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Effect of storage period and temperature on resistant starch and β-glucan content in cornbread

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Abstract

The objective of this study was to assess resistant starch development and β-glucan availability, as influenced by storage time and temperature. Cornbread was baked and freeze dried at −20 °C either whole or with the crust removed (crumb). Samples were stored in airtight containers at either 20 °C, 4 °C or −20 °C, for 2, 4 or 7 days, and analysed for resistant starch, β-glucans and starch damage. Resistant starch content in whole cornbread was 3.07 g/100 g [dry weight basis (dwb)] and 2.59 g/100 g (dwb) in the crumb. These levels increased with up to 4 days of storage, particularly at 4 °C. There was a decrease in resistant starch levels after 7 days of storage, with lowest levels at −20 °C. β-Glucan levels increased with storage. Starch damage percentage decreased with storage up to 4 days. These changes may be due to structural modification of components during storage.

Keywords: Resistant starch; β-Glucans; Starch damage; Cornbread; Storage temperature

1. Introduction

The formation of resistant starch and the availability of β-glucans are reported to be influenced by food processing and storage conditions. Colonic fermentation of these dietary fibre fractions produces short chain fatty acids including acetate, propionate and butyrate (Casterline, Oles, & Ku, 1997). Resistant starch, for instance, is a preferred fermentation substrate for colonic bacteria (Hylla, Gostner, & Dusel, 1998). This renders them physiologically beneficial as they have been associated with the prevention of colon cancer and other diet-related diseases.

Maize and maize-based products have been proposed as sources of beneficial carbohydrates, such as resistant starch and β-glucans. Resistant starch commonly occurs in maize, particularly in partially milled grains as physically entrapped starch. Maize (corn) starch is a common source of resistant starch, in part due to its amylose content. Corn-breads (arepas) made from regular dent corn with 25% amylose content have been shown to have high resistant starch and high fermentability in the hind gut of rats (Grandfeldt, Drews, & Bjorck, 1993).

Processing and storage conditions have impact on the physicochemical properties of food and food components. Modifications and changes in various oligosaccharides and starches with processing have been reported. High processing temperatures result in hydrolysis of various oligosaccharides (Voragen, 1998). This may also solubilize β-glucans and make these more available.

Heated and cooled starches in processed foods, such as breads and baked goods, typically contain retrograded, modified starch, which is resistant to gastrointestinal enzymic digestion, known as Type 3 resistant starch (Liljeberg, Akerberg, & Bjorck, 1996; Mangala, Udayasankar, & Tharanathan, 1999; Rabe & Sievert, 1992; Topping & Clifton, 2001; Tovar, Melito, Herrera, Rascon, & Perez, 2002). Retrograded starch, is the most prevalent form of resistant starch as it results from food processing techniques (Escarpa, Gonzalez, Manas, Garcia-Diz, & Sauro-Calixto, 1996). Cooling results in the formation of crystalline structures that contribute to this resistance to digestion (Garcia-Alonso, Jimenez-Escrig, Martin-Carron, Bravo, & Sauro-Calixto, 1999). Moist heat processes, such as autoclaving, increase

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resistant starch levels, and multi-cycle autoclaving is used for resistant starch formation and production (Escarpa et al. 1996). Processing of starchy foods results in damage of starch granules, further affecting their water absorption capability and hence ability to gelatinize and subsequently retrograde. Muir, Birken, Jones, and O'Dea (1995) showed that intestinal effluents, following test meals of high amylose maize-bread, contained bread fragments with intact starch granules, indicating that not all of the starch is gelatinized in baking. Accessibility to enzyme digestion is influenced by the extent of damage to the starch granules with processing.

Storage temperatures have a direct impact on retrogradation and starch restructuring. The most common form of resistant starch in processed foods being retrograded starch, storage conditions that either enhance or limit retrogradation will in turn influence resistant starch formation.

While storage of baked goods such as breads and muffins, is known to be highly sensitive, as they are susceptible to starch restructuring (including retrogradation and syneresis), the extent of corresponding resistant starch formation and availability of β-glucans has not been widely reported. Furthermore, the extent of starch damage in relation to resistant starch formation has not been clearly established. The objective of this study was therefore to determine resistant starch levels in cornbread, a maize-based, baked product, and assess the effect of storage period and storage temperature on resistant starch and β-glucan levels. Extent of starch damage was assessed to determine any relationship to resistant starch levels. This will provide insights into starch and β-glucan properties in food matrices, particularly as they are influenced or modified by storage conditions.

