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Nutritive value and chemical composition of pseudocereals as gluten-free ingredients

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Abstract
The only treatment available for patients with coeliac disease is a lifelong elimination of food products containing gluten. The gluten-free products currently available in the market are considered of low quality and poor nutritional value. In the present study, the pseudocereals amaranth, quinoa and buckwheat were studied as potential healthy ingredients for improving the nutritional quality of gluten-free breads. The pseudocereal seeds and pseudocereal-containing gluten-free breads were evaluated in terms of their protein, fat, total starch, dietary fibre, ash and mineral content as well as their fatty acid composition. The pseudocereal containing gluten-free breads showed significantly higher levels of protein, fat, fibre and minerals than the control bread. The attributes of these breads conform to the expert's nutritional recommendations for the gluten-free diet and gluten-free foods. These results suggest that the pseudocereals amaranth, quinoa and buckwheat can represent a healthy alternative to frequently used ingredients in gluten-free products.

Keywords: *Bread, coeliac disease, chemical composition, gluten-free, nutritive value*

Introduction

Coeliac disease (CD) is becoming an increasingly recognized disorder (Niewinski 2008). This disease is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains (wheat, barley, rye and possibly oats) in genetically susceptible individuals (Fasano and Catassi 2001). The consumption of gluten in CD causes self-perpetuating mucosal damage, whereas full mucosal recovery is observed in most patients upon elimination of gluten from their diets (Fasano and Catassi 2001). As a consequence of the intestinal mucosal injury in CD, gastrointestinal symptoms caused by malabsorption and maldigestion, as well as malnutrition, are common manifestations of the disease (See and Murray 2006). However, the symptoms of CD are presented in many different forms including non-specific symptoms such anaemia, disturbance in the bowel habit or osteoporosis (McGough and Cummings 2005).

CD is today known to be universal and affects all races. It is estimated that its mean prevalence is 1–2% of the general population (Rodrigo 2006). The only treatment to date for CD is the strict lifelong elimination of gluten from the diet (Niewinski 2008). In most patients diagnosed with CD, a gluten-free diet will lead to a symptomatic and

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histologic recovery of the disease and reduced risk of complications such as nutrient deficiencies, bone disease and lymphoma (See and Murray 2006).

To date, nutrition therapy for CD has centred on food allowed/not allowed in a gluten-free diet and little emphasis has been placed on the nutritional quality of the gluten-free diet (Thompson 2000; Thompson et al. 2005). Only a small number of studies have been conducted to assess the nutritional adequacy of the gluten-free diet and, although it has been shown to prove adequate within the first few years of treatment, uncertainty remains as to its long-term nutritional adequacy (Bardella et al. 2000; Hallert et al. 2002). In a study conducted over 10 years by Hallert et al. (2002) to assess the vitamin status of a series of coeliac patients living on a gluten-free diet, one-half of the patients showed signs of vitamin deficiency, which the authors attributed to dietary habits and food choices. Another study (Ciacci et al. 2002) showed how adults with CD following a strict gluten-free diet had significantly lower weight, body mass index, fat and lean body mass than control subjects. In addition, the bone mineral content in females diagnosed as coeliacs later in life was also significantly lower than in non-coeliac subjects. The authors also reported that the diet of these patients was unbalanced and had a higher percentage of calories from fat and less from carbohydrates. The authors attributed the inadequacy of the gluten-free diet to dietary habits and food products. Recently, Thompson (1999, 2000) studied the nutritional quality of gluten-free products and concluded that many of these products contain lower levels of thiamine, riboflavin, niacin, folate and iron compared with the wheat-containing products they are intended to replace. These findings are to be expected, as cereal based gluten-free breads and baking mixes usually consist of refined flours and/or starches, and in contrast to their wheat-containing counterparts, gluten-free products are not typically fortified (Thompson 2000). In addition, in a recent dietary survey conducted in the United States to assess the diets of adults with CD who were following a strict gluten-free diet, Thompson et al. (2005) reported inadequate intakes of fibre, iron and calcium in 46%, 44% and 31%, respectively, of the women studied. These findings suggest that the nutritional quality of the gluten-free products may be of concern, and point to the need for an improvement in the quality of the gluten-free products currently available on the market (Thompson 2000).

The pseudocereals amaranth, quinoa and buckwheat are currently emerging as healthy alternatives to gluten-containing grains in the gluten-free diet. Not only they are naturally gluten-free, they are also high in a wide range of nutrients (Kupper 2005; Pagano 2006). Cereal-based food products containing amaranth, quinoa and buckwheat as composites with wheat flour are already commercially available (pasta, noodles, breakfast cereals, biscuits, and breads) but few of these products are gluten free.

