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Folate fortification of white and whole-grain bread by adding Swiss chard and spinach. Acceptability by consumers

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ABSTRACT

The aim of this work was to study the effect of bread-making on the content of endogenous folate of white and whole-grain bread fortified with either Swiss chard or spinach at 20 g/100 g and 40 g/100 g measured by HPLC (H4-folate and 5-CH3-H4-folate); and to assess the sensory acceptability of folate-fortified breads compared to non-fortified breads. The fortification of breads with 20 g/100 g and 40 g/100 g vegetables significantly ($p < 0.001$) increased the total folate content (from 19.9 to 57.9 μg/100 g in white bread and from 37.4 to 75.5 μg/100 g in whole-grain bread). Moreover, 40 g/100 g Swiss chard- and spinach-fortified breads obtained higher scores in overall acceptability than 20 g/100 g fortified or control bread for both white and whole-grain breads. The consumption of two servings (56 g each) per day of 40 g/100 g fortified bread would meet the daily folate requirements by 14.3–21.8% in adults and 9.6–14.5% in some special states like women of child-bearing age.

1. Introduction

Folate is a generic way to name all natural iso-forms of vitamin B9, while folic acid refers to the fully oxidized synthetic form. Folate accepts one-carbon units from donor molecules and is involved in many metabolic pathways, such as methyl group biogenesis and synthesis of nucleotides, vitamins, and some amino acids (Ames, 1999). However, only plants and microorganisms are able to synthesize folate *de novo*, so both animals and humans have to intake it through their diet. Folate is widely spread in nature, mainly in vegetables, liver, and cereals. Despite this, lack of folates in the diet is one of the most common nutritional deficiencies in the world and has serious consequences on human health (Herbison et al., 2012). Traditionally, folate deficiency in humans has been associated with macrocytic or megaloblastic anaemia. Nowadays, however, it is known that marginal folate deficiency and change of its metabolism are associated with health problems such as cancer, cardiovascular diseases, and neural tube defects in newborns (Wang et al., 2007). To decrease the risk of neural tube defects in newborns, the increase in folate intake for woman in the periconceptional period is of vital importance to maintain an optimal folate status by considering the relationship between folate intake and blood folate status (Stamm & Houghton, 2013). The daily recommended intake (DRI) of folate in the European Union (EU) is set at 200 and 600 μg/day for adults and women in the periconceptional period, respectively (IOM, 2006). I must be noted that in the case of American population, as just as few 10% of women of childbearing age have folate levels in red blood cells enough to decrease the risk of neural tube disorders (Dietrich, Brown, & Block, 2005).

There are a number of ways to increase folate intake in the population: 1) consumption of foods naturally rich in folates, such as leafy vegetables, fruits and berries, beans, whole grain products, and liver; 2) use of folic acid supplements; and 3) consumption of foods fortified with synthetic folic acid or natural folates.

To date, no country in Europe has implemented mandatory folic acid fortification of flour, although it has been recommended by the UK Food Safety Authority, mainly to assist women of childbearing age with meeting folic acid intake recommendations for neural tube defect risk reduction. In Spain, the recommended dietary intakes (RDI) for folic acid have been set at 400 μg/day for women of childbearing age, 600 μg/day for the second half of pregnancy, and 500 μg/day for women who are breastfeeding (Moreiras, Carbajal, Cabrera, & Cuadrado, 2008).

Abbreviations: H4-folate, tetrahydrofolate; 5-CH3-H4-folate, 5-methyl tetrahydrofolate.

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Methods such as folic acid fortification have proven to be very useful in reducing health problems associated with a low folate intake. The addition of folic acid to food products in the USA and Canada has reduced the prevalence of neutral tube defects by 25% and 46%, respectively (De Wals et al., 2007). However, it was recently shown that high-level intake of folic acid (the folate isoform chemically synthesized) might have some adverse health effects (Smith, 2007). A too high intake of folic acid may mask the diagnosis of a vitamin B12 deficiency and possibly lead to irreversible neurologic damages but this is not expected to occur with the naturally occurring level of folates found in food (Morris, Jacques, Rosenberg, & Selhub, 2007). A recent report also has suggested that folic acid supplementation during pregnancy is associated with increased risks of respiratory infections in newborns (Håberg, London, Stigum, Nafstad, & Nystad, 2009). These researchers acknowledged that folic acid may act differently than naturally occurring folates and that regular intake of folic acid supplements could result in circulating unmetabolised folic acid, which in turn may have unknown effects on immune cells by epigenetic mechanisms (Kalmbach et al., 2008). For this reason, there recently has been growing interest in the fortification of foodstuff with natural folates. Cereals and cereal products are generally recognized as important food sources of folate; however, significant losses of this vitamin occur during the bread-making process (Gujska & Majewska, 2005).

Thus, the aim of this work was to study the effect of processing (fermentation and baking) on the final content of endogenous folates in white and whole-grain bread, both non-fortified and biofortified with either Swiss chard or spinach at two different percentages (20 g/100 g and 40 g/100 g). We also assessed the sensory acceptability of the folate-biofortified breads compared to the non-fortified breads (control breads).

2. Materials and methods

2.1. Food samples and bread-making

Food samples used in the present work are listed in Table 1. The staples (wheat flour, wheat bran and compressed baker's yeast (Levamax, Spain) containing approximately 70% water) and bread were provided by the bakery "La Colegiala" (El Raal, Murcia, Spain), while fresh spinach and Swiss chard were purchased at a local store.