2. Materials and methods

2.1. Sample preparation

Cornbread was prepared by an adaptation of a recipe from Blinn (1987) using the following ingredients: corn flour (200 g), baking powder (14 g), salt (4 g), eggs (96 ml), milk (159 ml), and vegetable oil (31 g). Ingredients were mixed for 60 s, and then baked at 425 °F (218 °C) for 30 min. The cornbread was cooled at ambient temperature for 45 min prior to freeze drying for 48 h. This was as indicated by Rabe and Sievert (1992), who showed that ground, dried wheat bread gave higher and possibly more measurable levels of resistant starch compared with freshly baked bread.

2.2. Determination of beta-glucan

Beta-glucan content was determined by the procedures of McCleary and Holmes (1985), McCleary and Codd (1991) and McCleary and Mugford (1992). Sample material was treated with 50% ethanol, sodium phosphate buffer (pH 6.5) was added and the samples incubated in a boiling water bath. The tubes were equilibrated at 50 °C, treated with lichenase enzyme (50 U/ml; Megazyme International Ireland Ltd) and then further incubated for 60 min at 50 °C. Acetate buffer (pH 4.0) was added and the tubes centrifuged for 10 min, after which aliquots were removed and treated with β-glucosidase (2 U/ml, Megazyme International Ireland Ltd) for another 10 min. The reaction mixture was incubated with glucose oxidase peroxidase reagent (GOPOD; Megazyme International Ireland Ltd) for 20 min and the absorbance read at 510 nm against a reagent blank.

2.3. Determination of resistant starch and soluble starch

2.3.1. General

These were determined by the procedure of McCleary and Monaghan (2002). Sample material (100 mg) was weighed out and sodium maleate buffer (pH = 6.0) containing pancreatic amylase and amyloglucosidase (3 U/ml, Megazyme International Ireland Ltd) added. Samples were thoroughly mixed and incubated for 16 h at 37 °C with agitation. The tubes were then treated with 50% ethanol, and centrifuged at 1000 *g* for 10 min. The supernatants were decanted, the pellets re-suspended in 50% ethanol, and the process repeated twice.

2.3.2. Determination of solubilized starch

The decanted supernatants from centrifugation were made up to volume in 100-ml volumetric flasks, and aliquots treated with GOPOD (Megazyme International Ireland Ltd.), for 20 min at 50 °C. The absorbance was read at 510 nm against a reagent blank.

2.3.3. Determination of resistant starch

The pellets from centrifugation were treated with potassium hydroxide (2 M) in an ice bath with stirring for 20 min. Sodium acetate buffer (pH = 3.8), and then amyloglucosidase (3300 U/ml, Megazyme International Ireland Ltd) were added and the tubes incubated for 30 mins at 50 °C. Aliquots were removed and treated with GOPOD reagent (Megazyme International Ireland Ltd). These were incubated at 50 °C for 20 min and the absorbance read at 510 nm, against a reagent blank. Glucose solution (1 mg/ml) was used as a standard.

2.4. Determination of starch damage

Percentage of starch damage in cornbread samples was determined by the procedure of Gibson, Al Qalla, and McCleary (1991) and Gibson, Kaldor, and McCleary (1993), using a reagent kit from Megazyme International Ireland, Ltd. Sample material was weighed out into centrifuge tubes, and fungal α-amylase solution (50 U/ml) added at 40 °C. The tube contents were vortexed and incubated for 10 min at 40 °C. Dilute sulphuric acid was then added and the tubes centrifuged at 1000 *g* for 5 min. Aliquots of the supernatant were removed and treated with amyloglucosidase solution (2 U), and incubated for 10 min at 40 °C. Glucose oxidase peroxidase solution was added to each tube, the mixtures incubated for 20 min at 40 °C and the absorbance read at 510 nm against a reagent blank.

2.4.1. Data analysis

Duplicate treatments were assessed. Absorbance data was converted by reference formula into g/100 g dry weight for β-glucans, resistant starch and percentage starch damage. Results are expressed as means ±standard deviation.