Recent studies in the area of gluten-free cereal-based products have mainly concentrated on the improvement of the structural properties of gluten-free bread (Gallagher et al. 2003, 2004; Moore et al. 2004; Schober et al. 2005). In the present study, the viability of the pseudocereals amaranth, quinoa and buckwheat as ingredients in gluten-free baking was studied with the ultimate aim of improving the nutritional quality of gluten-free breads. The nutrient composition of the pseducocereal seeds (i.e. before baking) and pseudocereal-containing gluten-free baked breads was analysed by means of chemical methods, and the nutritional adequacy of the formulations was assessed in relation to current dietary guidelines for

CD. Moreover, the information revealed in this study on the nutritional aspects of the pseudocereal seeds and breads is also applicable to the general population and need not necessarily be limited to CD patients.

Materials and methods

Seed materials

Amaranth seeds (*Amarantus caudatus*, grown in Peru) and Quinoa seeds (*Chenopodium quinoa*, grown in Bolivia) were obtained from Ziegler & Co., Wunsiedel, Germany. Buckwheat seeds (*Fagopyrum esculentum* Moench, grown in China) were sourced by Munster Wholefoods, Farranfore Killarney, Ireland. Wheat grains (*Triticum aestivum* L.), variety Raffles, grown in Ireland were provided by Gold Crop, Dublin, Ireland.

All seeds were delivered cleaned from dust and any other contaminants. Quinoa seeds were pre-processed by the manufacturer to partially remove the saponins and dust by washing, centrifuging and drying.

The samples were kept in insulated paper bags inside plastic drums in a dry and cool room until analysis. Prior to bread preparation and/or chemical analysis, the samples were ground using a Cemotec 1090 sample mill (FOSS Tecator, Hoganas, Sweden).

Bread ingredients

Pseudocereal and wheat seeds (as above), sprouted buckwheat achenes (as below), rice flour (S&B Herba, Orpington, Kent, UK), potato starch (Healy Chemicals Ltd, Dublin, Ireland), wheat flour (Odlum Group, Dublin, Ireland), 100% vegetable oil (Homestead, Dublin, Ireland), sunflower oil (Euro Shopper, Dublin, Ireland), bakers fat (Irish Bakels Ltd, Dunshaughlin, Co. Meath, Ireland), linseed oil (FMD Ltd, Burnaby, British Columbia, Canada), xanthan gum (AllInAll Ingredients, Dublin, Ireland), fresh yeast (Yeast Product, Dublin, Ireland), salt (Imeos Enterprises, Runcorn, Cheshire, UK) and caster cane sugar (Tate & Lyle, London, UK) were used.

Sprouted buckwheat seeds, buckwheat achenes (as above), were soaked and germinated using a Micro Malting Machine (Joe White Malting Systems, Perth, Australia). During the steeping step, 1 kg buckwheat seeds were subjected to alternating wet and dry cycles of 3 h duration over a 24-h period, and the temperature was kept at 15°C. The steeped grains were then germinated at 18°C and the germination time was 96 h. During steeping and germinating, the seeds were turned every 30 min. Germinated seeds were then freeze-dried (Ima Edwards, Dongen, The Netherlands) and kept frozen at −20°C until analysis.

Preparation of breads

The different bread formulations are presented in Table I.

The batters/doughs were prepared as follows:

(i) *Gluten-free batter*: dry ingredients were mixed together for 1 min using an A120 Hobart mixer (Hobart, Hobart Food Equipment, Sydney, Australia) at speed 1, yeast was dissolved in the water and added to the dry ingredients together with the oil, and the batter formed was mixed for a further minute. After scraping the base of the bowl, the batter was further mixed for 2 min at speed 2.

(ii) *Wheat dough*: the yeast was dissolved in water, added to the rest of the ingredients and mixed (A120 Hobart mixer; Hobart) for 3 min at speed 3. Optimal mixing time was recorded from the Farinograph (Brabender, Duisberg, Germany).

Batter or dough (for gluten-free breads and wheat breads, respectively) was then scaled into pup loaves tins (65 g) and placed in a proofer (Koma, Roermond, The Netherlands) for 45 min at 35°C and 80% relative humidity. The loaves were baked in a deck oven (Tom Chandley Ovens, Manchester, UK) at 220–225°C for 20 min. They were then cooled to room temperature, sliced and freeze-dried (Ima Edwards).