The processing starts mixing all staples (including flour, baker's yeast, salt and the appropriate amount of fresh vegetables to provide 20 g/100 g and 40 g/100 g in each case) making the dough that was kneaded and then, samples were collected. The bread rolls were allowed to stand for 30 min, and the final desired form was given to the bread. Then, four breads were baked in the same day, for each sample, in a deck oven (MIWE condo, Arnstein, Germany) at 200–225 °C until the temperature in the centre of the product reached 96 °C, which occurred after 20 min for 60 g dough rolls. Finally, the rolls of bread were cooled in darkness at 8 °C for 2 h ending with processing.

For each kind of bread, four independent bread rolls were selected to determine the folate content of each one within the next 2–4 h once bread was cooled. A total of 70 bread rolls of each type were made for the sensory evaluation test. Both white bread and whole-wheat bread were provided by the same bakery, as final product, in order to compare the results obtained from the vegetable-fortified breads with the same products without folate fortification.

2.2. Folate standards

Individual folate standards, (6R,S)-5,6,7,8-tetrahydrofolic acid calcium salt (H4-folate) and (6R,S)-5-methyl-5,6,7,8-tetrahydrofolic acid sodium salt (5-CH3-H4-folate) were obtained from Dr. Schirck's Laboratory (Jona, Switzerland).

2.3. Extraction and deconjugation of folates from samples

Folates were extracted from the staples and breads following the procedure described by Konings (1999) and Pfeiffer, Rogers, and Gregory (1997). Briefly, one gram of sample was mixed with 25 mL of extraction buffer (50 mmol/L CHES, 50 mmol/L HEPES, containing 2 g sodium ascorbate/100 mL and 10 mmol/L 2-mercaptoethanol, pH 7.85) under a nitrogen atmosphere. The extraction mixtures, in screw-capped tubes, were placed in a boiling water bath for 10 min, cooled on ice, and homogenized using an Omnimixer Model 17106 (OMNI, Inc., Waterbury, CT, USA). Then, the pH was adjusted to 4.9 with 60 mmol/L HCl, and the samples were made up to a final volume of 50 mL with extraction buffer. Enzymatic deconjugation and purification of samples were carried out following the methodology described by Vahteristo, Ollilainen, Koivistoinen, and Varo (1996). Briefly, an aliquot of 5 mL was incubated for 3 h at 37 °C under a nitrogen atmosphere with 1 mL of hog kidney conjugase prepared from fresh pig kidneys, as described by Gregory, Sartain, and Day (1984). In addition, 1 mL of α-amylase preparation (20 mg/mL in 1 g Na ascorbate/100 mL) from *Aspergillus oryzae* (Sigma Chemical Co., St. Louis, MO, USA) was filtered through a 0.22 μm microfilter and jointly added with conjugase to the bread and flour samples. To inactivate the enzymes, the samples were boiled at 100 °C for 5 min and then cooled on ice. The samples were then filtered through 0.45 μm pore size, 25 mm diameter nylon disposable filters (Whatman, Florham Park, NJ, USA) and purified in strong anion-exchange (SAX) cartridges (3 mL/500 mg of quaternary amine N^+, counter ion Cl^-, no. 52664-U, Bellefonte, PA, USA) connected to a Supelco 12-port vacuum manifold (Supelco, Bellefonte, PA, USA). First, the cartridges were conditioned with 3 mL of n-hexane (twice), methanol, and Milli-Q water and then equilibrated with 3 mL of purification buffer

Table 1
Food samples and the ingredients used in the present study.

Food	Ingredients (g/100 g)
White bread	Wheat flour (49), dough (14.7), baker's yeast (0.9), salt (0.9)
Whole-grain bread	Wheat flour (42.7), wheat bran (8.5), dough (12.8), baker's yeast (0.85), salt (0.8)
20 g/100 g Spinach-fortified white bread	Wheat flour (35.2), dough (10.5), baker's yeast (0.7), spinach (20), salt (0.9)
40 g/100 g Spinach-fortified white bread	Wheat flour (35.2), dough (10.5), baker's yeast (0.7), spinach (40), salt (0.9)
20 g/100 g Swiss chard-fortified white bread	Wheat flour (35.2), dough (10.5), baker's yeast (0.7), Swiss chard (20), salt (0.9)
40 g/100 g Swiss chard-fortified white bread	Wheat flour (35.2), dough (10.5), baker's yeast (0.7), Swiss chard (40), salt (0.9)
20 g/100 g Spinach-fortified whole-grain bread	Wheat flour (31.8), wheat bran (6.3), dough (9.5), baker's yeast (0.6), spinach (20), salt (0.6)
40 g/100 g Spinach-fortified whole-grain bread	Wheat flour (28.2), wheat bran (5.6), dough (8.4), baker's yeast (0.5), spinach (40), salt (0.5)
20 g/100 g Swiss chard-fortified whole-grain bread	Wheat flour (31.8), wheat bran (6.3), dough (9.5), baker's yeast (0.6), Swiss chard (20), salt (0.6)
40 g/100 g Swiss chard-fortified whole-grain bread	Wheat flour (28.2), wheat bran (5.6), dough (8.4), baker's yeast (0.5), Swiss chard (40), salt (0.5)

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