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Research paper

Folates in quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus* sp.) and buckwheat (*Fagopyrum esculentum*): Influence of cooking and malting

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ABSTRACT

Effects of processing on the contents of five folate vitamers in quinoa, amaranth and buckwheat were analysed using a trienzymatic extraction method followed by LC–MS/MS. Total folate (TF) content, corresponding to the sum of folic acid (FA), 5-methyltetrahydrofolate (5-MTHF) and 10-formyltetrahydrofolate (10-CHOTHF) expressed as folic acid equivalent, in raw quinoa, amaranth and buckwheat were 309 ± 8.07, 228 ± 24.2 and 153 ± 12.4 μg/100 g dw, respectively, being dominantly 5-MTHF. Boiling and steaming reduced the TF in amaranth by 58% and 22%, respectively, whereas up to a 10–15% increase was observed in quinoa. Boiling and steaming did not significantly alter the TF content in buckwheat although significant changes were observed in some individual folate vitamers. Malting, on the other hand significantly increased TF content in amaranth by 21% (276 ± 14.2 μg/100 g dw) and buckwheat by 27% (193 ± 20.0 μg/100 g dw), whereas no significant change in quinoa was observed. Based on the EFSA recommendations, a portion of amaranth and quinoa (either boiled, steamed or malted) may contribute up to more than 25% of the dietary reference value for folates, whereas buckwheat may contribute only 14% when cooked and 19% when malted. Results demonstrate that quinoa, amaranth and buckwheat are good sources of folates, regardless of processing.

1. Introduction

Folate is a generic term used for different water-soluble vitamins of the B-complex group. It is an essential micronutrient needed for optimal health, growth, and development. Dietary folate deficiency is common around the world, and deficiency of this vitamin is directly or indirectly associated with other metabolic disorders and pathophysiological conditions such as inflammatory bowel disease and celiac disease (Wani et al., 2008). Pregnancy and lactation also increase the risk of folate deficiency due to the high requirement to support optimal growth and development of the fetus (Stamm and Houghton, 2013). According to the European Food Safety Authority, the average requirement (AR) for healthy adult men and women is 250 μg of dietary folate equivalent (DFE)/day and the Population Reference Intake (PRI) is 330 μg DFE/day (European Food Safety Authority, 2014). Dietary reference values are in agreement with controlled studies showing that folate intake of around 200–300 μg/day may be sufficient to maintain folate concentration in serum and red blood cells (European Food Safety Authority, 2014).

Naturally occurring folates are present in a wide range of foods. However, some staple diets, especially those consisting of polished cereal grains and tubers, are very poor in folate but can be improved by the addition of legumes or green leafy vegetables (FAO and WHO, 2001). Thus, to achieve the recommended folate intake, efforts to fortify staple foods with folates have been explored. Alternatively, consumption of foods naturally high in folates could also serve as a good strategy to achieve recommended folate intake.

Pseudocereals, such as quinoa, amaranth and buckwheat, differ from true cereals (poaceae botanic family) like wheat and rice due to their differences in seed physiology and absence of gluten (Alvarez-Jubete et al., 2010; Saturni et al., 2010). Hence, amaranth, quinoa and buckwheat have been recommended by the World Gastroenterology Organization for celiac disease patients and as a base ingredient for baby foods due to their low allergenicity (WGO, 2012). However,

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Table 1
Conditions in malting process.

	Steeping		Germination		Kilning	
Pseudocereals	Time (Hours)	Temperature (°C)	Time (Hours)	Temperature (°C)	Time (Hours)	Temperature (°C)
Amaranth	8	30	24	23	4	42
Quinoa	8	30	24	23	6	42
Buckwheat	10	30	40	23	8	42

although much has been said about the nutritive value of these pseudocereals, their folate content has not been elaborately reported in the literature. Since folates are unstable to heat, light and pH conditions, the content of folates in processed and stored food may be lower than in raw food. Several studies previously reported negative effects of cooking processes to folate in foods, especially in legumes and vegetables (Delchier et al., 2013, 2012; Stea et al., 2007). This effect is more pronounced with longer processing times and higher temperatures (Witthoft et al., 1999). On the contrary, other means of processing, like malting, have been reported to increase the physicochemical accessibility and compounds that intensify bioavailability of micronutrients (Platel and Srinivasan, 2016). Malting has also been shown to decrease the content of antinutrients such as phytates (Hotz and Gibson, 2007). The germination phase, which proceeds after the malting process in food products, has been reported as a way to increase the nutritional and bioactive profile of cereal grains (Hefni and Witthöft, 2011; Nelson et al., 2013; Shohag et al., 2012). Furthermore, malted grains are recommended as an ingredient for medicinal foods to increase their nutritional density without further processing (Kaur, 2009). In large-scale service systems, for example hospitals, knowledge of the content of folate in foods can be critical because of special or increased requirements for many patients. The content of folate after processing must be taken into consideration when calculating the real total folate intake from food. Thus, the effect of boiling, steaming and malting on the folate profile of these pseudocereals was also investigated. Lastly, the contribution of each portion of cooked or malted quinoa, amaranth and buckwheat to the recommended daily intake of folates was assessed.