Chemical analysis

Macronutrient analysis of the seeds and baked loaves. Protein was determined by the combustion method based on the Dumas principle using a nitrogen analyser (FP-328 Leco Instrument; Leco Corporation, St Jodeph, Michigan, USA). Blank and the standard compound ethylenediamine tetraacetic acid (9.57% N) were run prior to the samples. Combustion of the samples (30 ± 2 mg) took place in a sealed furnace at 1,150°C. The nitrogen to protein conversion factors used were 5.85, 5.96 and 5.70 for amaranth, quinoa and buckwheat seeds, respectively, and 5.7 for wheat grain (Bonafaccia et al. 2003b; Guzman-Maldonado and Paredes-Lopez 1998; Berghofer and Schoenlechner 2002; Ragaee et al. 2006).

Moisture was measured following a procedure based on the ICC method 110.1 (ICC 1976) using a Brabender moisture oven (Brabender).

Fat was quantified by acid hydrolysis according to the AOAC method 922.06 (AOAC 1990) with slight modifications. Briefly, 2 g sample were placed in a graduated cylinder, 5 ml ethanol were added, followed by 15 ml diluted hydrochloric

Table I. Bread formulations.

			50% pseudobreads			100% pseudobreads	
Ingredient (%)	GFC bread	WC bread	A bread	Q bread	B bread	100%Q bread	SpB bread
Wheat flour	–	✓	–	–	–	–	–
Rice flour	✓	–	✓	✓	✓	–	–
Potato starch	✓	–	–	–	–	–	–
Amaranth seeds	–	–	✓	–	–	–	–
Quinoa seeds	–	–	–	✓	–	✓	–
Buckwheat seeds	–	–	–	–	✓	–	✓
Wheat grain	–	✓	–	–	–	–	–
Buckwheat sprouts	–	–	–	–	–	–	✓
Yeast	✓	✓	✓	✓	✓	✓	✓
Sugar	✓	–	✓	✓	✓	✓	✓
Salt	✓	✓	✓	✓	✓	✓	✓
Xanthan gum	✓	–	✓	✓	✓	✓	✓
Vegetable oil	–	–	✓	✓	✓	–	–
Sunflower oil	✓	–	–	–	–	–	–
Linseed oil	–	–	–	–	–	✓	✓
Bakers fat	–	✓	–	–	–	–	–
Water	✓	✓	✓	✓	✓	✓	✓

acid (250 ml concentrated HCl diluted in 110 ml distilled water). The cylinders were then placed in a water bath (70°C) overnight. The fat in the digested sample was then extracted with equal volumes (25 ml) of ether and petroleum spirit on three consecutive extractions, solvents removed by evaporation on a water bath, and the fat was determined gravimetrically.

Ash was determined according to Kent-Jones and Amos (1967).

Total starch content was established by the amylogluscosidase/α-amylase method according to AOAC 996.11 (AOAC 2000) using a Megazyme Total Starch assay kit (Megazyme Int. Ireland Ltd, Bary, Co. Wicklow, Ireland).

The total dietary fibre was determined using the enzymatic gravimetric procedure according to AOAC Method 985.29 (AOAC 1990).

Fatty acid analysis of the seeds and baked loaves. The fatty acid methyl esters were prepared from the milled whole seeds, breads and oils according to Garces and Mancha's method (1993) and were analysed by gas chromatography (GC). The GC analysis was performed on a Star 3400 cx Varian GC/MS (Varian Inc., Palo Alto, California, USA) equipped with a flame ionization detector using a BPX70 GC column (SGE Europe Ltd, Milton Keynes, UK) of dimensions 120 m × 0.25 mm × 0.25 μm. The temperature gradient programme used was as follows, initial temperature 50°C, hold time 1 min, 20°C/min to reach 160°C, 4°C/min to 220°C, hold time 5 min, 4°C/min to 240°C, hold time 10 min. The carrier gas was hydrogen at a flow rate of 2 ml/min. During the analysis, the temperatures of the injector and detector were maintained at 270°C and 300°C, respectively. Fatty acids were identified by comparison with the standards Fatty Acid Methyl Ester Mix (C14–C22), Linoleic Acid Methyl Ester Isomer Mix and Linolenic Acid Methyl Ester Isomer Mix purchased from Sigma-Aldrich Chemical Co. (St Louis, MI, USA).