3. Results and discussion

3.1. Resistant starch content

Resistant starch content of cornbread crumb (crust removed) was 2.59±0.6 g/100 g dry weight, while the resistant starch content of the whole bread was 3.07±0.9 g/100 g dry weight (Table 1). This is an increase from raw corn flour with resistant starch content of 1.96 g/100 g on a dry weight basis (dwb; Garcia-Alonso et al., 1999). This also corresponds to measures of resistant starch in most flour-based breads which are generally below 2% (Englyst, Kingman, & Cummings, 1992; Liljeberg & Bjorck, 1994). The comparatively low difference between the whole bread and the crumb could possibly be due to caramelization that occurs with baking, as has been reported in wheat bread by Rabe and Sievert (1992). Resistant starch formation and its levels in processed foods is influenced by various factors, including temperature (Mangala et al., 1999). Cooling at room temperature appears to enhance resistant starch formation, while refrigeration increases resistant starch in potato, but decreases the levels in cereals (Garcia-Alonso et al., 1999).

Table 1
Resistant starch content of cornbread

Storage period	Storage temperature/conditions		
	Ambient temperature (20 °C) g/100 g[a]	Refrigerated (4 °C) g/100 g[a]	Freezer (−20 °C) g/100 g[a]
Crumb			
0 days	2.59±0.6	2.59±0.6	2.59±0.6
2 days	7.65±1.3	7.36±1.4	8.72±2.4
4 days	4.52±0.1	4.89±0.1	5.41±1.1
7 days	4.39±0.0	2.18±0.3	2.29±0.2
Whole cornbread (with crust)			
0 days	3.07±0.9	3.07±0.9	3.07±0.9
2 days	5.41±2.3	5.73±2.8	5.42±1.3
4 days	4.74±0.3	5.83±2.0	5.65±1.4
7 days	3.48±0.1	2.50±0.2	2.18±0.4

Data expressed on dry weight basis.
[a] Mean of two samples; data expressed as mean±standard deviation.

Heat-treatment followed by cooling results in the formation of resistant starch (Type 3 resistant starch), which is mainly retrograded starch (Liljeberg et al., 1996; Namratha, Asna, & Prasad, 2002; Topping & Clifton, 2001). Process pH also influences resistant starch formation of corn starch, with higher pHs of 7–10.5 yielding more resistant starch (Garcia-Alonso et al., 1999).

Incomplete gelatinization of starch during bread baking may also account for resistant starch levels as was the case when high amylose maize flour was substituted in wheat bread (Hoebler, Karinthi, Chiron, Champ, & Barry, 1999).

Storage increased the resistant starch content in crumb and whole bread, but only up to 4 days of storage (Table 1). This increase corresponds to similar increases in wheat bread in which there was an increase in resistant starch after 6 days of storage at 4 °C (Rabe & Sievert, 1992). Namratha and colleagues (2002) also showed that storage of processed ready-to-eat foods for up to 4 months increased resistant starch content, and attributed this to the formation of starch–lipid or starch–protein complexes, reducing starch digestibility. The increase in resistant starch content with storage at freezing temperatures supports findings by Garcia-Alonso and colleagues (1999), who showed that cooling gelatinized corn flour to room temperature, then freezing increased resistant starch levels.

Resistant starch formation with storage notably involves amylose re-crystallization (Namratha et al., 2002). Re-crystallization of amylopectin is much slower and is involved to a much lesser extent in resistant starch formation (Garcia-Alonso et al., 1999; Mangala et al., 1999). However, resistant starch formed by multicycle-autoclaving of potato was shown to yield up to 7.61% amylopectin, a relatively significant percentage (Escarpa et al., 1996). In breads, the crumb structure firms with amylopectin retrogradation (Mangala et al., 1999).

After 7 days of storage, there was a considerable drop in resistant starch content. The decrease in resistant starch levels with prolonged storage may be a result of reverse in retrogradation of amylopectin. While amylopectin contributes only minimally to retrogradation and resistant starch formation, this retrogradation of amylopectin is reversible, particularly with reheating (Muir et al., 1995). Storage of amylopectin gels also results in firmer, more retrograded gels than amylose gels (Mua & Jackson, 1997). The decrease in resistant starch with prolonged storage could also be due to instability of

