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The effect of fermentation and addition of vegetable oil on resistant starch formation in wholegrain breads

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ABSTRACT

Resistant starch has potential health benefits but the factors affecting its formation in bread and baked products are not well studied. Here, the formation of resistant starch in wholemeal bread products was evaluated in relation to the processing conditions including fermentation time, temperature and the inclusion of palm oil as a vitamin source. The effects of each the factor were assessed using a full factorial design. The impact on final starch content of traditional sourdough fermentation of wholemeal rye bread, as well as the bulk fermentation process of wheat and wheat/oat blends of wholemeal bread, was also assessed by enzyme assay. Palm oil content was found to have a significant effect on the formation of resistant starch in all of the breads while fermentation time and temperature had no significant impact. Sourdough fermentation of rye bread was found to have a greater impact on resistant starch formation than bulk fermentation of wheat and wheat blend breads, most likely due the increased organic acid content of the sourdough process.

1. Introduction

For many years starch was considered to be a completely digestible carbohydrate. However, this assumption was recently proved incorrect when starch remnants were detected in human faeces after consumption of a high starch meal (Englyst, Kingman, & Cummings, 1992). It is now known that significant amounts of starch escape digestion and absorption in the small intestine of healthy individuals and can be fermented in the large intestine where it encourages the growth of health-promoting bacteria, reduces pH and increases the production of butyric acid, which has been shown to have potential health benefits (Gao et al., 2009), through fermentation; this type of starch is known as resistant starch (RS) (Perera, Meda, & Tyler, 2010).

RS has now been identified as a bioactive component of food that can be formed in cereal fermentations (Perera et al., 2010). It involves the conversion of carbohydrates to alcohol and carbon dioxide, and/or organic acids, using yeast, bacteria or a combination of both, under anaerobic conditions. It is hypothesised that the time-intensive bulk fermentation and sourdough bread production processes promote retrogradation of starch via realignment of amylose and amylopectin chains, thereby increasing RS formation in the finished products. The fermentation process and its potential nutritional benefits may therefore contribute to an enhanced health status, as well as reduction of starch digestibility (Minervini et al., 2010; Poutanen, Flander, & Katina, 2009).

The increase in consumer demand for healthy, high quality foods in recent years has led to a growth in the use of new technologies as well as novel ingredients including RS. Wholegrain flour contains more RS than white (refined) flour and the health benefits of consumption of RS in the form of wholegrain products include its role as a prebiotic (Fuentes-Zaragoza et al., 2011; Slavin, 2013), a reduction in the incidence of chronic diseases, particular diabetes and colon cancer (Okarter & Liu, 2010) and weight loss (Higgins et al., 2011).

Previous studies have indicated that processing conditions can affect the formation of RS by influencing the gelatinisation and retrogradation of normal starch (Johansson, Siljestrom, & Asp, 1984; Thompson, 2000). It has also been reported that one can make a physically functional RS ingredient by the application of physical processes to a starch suspension (Augustin, Sanguansri, & Htoon, 2008). It is also possible to increase the RS content in foods by modifying certain processing conditions including pH, heating temperature and time, number of heating and cooling cycles as well as by freezing and drying (Sajilata, Singhal, & Kulkarni, 2006).

To the best of our knowledge, an investigation into the formation of RS using either wholegrain flours for the bulk fermentation

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of wheat doughs or rye sourdough fermentation has not previously been reported. The objective of the present study was to investigate the effect of fermentation of doughs prepared with selected wholegrain cereal meals on the levels of RS during breadmaking; this included the optimisation of suitable fermentation conditions, including time and temperature in relation to various blends of cereal grains. Since fats and oils are commonly used to enhance volume and softness of wholegrain bread the effect of incorporating different amounts of palm oil on RS levels was also investigated.

2. Materials and methods

2.1. Preparation of bread samples

2.1.1. Milling process

For all bread varieties, grains were milled to provide wholemeal flour on the day of the experiment. The mill used was a Grain Master Whisper Mill bench top unit (Retsel Dandenong, Victoria, Australia), which uses upright blades spinning at high speed (10,000 rpm) to produce a relatively fine meal with small particle size and a large surface area for microbiota to interact with, thus increasing the fermentability of the meal.