Mineral analysis of the seeds and baked loaves. The mineral content of the samples was analysed by atomic absorption spectrometry in previously dry-ashed samples as detailed in *Analytical Methods for Atomic Absorption Spectrometry* (Perkin-Elmer 1994). Analyses were performed on a 3110 Perkin-Elmer Atomic Absorption Spectrometer (The Perkin-Elmer Corporation, Norwalk, CT, USA) with standard conditions for each of the elements as described by the instrument manufacturer (Perkin-Elmer 1994).

Statistical analysis

All analyses were conducted in triplicate. Results were reported as means ± standard deviation. Statistical analysis was conducted for each of the measured traits by one-way analysis of variance, and the means were separated by the Tukey–Kramer test using the Statistics Toolbox of the software Matlab 7.6 R2008a (Mathworks, Nattick, Massachusetts, USA). Differences of $P < 0.01$ were considered significant.

Results

Macronutrient analysis of the seeds and baked loaves

Seeds. Results for the macronutrient analysis of amaranth, quinoa, buckwheat and wheat seeds are presented in Table II.

Table II. Chemical composition of amaranth, quinoa, buckwheat and wheat seeds.

Seed	Protein[a]	Fat	Total starch	Dietary fibre	Ash
Amaranth	16.5 ± 0.3	5.7 ± 0.3	61.4 ± 0.8	20.6 ± 1.1	2.8 ± 0.0
Quinoa	14.5 ± 0.3	5.2 ± 0.1	64.2 ± 1.3	14.2 ± 0.6	2.7 ± 0.0
Buckwheat	12.5 ± 0.3	2.1 ± 0.1	58.9 ± 1.3	29.5 ± 1.2	2.1 ± 0.0
Wheat	12.0 ± 0.1	2.5 ± 0.1	63.0 ± 1.4	17.4 ± 1.2	1.5 ± 0.0

Data presented on a percentage dry-weight basis ± standard deviation. [a]Nitrogen-to-protein conversion factors are 5.85, 5.96, 5.70 and 5.70 for amaranth, quinoa, buckwheat and wheat milled whole seeds, respectively.

The protein content was significantly higher for amaranth (16.5%) and quinoa (14.5%) seeds compared with wheat (12.0%). The fat content for amaranth and quinoa seeds was 5.7% and 5.2%, respectively, more than twice that of wheat (2.5%). Buckwheat, however, was the leanest of the seeds studied, with a fat content of 2.1%. This seed also had the highest dietary fibre content ($P<0.01$), with a measured value of 29.5%. The dietary fibre content of wheat was 17.4%, higher than quinoa but lower than that of amaranth ($P<0.01$). The ash content was significantly higher for all pseudocereal seeds in comparison with wheat (1.5%), where the highest values obtained were for amaranth (2.8%) and quinoa (2.7%, $P<0.01$).

A compilation of some previously published data by other authors on the chemical composition of similar seeds is presented in Table III.

The values obtained in the present study are comparable with the published data (Becker et al. 1981; Teutonico and Knorr 1985; Pedersen et al. 1987; Singhal and Kulkarni 1988; Chauhan et al. 1992; Koziol 1992; Ranhotra et al. 1992; Prakash et al. 1993; Bressani 1994, 2003; Guzman-Maldonado and Paredes-Lopez 1998; Steadman et al. 2001a; Berghofer and Schoenlechner 2002; Taylor and Parker 2002; Bonafaccia et al. 2003b), where any slight variations observed can most probably be attributed to differences in the varieties of the seeds tested and, to a lesser extent, the climatic conditions and cultural practices (Koziol, 1992; Bressani, 1994).

For amaranth, in the present study the protein and fibre contents were higher, and the fat content was lower, in comparison with the average composition for *A. caudatus* published by Bressani (1994). In his review, Bressani reports protein content ranges of 13.9–18.1% and 11.7–14.8% (N $\times$5.85, dry weight basis) among amaranth species and between selections of the same species, respectively. Variability in the fat content is also relatively high, with content ranges of 7.2–13.1%, 8.8–12.1% and 10.6–14.6% (dry weight basis) for 25 selections of *A. caudatus*, seven selections of *Amarantus hypochondriacus* and three selections of *Amarantus cruentus*, respectively. The starch content for the main amaranth species was found to be in the range between 48% and

Table III. Published chemical composition of amaranth, quinoa and buckwheat seeds (Koziol 1992; Bressani 1994; Bonafaccia et al. 2003b).