2. Materials and methods

2.1. Reagents and chemical standards

Deionized water employed in all solutions was obtained by a Milli-Q purifier (Millipore, Eschborn, Germany). Folic acid (FA), 5-methyltetrahydrofolate (5-MTHF), tetrahydrofolate (THF), 5-formyltetrahydrofolate (5-CHOTHF) and 10-formyltetrahydrofolate (10-CHOTHF) standards were supplied by Schircks Laboratories (Jona, Switzerland). Stable isotope labelled ($^{13}C_5$) FA and ($^{13}C_5$) 5-MTHF, used as internal standard (I.S.), were purchased from Merck-Eprova (Schaffhausen, Switzerland) and stored at −80 °C. LC–MS grade acetonitrile and formic acid were from Merck Millipore (Germany). α-Amylase (Type I-A, from porcine pancreas, 23.5 units/μL, EC no. 232-565-6) and protease (Type XIV, from Streptomyces griseus, ≥3.5 units/mg, EC no. 232-909-5), ammonium bicarbonate, DL-Dithiothreitol (DTT) and L-ascorbic acid were purchased from Sigma (St. Louis, MO USA). Rat whole serum, lyophilized was purchased from Invitrogen Corporation (Waltham, MA USA).

2.2. Samples and sample preparation

All pseudocereal samples white quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus sp.*) and buckwheat (*Fagopyrum esculentum*) seeds were purchased from local organic markets in Lisbon, Portugal, between March 2014 and September 2015. Sampling plan for primary samples (three with 0.5 to 1 kg each), laboratory samples and test portions was designed according to the protocol desbribed in Mota et al. (2016) to guarantee representativeness.

Sample preparation for cooking methods (boiled and steamed) were conducted three times according to Mota et al. (2016). Briefly, 50 g of raw pseudocereals were boiled for 15 min in 100 °C using a Thermomix® TM31 food processor (Vorwerk, Germany) or steamed for 30 min using the same food processor.

For the malting process steeping phase, 50 g of each sample, weight in triplicate, was mixed with distilled water in a ratio of 1:10 and the samples were kept at 30 °C for 48 h until the moisture content is constant (with CV < 0.01%). The germination process took place at 23 °C for 48 h until the germination rate (number of germinated seeds/number of non germinated seeds) was maximum. Steeping and germination were performed in dark conditions using a temperature controlled oven (Infors, Ecotron, Switzerland). The sprouts were dried in a food dehydrator (Excalibur®, California, USA) at 42 °C for 10 h until moisture content were below 5%. Table 1 showed the malting optimization process conditions for all pseudocereals under study.

Raw, boiled, steamed and malted samples were milled in a GRINDOMIX GM 200, high speed grinder from Retsch (Germany). After milling process samples were pooled and stored separately in aluminum foils in vacuum bags at −20 °C until use. For each pooled sample four test portions were take to perform the assays (quadruplicate, n = 4).

2.3. Moisture analysis

Moisture content was determined according to AOAC 952.08 (2000). The method was performed using a dry air oven (Heraeus Instruments, Hanau, Germany) at 102 °C ± 2 °C for 2 h, using test portions (3 g) of each sample, until constant weight.

2.4. Calibration standards

Folates stock standard solutions for FA, 5-MTHF, THF, 5-CHOTHF and 10-CHOTHF (100 μg/mL) were prepared under subdued light in ammonium bicarbonate buffer 0.5 mM, pH 7.2 with 0.5% of DTT and 1% of ascorbic acid as antioxidants. Working solutions were prepared by diluting the stock solution (25 μg/mL, pH 7.2) with water and reading in a UV-spectrometer (Thermo Scientific™ Evolution 300, England) in a wave length of 200–400 nm. The concentration of each folate was calculated at a maximum absorvance of 307 nm, and a molar absorptivity of 250 M, according to the Eq. (1).

$$A\lambda = \varepsilon \times c \times L \quad (1)$$

where Aλ is absorbance in a specific wavelength, ε is molar absorptivity for the dissolved substance, c is the molar concentration and L is light path length in centimeters. An acceptance criteria of 80% to 120% was established for the standard solutions.

2.5. Folate extration

The extraction of folates was performed with a tri-enzyme treatment (α-amylase, protease and rat serum). One gram of each pseudocereal was diluted in ten milliliters of ammonium bicarbonate buffer (0.5 mM, pH 7.2 with 0.5% of DTT and 1% of ascorbic acid), containing all I.S. (40 μg/mL of stable isotope labelled ($^{13}C_5$) FA to quantify FA and ($^{13}C_5$) 5-MTHF to quantify 5-MTHF, THF, 5-CHOTHF and 10-CHOTHF). After