2.1.2. Optimisation of fermentation time and temperature

In a preliminary experiment to establish the fermentation time for the bulk fermented bread freshly prepared wheat and wheat/oat blends doughs were placed in glass beakers, plastic wrapped and placed in an incubator at 37 °C (the proving temperature used in commercial bakeries) or at room temperature (23 °C). The three fermentation times for further study were selected as those when the prepared dough started to increase in size, after ~3 h (minimum time), when it started to collapse, which was after ~7 h and a midpoint of five h. These fermentation times were then used in a full factorial design to study the effects of fermentation time, temperature and palm oil content on levels of RS formation. The settings used in the design are shown in Table 1. The design was set up and analysed using Minitab software (version 16, Minitab Pty, Sydney, NSW, Australia) and the centre points of each data set were used to estimate reproducibility of the method and to check whether the response surface had curvature, calculated at 5 h and 30 °C respectively. The temperatures, but not the fermentation times, were applied to the rye sour dough fermentation. The fermentation time for rye sour dough was set at 24 h (which is the minimum time needed for traditional rye sourdough fermentation).

2.1.3. Preparation of breads

The dough ingredients for the wheat and wheat-/oat blend flours were the same: 100% wheatmeal, 70% water, 2%, 5%, or 8% red palm oil, 2% salt, 0.2% instant dry yeast (note: all percentage values are relative to the total flour weight). These were first mixed using a Kitchen Aid heavy duty bench mixer with 10 different speeds (Model 5KPM50, Benton Harbor, USA). Mixing was carried out initially at slow speed (setting 2) for 4 min and followed by fast speed (setting 4) for 6 min until full dough development was achieved – defined as the time when the dough could be readily removed from the dough hook and the mixing bowl (Suas, 2008). The wheatmeal dough was mixed and then bulk fermented for 5 h at a temperature of 30 °C. The dough was weighed out into 180 g sections and placed into bread tins prior to the final proof at 37 °C for 45 min. Baking was at 230 °C for 10 min followed by 15 min at 200 °C.

The bread making procedure for rye differed from that for wheatmeal since the first step involved sourdough fermentation. For this, 35% of the total rye meal weight was fermented with 10% starter culture. This step is necessary due to the different biochemistries of rye and wheat flours. Wheat amylases are generally not heat-stable and break down at temperatures over 40 °C, so can not affect the wheat glutens that give wheat bread its structure. In contrast, rye amylase remains active up to 50 °C (Ase, 2005), and rye gluten is not particularly stable. In sourdough fermentation the acidic *Lactobacillus* culture and an acid-tolerant yeast strain are added to the flour to lower the pH which inactivates the rye enzymes and helps gelatinize starches in the dough matrix. The production of starter culture involved a 24 h incubation of rye meal dough with the ratio of rye meal to water 1:1, activation with 1% of the ripe sourdough, and this was repeated every 24 h over 72 h to allow the microbial community to develop fully; this was assessed by the presence of a characteristic sourdough aroma (Salim ur et al., 2006).

The rye bread formulation consisted of 90% rye meal, 10% wheatmeal, 100% water, 2% salt and 1% instant dry yeast. Again, all percentage values are relative to the total flour weight. Three treatment levels of red palm oil were used (2%, 5% and 8%). Following the 24 h incubation of the sourdough, the remaining 65% flour and the other ingredients were incorporated into the dough which was mixed for 10 min at slow speed (speed setting 2) This was then divided into 250 g portions and placed into bread tins prior to the final proofing stage which was at 37 °C for 45 min. Baking was then carried out at 230 °C for 10 min followed by a further 15 min at 200 °C.

2.1.4. Freeze drying of samples

All samples of dough and baked breads were frozen immediately after baking at −30 °C in a blast freezer and placed in a controlled freeze dryer (VirTis SP Industries, Gardiner, Montana, USA). Each freeze dried samples was ground in a mortar to form a powder and samples were stored in air-sealed containers at −18 °C prior to analysis.

2.2. Characterisation of samples

2.2.1. Moisture content

The moisture content of samples was measured according to the American Association of Cereal Chemists (AACC) International air oven method (AACC International, 2014a). Briefly, empty aluminium moisture dishes were placed into a pre-heated oven set at 130 ± 3 °C. After 1 h, the empty dishes were taken from the oven and cooled in a desiccator containing active silica gel desiccant for a period of 30 min and then weighed. Sub-samples (~5 g) were weighed into the pre-weighed dishes. The dishes were placed into the oven and dried at 130 ± 3 °C for 1 h. The process of drying, cooling and weighing was repeated three times so that a constant weight was attained for each bread sample.

2.2.2. Determination of resistant starch

The analysis of RS was undertaken using an enzymatic assay (Megazyme International, Bray, Co. Wicklow, Eire) following the procedure outlined by the manufacturer. The samples were incubated in a shaking water bath with pancreatic α-amylase and amy-

Table 1
Levels of the factors used for the 2 level full factorial design.

	Fermentation					
	Time (h)		Temperature (°C)		Palm oil %	
Actual values and coded values						
Low	3	−1	23	−1	2	−1
High	7	+1	37	+1	8	+1
Centre points	5	0	30	0	5	0