Seed	Protein	Fat	Carbohydrate	Starch	Crude fibre	Dietary fibre	Ash
Amaranth	14.9	9.1	70.3	na	2.8	12.0	2.9
Quinoa	15.0	6.3	70.5	na	3.8	13.3	3.8
Buckwheat	11.7	2.9	na	55.8	na	27.4	2.2

Data presented on a percentage dry-weight basis ± standard deviation. na, data not available.

69%, and data for total dietary fibre, although limited, showed variability among species (7.1–16.4%) and within selections of the same species (7.6–16.5% for *A. caudatus*). The ash content between species falls in the range 2.6–4.4%, with little variability within species. The global means for the proximate analyses of different Andean varieties of quinoa (Koziol 1992) are also presented in Table III. The average ash content reported by Koziol (1992) was higher than the value obtained for the quinoa samples used in this study (3.8% versus 2.7%) and was the only difference found between the two quinoa data-sets. The chemical composition of common buckwheat grown in Slovenia in 1999 (Bonafaccia et al. 2003b) is also shown in Table III. Protein and the total dietary fibre content were slightly lower, and fat and ash contents were higher, in comparison with the values as determined in the present study.

Breads. The results of the chemical analysis of gluten-free control (GFC), wheat control (WC), amaranth (A), quinoa (Q), buckwheat (B), 100% quinoa (100%Q) and sprouted buckwheat (SpB) breads are presented in Table IV.

The 50% replacement of potato starch by milled amaranth, quinoa and buckwheat pseudocereals respectively produced breads that were generally characterized by a higher nutrient content. The protein content of these pseudocereal-containing breads (50% pseudobreads) was, in all cases, at least double the value of the control bread (GFC). Among the 50% pseudobreads, the highest protein value was obtained for those containing amaranth (11.6%), followed by quinoa (10.6%) and buckwheat (8.4%). This compares with 4.2% for the GFC bread ($P<0.01$). Similar significant increases were observed for fat and ash contents. The total starch content, as expected, was significantly lower for A, Q and B breads. The fibre content also increased significantly. In particular, the fibre content of bread B, at 23.3%, was more than three times the value obtained for the GFC bread ($P<0.01$). For A and Q breads, the fibre contents were 17.2% and 16.1%, respectively, more than double that of the GFC bread. In comparison with the WC bread, A and B breads had significantly higher fat and fibre contents.

A further increase in the nutrient content was observed when the rice flour and potato starch in the control gluten-free formulation were totally replaced by a pseudocereal flour. Two types of breads were produced, 100%Q bread and SpB bread. As expected, the protein, fibre and ash contents were increased ($P<0.01$) for 100%Q and SpB breads in comparison with Q and B breads, whereas the total starch

Table IV. Chemical composition of gluten-free control, wheat control, amaranth, quinoa, buckwheat, 100% quinoa and sprouted buckwheat breads.

Bread type	Protein[a]	Fat	Total starch	Dietary fibre	Ash
GFC	4.2±0.0	6.7±0.3	75.4±0.8	7.6±0.9	2.3±0.0
WC	11.9±0.1	2.6±0.2	77.5±0.0	13.4±0.8	2.8±0.1
A	11.6±0.0	8.8±0.4	73.2±1.1	17.2±0.8	3.3±0.0
Q	10.1±0.1	8.6±0.4	72.6±1.1	16.1±0.6	3.1±0.0
B	8.4±0.4	7.5±0.5	68.9±1.6	23.3±0.7	2.6±0.1
100%Q	12.5±0.0	10.4±0.5	55.9±0.4	20.4±2.0	3.9±0.1
SpB	11.6±0.0	7.0±0.2	51.1±1.6	27.5±0.3	3.6±0.0

Data presented on a percentage dry-weight basis±standard deviation. [a]Nitrogen-to-protein conversion factors are 5.70 for GF, WC, B and SpB breads, 5.85 for A bread, and 5.96 for Q and 100%Q breads.

content was decreased ($P < 0.01$). The nutrient content of these breads was also higher than in the WC bread.

Fatty acid composition of the seeds and baked loaves

Seeds. Table V presents the fatty acid composition in amaranth, quinoa, buckwheat and wheat seeds. Results indicate that all of the studied seeds are rich in unsaturated fatty acids, with the highest unsaturated/saturated ratio observed from quinoa (6.2) ($P < 0.01$). Linoleic acid was the most abundant fatty acid in amaranth, quinoa and wheat. In buckwheat, the percentage of linoleic acid is equal to that of oleic acid. Oleic acid is also the second most abundant fatty acid in amaranth and quinoa, and the third most abundant in wheat ($P < 0.01$). Palmitic acid is the main saturated fatty acid for all the seeds and the third most common fatty acid in amaranth, quinoa and buckwheat ($P < 0.01$). With regards to the α-linolenic content, quinoa (8.3%) represents the richest source ($P < 0.01$) among the four seeds analysed, followed by wheat (3.8%), buckwheat (2.2%) and amaranth (0.9%).