Table 2
Soluble starch content of cornbread

Storage period	Storage temperature/conditions		
	Ambient temperature (20 °C) g/100 g[a]	Refrigerated (4 °C) g/100 g[a]	Freezer (−20 °C) g/100 g[a]
Crumb			
0 days	60.3±1.9	60.3±1.9	60.3±1.9
2 days	52.1±3.7	52.6±1.6	53.3±6.8
4 days	47.0±0.6	48.3±3.4	45.8±6.6
7 days	48.8±1.7	49.1±0.0	44.6±0.0
Whole cornbread (with crust)			
0 days	64.9±1.7	64.9±1.7	64.9±1.7
2 days	53.2±0.0	56.1±2.3	52.3±0.0
4 days	47.4±0.0	46.1±1.7	45.4±3.4
7 days	51.6±5.4	49.3±7.8	42.2±0.0

Data expressed on dry weight basis.
[a] Mean of two samples; data expressed as mean±standard deviation.

Table 3
β-Glucan content of cornbread

Storage period	Storage temperature/conditions		
	Ambient temperature (20 °C) g/100 g[a]	Refrigerated (4 °C) g/100 g[a]	Freezer (−20 °C) g/100 g[a]
Crumb			
0 days	0.36±0.00	0.36±0.00	0.36±0.00
2 days	0.38±0.05	0.42±0.00	0.48±0.03
4 days	0.35±0.01	0.41±0.03	0.37±0.00
7 days	0.45±0.04	0.49±0.06	0.46±0.01
Whole cornbread (with crust)			
0 days	0.35±0.01	0.35±0.01	0.35±0.01
2 days	0.46±0.00	0.48±0.00	0.48±0.08
4 days	0.45±0.05	0.45±0.00	0.43±0.10
7 days	0.49±0.04	0.52±0.05	0.46±0.07

Data expressed on dry weight basis.
[a] Mean of two samples; data expressed as mean±standard deviation.

amylopectin crystals formed during baking. Another possible explanation could be general unavailability of starch as a result of the formation of starch–protein or starch–lipid complexes, in conjunction with the depletion of water needed for recrystallization. Availability of water is essential in the formation of resistant starch, as it is involved in re-crystallization of amylose (Rabe & Sievert, 1992). Mangala and colleagues (1999) and Garcia-Alonso et al. (1999), showed that moisture content facilitates swelling which in turn would influence resistant starch formation. Prolonged storage of baked goods (for up to 7 days in this case), particularly at refrigeration temperatures, often results in syneresis, which occurs as a consequence of retrogradation. The occurrence of syneresis is not an absolute predictor of resistant starch formation, particularly in maize starch (Tovar et al., 2002). Resistant starch formation and degradation during storage may therefore be influenced by other structural associations.

3.2. Soluble starch content

Soluble starch content of cornbread crumb was 60.3±1.9 g/100 g, and 64.9±1.7 g/100 g in whole cornbread (Table 2). This decreased with storage. Soluble starch was quantified by the procedure of McCleary and Monaghan (2002), as starch hydrolyzed after 16 h incubation at 37 °C with pancreatic amylase and amyloglucosidase, and this therefore represents digestible or non-resistant starch. The decrease in soluble starch could therefore be as a result of the restructuring and rearrangement of amylose to become resistant to amylase hydrolysis, which hence requires the subsequent alkaline hydrolysis step in resistant starch analysis. Resistant starch has been shown to be primarily recrystallized amylose (Rabe & Sievert, 1992; Tovar et al., 2002). In addition, other structural associations with other components in the food matrix could modify the availability of starch to enzyme hydrolysis. Storage of maize flour, for instance, has been shown to result in fatty-acid–amylose complexes that decrease amylose solubility (Mestres, Nago, Akissoe, & Matencio, 1997). Similar associations in cornbread may have reduced soluble starch content over the duration of storage.

3.3. β-Glucan content

There was an increase in β-glucan content of cornbread with storage (Table 3). The distribution of β-glucans could occur in various dietary fibrr fractions, as is the case in mushrooms (Manzi & Pizzoferrato, 2000). Processing and storage, which involve structural breakdown and restructuring, may therefore increase their

