Journal of Cereal Science 59 (2014) 3–8

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

The influence of fermentation processes and cereal grains in wholegrain bread on reducing phytate content

Oliver Buddrick*, Oliver A.H. Jones, Hugh J. Cornell, Darryl M. Small

School of Applied Sciences, RMIT University, PO Box 2476, Melbourne, VIC 3001, Australia

ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form
6 November 2013
Accepted 14 November 2013

Keywords:
Wholegrain
Dough fermentation
Phytate
Phytic acid

ABSTRACT

Wholegrain bread is generally thought of as being more healthy than white bread due to it having a higher content of dietary fibre, vitamins (especially vitamin B and E) and many important minerals. However, wholegrain bread also contains high levels of phytate (myo-inositolhexakisphosphate, InsP-6) which may bind desirable nutrients, preventing their absorption in the gut and thereby reducing the nutritional value of the end product. In order to evaluate factors influencing phytate levels, the effects of fermentation and selected wholemeal flours from rye, oats and wheat were investigated. Phytate levels were assessed using a spectrophotometric assay based on the measurement of iron with 2,2′-bipyridine. Phytate decreased in freshly ground wholegrain flour dough during the fermentation process with time of fermentation being the most important factor. Fermentation temperature was found to make only a small difference to the process of phytate reduction. Since the potential benefits of wholemeal breads incorporating various grains (e.g. oats and rye) are increasingly evident, this research has important implications for human health.

1. Introduction

The milling of grain to give white flours reduces the levels of many nutrients as well as phytate (*myo*-inositolhexakisphosphate, InsP-6). In contrast, wholegrain products are known to have both high phytate levels and high nutrient content (Fardet, 2010; Stevenson et al., 2012). Wholegrain products also have a wide range of benefits to human health, for example they are associated with reduced risk of many chronic diseases including heart disease and diabetes (Jones, 2011; Mellen et al., 2007). Wholegrain cereals and legumes are the major sources of dietary phytate intake. The interaction of phytate and dietary minerals and beneficial health effects of phytate have been the subject of a review (Kumar et al., 2010). Phytates can chelate and bind minerals, resulting in insoluble complexes that may lead to a decrease in mineral absorption and bioavailability, and therefore the removal of phytates from baked goods has long been considered desirable. Evidence demonstrating a diverse range of benefits to health and wellbeing is now accumulating (Kumar et al., 2010).

Fermentation has been shown to decrease the amount of phytate in wholegrain cereals (Liukkonen et al., 2003; Sanz-Penella et al., 2012). For example, Leenhardt et al. (2005) assessed changes in phytate hydrolysis brought about by sourdough fermentation or exogenous organic acid addition using an *in vitro* trial. They found that a slight acidification (pH ~ 5.5) of the dough with either sourdough or via the addition of lactic acid caused a significant phytate reduction (~35%).

A direct comparison of the reduction in phytate using wholegain for the bulk fermentation of wheat doughs and rye sourdough fermentation has not previously been reported. Therefore, the objective of the present study has been to thoroughly investigate the effect of fermentation of doughs prepared with selected wholegrain cereal meals on the levels of phytate during breadmaking. This includes the optimisation of suitable fermentation conditions, including time and temperature in relation to various blends of cereal grains. The effect of different amounts of palm oil on phytate levels was also investigated as fats and oils are commonly used to enhance volume and softness of wholegrain bread.

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 bench top unit (Grain Master Whisper Mill, Retsel Dandenong, Victoria, Australia), which uses upright blades spinning at high speed

* Corresponding author. Tel.: +61 399252124; fax: +61 399253747.
E-mail address: s3190486@student.rmit.edu.au (O. Buddrick).

0733-5210/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jcs.2013.11.006

(10,000 rpm) to produce a relatively fine meal with small particle size and a large surface area. This gives a greater area that microbiota can interact with and thus increases the fermentability of the meal.

2.1.2. Preparation of bread

For all baking trials, a central composite design was developed using Minitab software (version 16). The centre points of each were used to estimate reproducibility of the method and to check whether the response surface had curvature.

For the dough preparation, 100% wheatmeal, 70% water, 2, 5, 8% red palm oil, 2% salt, 0.2% instant dry yeast (NB: All percentage values are relative to the total flour weight) were first mixed using a bench mixer with 10 different speeds (Kitchen Aid Heavy Duty, Model 5KPM50, Benton Harbor, USA) at slow speed (setting 2) for 4 min and followed by fast speed (setting 4) for 6 min until full dough development was achieved – corresponding to 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 (180 g) 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 in order to bake the bread evenly without causing an increase in crust colour. The oat blend breads were also prepared using the procedure described above to facilitate direct comparisons.

