACC) International air oven method. Empty aluminium dishes with lids were placed in a pre-heated oven set at 130 °C. After 1 h the dishes were removed and allowed to cool for 30 min in a desiccator containing active silica gel desiccant, then weighed on a four-figure balance. Subsamples (5 g) were accurately weighed into these preheated, cooled and weighed dishes.

The samples were then dried at 130 °C for 1 h, cooled in a desiccator for 30 min with subsequent weighing. This procedure of drying, cooling and weighing was repeated three times until a constant sample weight was achieved and then the final moisture content was determined by:

$$\textit{Moisture content}\ (\%) = \frac{\textit{Total weight} - \textit{weight postdrying}}{\textit{Sample weight}} \times 100$$

2.2.3. Resistant starch content

The RS content of the samples was determined according to the AACC International method (AACC 32-40.01), using an enzyme assay kit obtained from Megazyme International (Bray, Ireland). Subsamples (100 mg, accurately weighed) were exposed to both pancreatic α-amylase and amyloglucosidase at 37 °C for exactly 16 h in a shaking water bath.

The combination of these enzymes hydrolysed the non-resistant portion of the starch into D-glucose; further reaction was terminated by the addition of ethanol. After centrifuging and decanting (three repetitions), a pellet was obtained containing the RS. This was then dissolved in 2 M KOH in an iced water bath with vigorous magnetic stirring.

The solution was then neutralised with an acetate buffer and the starch of the redissolved pellet was hydrolysed to D-glucose using amyloglucosidase. The D-glucose was treated with the oxidase/peroxidase reagent (GOPOD) and A_{510} measured for