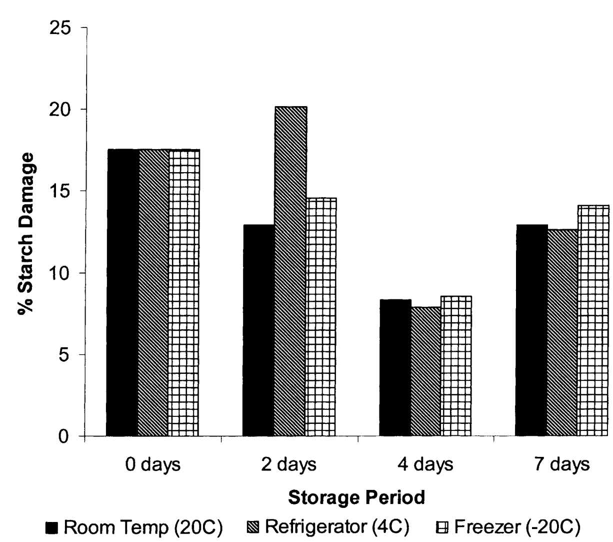

Fig. 1. Mean percentage starch damage in cornbread crumb.

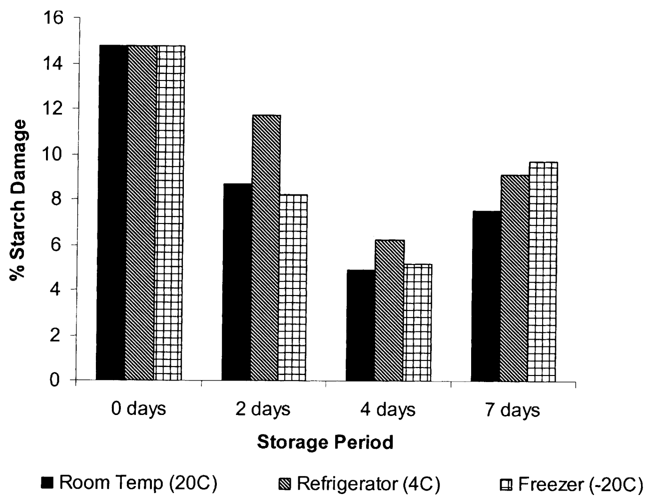

Fig. 2. Mean percentage starch damage in whole cornbread.

availability and solubility. β-Glucan availability and extractability in barley, for instance, is shown to be influenced by various factors, including processes such as steaming and autoclaving that involve water, as well as β-glucanase activity and agitation (Izydorczyk, Storsley, Labossiere, MacGregor, & Rossnagel, 2000). High temperatures result in degradation of barley β-glucans (Wallwork, Jenner, Logue, & Sedgley, 1998), while dry heat processes, such as roasting, increase extractability of β-glucans in oat grains (Zhang, Doehlert, & Moore, 1998). Prolonged storage would therefore appear to increase extractability and measurability of β-glucans in cornbread.

3.4. Starch damage

There was a decrease in the proportion of starch damage in cornbread, up to 4 days of storage (Figs. 1 and 2). This decrease in starch damage (in conjunction with changes in resistant and soluble starch levels) may be indicative of retrogradation and inaccessibility to enzyme digestion of the starch, and possible re-association of gelatinized starch granules. Baking temperatures often do not guarantee complete gelatinization of many granular starches. Bread made from high-amylose maize grains found in intestinal effluents showed that not all of the starch is gelatinized and remains intact, even with baking and processing (Muir et al., 1995). These granules contribute to resistant starch levels. It has been suggested that high-amylose maize granules require much higher temperatures than the regular temperature of baking for adequate gelatinization. There was an increase in starch damage proportions after 7 days of storage. This increase could result from structural breakdown and degradation of starches and hence the concomitant decrease in resistant and soluble starch levels.

4. Conclusion

Resistant starch and soluble starch contents in cornbread are considerably affected by storage duration and storage temperature. This may be due to structural modifications in the cornbread over time, as there is depletion of resistant starch and soluble starch after seven days of storage. β-Glucan levels increase with storage. This is possibly a result of increased extractability and solubility as a consequence of structural changes in bread with storage, regardless of storage temperature. Starch damage decreases with storage as resistant starch increases (up to 4 days of storage). There is however, an increase in starch damage with decrease in resistant and soluble starch after 7 days of storage. It would appear therefore, that possible structural changes during storage influence resistant starch in cornbread. This indicates a possible relationship between starch structure, susceptibility to digestion and resistant starch content. Storage of grain-based baked foods, such as cornbread, for up to 4 days would therefore increase resistant starch levels, but these are depleted with prolonged storage, regardless of storage temperature. β-Glucan extractability and availability are, however, enhanced.

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