The rye bread making procedure differed from that for wheatmeal bread since the first step involved sourdough fermentation where 35% of the total rye meal weight was fermented with 10% starter culture. 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 three days after which time the microbial community had developed fully, assessed by the presence of a characteristic sourdough aroma. The rye bread formulation consisted of 90% rye meal, 10% wheatmeal, 100% water, 2, 5 and 8% red palm oil, 2% salt and 1% instant dry yeast; again all percentage values are relative to the total flour weight. Following the 24 h incubation of the sourdough, the remaining 65% flour and the other ingredients were incorporated. The dough was mixed for 10 min at slow speed (speed setting 2), and then dough was scaled (250 g) 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.

2.1.3. Sampling

During the making of the wheatmeal bread and wheat blends, samples were taken from the dough at four stages; after mixing, then after fermentation, after the final proof (just before entering the oven) and finally, after baking. In the rye bread making procedure, the samples were taken after inoculation, fermentation, mixing, final proof and baking.

2.1.4. Freeze drying

All samples were immediately frozen at −40 °C in a blast freezer and placed in a controlled freeze dryer (VirTis SP Industries Company, Gardiner, USA) to obtain low-moisture-content samples for further analysis. Freeze dried samples were ground in a mortar to form 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 AACC International air oven method (AACC International, 2010b). 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 accurately weighed into pre-weighed dishes. The dishes containing the samples were then placed into the oven and dried at 130 ± 3 °C for 1 h. The process of the drying, cooling and weighing was repeated three times, until a constant weight was attained.

2.2.2. Determination of phytate

The phytic acid was determined by the method described by Haug and Lantzsch (1983), in which the phytic acid is precipitated with an iron-III solution of known iron content and the decrease in iron in the supernatant is taken as a measure of phytic acid content. Phytate reference solution containing phytic acid sodium salt hydrate from rice (type V 94% purity and ~6% water) was obtained from Sigma–Aldrich, Sydney, Australia. Stock solutions were prepared with 1.3 mg/mL phytic acid. Ferric ammonium sulphate solution was prepared by dissolving 0.2 g $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 100 mL 2 mol/L HCl and the volume was made to 1000 mL with distilled water. The 2,2′-bipyridine solution was prepared by dissolving 1.0 g 2,2′-bipyridine and 1.0 mL thioglycollic acid in distilled water and making up the volume to 1000 mL.

For the analysis of the cereal grains (wheat, rye and oat) and bread doughs as well as bread, 0.5 g of sample was extracted with 50 mL of 0.5 mol/L HCl for 3 h followed by centrifugation for 30 min at 3000 rpm. The extract (0.5 mL) was pipetted into a 15 mL centrifuge tube. Then 1 mL of ammonium iron (III) sulphate solution was added. The tubes were incubated in a boiling water bath for 30 min then cooled in ice water to adjust to room temperature. Once the tubes had reached room temperature, 1.5 mL of (1% v/v) 2,2′-bipyridine solution was added. The absorbance was immediately measured at 519 nm against distilled water and the test method was calibrated with the reference solutions prepared by diluting the stock solution with 0.2 mol/L HCl in a range of 0.1–1.0 mL (3.12–31.2 μg/mL phytate phosphorus).

2.2.3. Determination of pH and total titratable acidity (TTA)

The pH values of all samples were measured according to the AACC International hydrogen ion activity (pH)-electrometric method (AACC International, 2010a). For this, 15 g of samples were weighed and agitated with 100 mL distilled water using a bath mixer until an even suspension, free of lumps, was obtained. The suspension was rested (25 °C) for 30 min, then agitated continuously to keep particles in suspension, then rested for a further 10 min. The supernatant liquid was placed into an electrode vessel and the pH value was immediately determined using a potentiometer and electrode that had been calibrated against known buffer solutions. After the pH value was obtained and recorded, 0.1 mol/L NaOH for rye bread varieties and 0.01 mol/L for the wheat bread varieties was slowly added from a burette and stirred constantly until a constant pH of 6.6 was obtained. The volume of NaOH (mL) used is reported as the TTA.

3. Results

3.1. pH profile and TTA in sourdough

The acidity of the sample during various processing stages affects the phytate levels. The pH and phytate content for 100% wholemeal, a blend of wholemeal 70% wheat and 30% oat and rye bread during various processing stages are summarized in Tables 1–3, respectively.