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Original Research Article

Thiamin fortification of bread-making flour: Retention in bread and levels in Australian commercial fortified bread varieties

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ABSTRACT

In Australia, thiamin is mandatorily added to bread-making flour with the main purpose of fortification and reducing the prevalence of Wernicke–Korsakoff syndrome. This study aims to measure the retention of added thiamin through laboratory-scale bread processing and provide an update on thiamin contents in commercially fortified bread and flatbread varieties since the introduction of the program in 1991. Even though baking caused degradation loss of thiamin (approximately 16%), the laboratory fortified white bread had a 25% higher thiamin content than its corresponding flour, and laboratory fortified wholemeal bread showed a 16% increase ($p < 0.05$). Thiamin levels in commercially fortified bread and flat bread varieties ranged between 0.24 and 1.9 mg/100 g (dry weight basis). It can be suggested that most of the bread varieties were made from flour fortified at the minimum mandated level (0.64 mg/100 g flour). Samples of flat bread varieties (white without yeast, wholemeal with yeast and wholemeal without yeast) showed low thiamin levels (0.24–0.49 mg/100 g, dry weight basis). The results suggest that the flat bread varieties were likely made from either commercially under-fortified flour or unfortified general-purpose flour, as only bread-making flour is fortified with thiamin.

1. Introduction

Thiamin, a common term for 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium, is a water soluble B_1 vitamin (Combs, 2012). In plant based foods, thiamin predominantly occurs in free form, while in animal based foods it exists in phosphorylated forms as thiamin monophosphate, thiamin diphosphate and thiamin triphosphate. Thiamin diphosphate, also known as thiamin pyrophosphate, is the biologically active form. It plays an important role as a co-factor for several key enzymes involved in the carbohydrate metabolism and defence mechanisms (Martin et al., 2003). Thiamin triphosphate is essential in neural function (Combs, 2012). Thiamin found in food is sensitive to pH and high temperatures (Butterworth, 2003). It is stable between pH 2.0 and 4.0, but unstable in alkaline solutions (Mihhalevski et al., 2013). Several studies reported that heat during baking caused loss of endogenous thiamin that ranged from 20% to 56% (Batifoulier et al., 2005; Martinez-Villaluenga et al., 2009; Mihhalevski et al., 2013).

Whole grain cereals are typically rich in thiamin. However, the scutellum and germ are removed during milling, which results in the production of grains and flour with lower thiamin content. Beri-beri, a condition of severe thiamin deficiency, has been documented to be prevalent in countries where polished rice was a staple (Lonsdale, 2006). Severe thiamin deficiency leads to cardiovascular and neurological disease (Harper, 2006; Rapala-Kozik, 2011) including Wernicke–Korsakoff Syndrome (WKS), which is commonly associated with alcohol abuse (Harper et al., 1989). Australia had a higher incidence of WKS (2.1%) than other comparable countries (Truswell, 2000). Based on this evidence, in 1991 the mandatory thiamin fortification program was introduced with the purpose of reducing the prevalence of WKS. This is unlike other countries, where fortification programs are being implemented to replace the thiamin loss due to milling (Truswell, 2000). The Australian fortification program mandates the addition of 0.64 mg (minimum) thiamin hydrochloride per 100 g bread-making flour.

The objective of this study was to determine the actual thiamin levels in selected commercially fortified bread and flat bread varieties being sold in the Sydney metropolitan area (Australia). Specifically, the study measured thiamin in bread samples made from laboratory-scale fortified flour. The results presented here would provide an update on thiamin levels after 22 years of the

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fortification program, as thiamin values are not being declared on the nutrition labels of most bread and flat-bread varieties.

2. Materials and methods

2.1. Reagents and plant materials

Thiamin hydrochloride was obtained from DSM Nutritional Products (Kaiseraugst, Switzerland). Potassium ferricyanide used to oxidise thiamin hydrochloride was purchased from Ajax Finechem (Sydney, NSW, Australia). HPLC grade acetonitrile and methanol were obtained from Honeywell Burdick and Jackson (Sydney, NSW, Australia). Enzyme taka-diastase (100 U/mg, product no 86247) from *Aspergillus oryzae* was acquired from Sigma–Aldrich (Sydney, Australia). Purified water (conductance$\leq$ 0.1 μS/cm) was sourced from a Milli-Q-system (Millipore, Bedford, MA, USA).

Commercial thiamin-fortified flour (white and wholemeal), unfortified white flour, wheat bran and wheat germ were provided by a flour miller in Australia and stored in 20 L air-tight containers. Bread ingredients including instant dry yeast, canola oil, table salt and bread improver were purchased from a local supermarket in Sydney.

2.2. Laboratory-made fortified flour

Wholemeal flour was prepared by substituting 20% of unfortified white flour with wheat bran (10%) and wheat germ (10%). To unfortified flour (white and wholemeal) thiamin hydrochloride was added at the minimum fortification level of 0.64 mg/100 g flour. Both types of flour were then homogenised using a rotary shaker for at least 18 h. Flour samples (white flour, $n = 4$ and whole meal flour, $n = 4$) were then obtained for thiamin analysis and moisture determination according to AOAC 925.09-1925 method (AOAC, 2000). The laboratory-fortified flour was used for bread making.

2.3. Laboratory-made fortified bread

Bread loaves (wholemeal, $n = 2$ and white, $n = 2$) were made from the laboratory fortified flour previously described. Baking of these bread types was repeated on different day to obtain a total of 8 bread loaves. Initially, bread ingredients consisting of 1 kg flour, 15 g table salt, 15 g bread improver, 20 g canola oil, 13 g instant dry yeast and 600 g water (Chandra-Hioe et al., 2013b) were mixed using Vorwerk Thermomix (Wuppertal, Germany). The dough was hand-kneaded, proofed for 60 min at 37 °C and then baked at 200 °C in a pre-heated oven for 30 min. Samples were collected after mixing (dough), 60 min of proofing and stored in the freezer. On the day of baking, the samples including bread were cut and ground (Vorwek Thermomix, Wuppertal, Germany) for moisture determination and thiamin analysis. Ingredients used for bread making in this study (excluding salt, oil and water) were also analysed for thiamin contents.

2.4. Bread sampling

A variety of bread was selected according to the A.C. Nielsen Top Brands Report 2009 that achieved sales exceeding 66 million Australian dollars since 2003 (Nielsen, 2010). Bread varieties from leading brands including private labels ($n = 84$ loaves) were purchased in August 2013 from the supermarkets and local bakeries from 2 different suburbs of Sydney. They were then grouped into 10 types: white bread ($n = 14$ loaves), white bread with added soy fibre ($n = 4$ loaves), white bread containing soy fibre and resistant starch ($n = 3$ loaves), wholemeal bread ($n = 9$ loaves), multigrain bread ($n = 16$ loaves), white flatbread with yeast ($n = 6$ packs) and without yeast ($n = 6$ packs), white flatbread (wheat fibre added) with yeast ($n = 3$ packs), wholemeal flatbread with yeast ($n = 6$ packs) and without yeast ($n = 6$ packs). In the flatbread category sample composites were prepared from Lebanese bread, pita and wraps. On the day of purchase, 6 slices were taken from each pack (different brands within the same category), then diced, ground and homogenised using Vorwerk Thermomix (Wuppertal, Germany) to obtain bread composites.

2.5. Preparation of standard solutions

The standard stock solution (100 μg/mL) was prepared by dissolving thiamin hydrochloride in Milli-Q water. Fresh working standard solution (5 μg/mL) was made on the day of use by dilution of the stock solution in Milli-Q water. For the purpose of detection, thiochrome was prepared by oxidising the working standard solution (4 mL) with alkaline potassium ferricyanide (3 mL). The concentrations of the calibration curve (10 points) ranged between 0.025 and 1.75 μg/mL.

2.6. Thiamin extraction

Extraction of thiamin was performed according to a published method (Ndaw et al., 2000). Samples (at least in duplicate) of instant dry yeast (0.5 g), bread improver, wheat bran, wheat germ, flour, dough and bread (2.5 g) were weighed in the centrifuge tubes. After adding 0.1 M hydrochloric acid (25 mL) samples were placed in a shaking water bath (100 °C) for 30 min, and then cooled down. Hot acid digestion was carried out to denature protein complexes and release protein-bound thiamin. The pH of the samples was then adjusted to 4.5 with 2.5 M sodium acetate, followed by taka-diastase (250 mg) treatment in a 37 °C shaking water bath for 18 h. Taka-diastase was used to dephosphorylate thiamin to its free form for quantitative analysis (Ndaw et al., 2000; Tang et al., 2006). Milli Q water was consequently added into the samples to make up to 50 mL volume. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C and the supernatants were filtered through a 0.45 μm PTFE syringe filter (Grace Davison, Chicago, IL, USA). Similar to the working standard solutions, to 4 mL of the filtered samples 3 mL of alkaline potassium ferricyanide (1:25 of 1% (w/v) potassium ferricyanide in 15% (w/v) sodium hydroxide) was added, vortexed and equilibrated for exactly 1 min (Arella et al., 1996).

Solid phase extraction was performed according to Arella et al. (1996) using a vacuum manifold (Supelco, St. Louis, MO, USA). Initially, Sep-Pak C_{18} cartridges (Waters, Milford, MA, USA) were activated with 5 mL of Milli-Q water and conditioned with 2 mL of methanol. The oxidised samples (7 mL) were then passed through the cartridges. After washing with 10 mL of 0.05 M sodium acetate (pH 6.0), the analyte was eluted with 8 mL of 70% (v/v) methanol in Milli-Q water. The eluents were then made up to 10 mL and filtered prior to chromatographic analysis.

2.7. Liquid chromatography

The liquid chromatography system (Shimadzu Co., Kyoto, Japan) was coupled with a fluorescence detector (RF-10A XL) and employed a Synergi Hydro-RP column (150 mm × 4.6 mm, 4 μm, Phenomenex, Torrance, CA, USA). The method was adopted from Arella et al. (1996) with some modifications. The mobile phase consisted of 70% 0.05 M sodium acetate (pH 6.0) and 30% methanol (isocratic), the flow rate and the injection volume were 1 mL min^{-1} and 20 μL, respectively. The column temperature was set at 30 °C. The excitation and emission wavelengths used for detecting thiochrome were set at 365 nm and 435 nm, respectively.