The fatty acid composition of amaranth, quinoa, and buckwheat seeds in previously published work is presented in Table VI. Findings are similar to the present study and the little variation observed can be explained, again, on the basis of different genetic and and/or environmental factors (Bressani 2003), or differences in the methods of analysis. From a nutritional point of view, the most relevant difference observed could be the lower linoleic/α-linolenic ratio for quinoa and buckwheat in the samples in the present study compared with the published data.

Breads. The results for the fatty acid analysis of the breads including the two control breads, GFC and WC, the 50% pseudobreads (A, Q and B) and the 100% pseudobreads (100%Q and SpB) are presented in Table VII.

The following fat sources were used in the production of the breads:

(i) GFC bread: sunflower oil (58.4% linoleic acid, 28.5% oleic acid, 6.4% palmitic acid, 3.7% stearic acid and 0.9% total C18 trans-fatty acids).

Table V. Fatty acid composition of fat in amaranth, quinoa, buckwheat and wheat seeds.

Fatty acid	Amaranth	Quinoa	Buckwheat	Wheat
Palmitic (C16:0)	20.9 ± 0.3	11.0 ± 0.3	20.5 ± 0.3	23.7 ± 0.6
Stearic (C18:0)	4.1 ± 0.1	1.1 ± 0.2	2.9 ± 0.1	2.8 ± 0.9
Oleic (C18:1)	23.7 ± 0.1	26.7 ± 0.2	33.6 ± 0.2	13.2 ± 0.5
Linoleic (C18:2)	47.8 ± 0.2	48.2 ± 0.2	33.7 ± 0.2	55.1 ± 1.2
α-Linolenic (C18:3)	0.9 ± 0.0	8.3 ± 0.1	2.2 ± 0.0	3.8 ± 0.1
Arachidonic (C20:0)	0.8 ± 0.0	0.6 ± 0.0	1.1 ± 0.1	0.3 ± 0.2
Cis-11,14-Eicosadienoic (C20:2)	0.3 ± 0.0	1.4 ± 0.1	2.0 ± 0.1	0.5 ± 0.1
Behenic (C22:0)	0.4 ± 0.0	0.7 ± 0.0	1.1 ± 0.1	0.2 ± 0.2
Erucic (C22:1)	nd	1.2 ± 0.1	nd	nd
Lignoceric (C24:0)	0.4 ± 0.0	0.4 ± 0.0	1.0 ± 0.1	nd
Saturated	26.9 ± 0.2	14.0 ± 0.5	27.6 ± 0.4	27.3 ± 1.7
Monounsaturated	23.9 ± 0.1	28.1 ± 0.3	34.7 ± 0.2	13.4 ± 0.5
Polyunsaturated	49.1 ± 0.2	57.5 ± 0.4	37.9 ± 0.6	59.4 ± 1.4
Unsaturated/saturated	2.7 ± 0.0	6.2 ± 0.2	2.6 ± 0.1	2.7 ± 0.2
Linoleic/α-linolenic	52.4 ± 1.2	5.8 ± 0.0	15.6 ± 0.2	14.5 ± 0.2
Total C18 *trans*	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0

Data presented as g/100 g total fatty acids ± standard deviation. nd, not detected.

Table VI. Published fatty acid composition of fat in amaranth, quinoa, buckwheat and wheat seeds (Ruales and Nair 1993; Bruni et al. 2001; Bonafaccia et al. 2003b).

Fatty acid	Amaranth	Quinoa	Buckwheat
Palmitic (C16:0)	16.5	9.9	15.6
Stearic (C18:0)	3.6	0.6	2.0
Oleic (C18:1)	25.5	24.5	37.0
Linoleic (C18:2)	47.5	52.3	39.0
α-Linolenic (C18:3)	na	3.8	1.0
Arachidonic (C20:0)	0.7	0.4	1.8
Cis-11-Eicosaenoic (C20:1)	na	1.4	2.3
Behenic (C22:0)	0.3	0.6	1.1
Erucic (C22:1)	na	1.5	na
Lignoceric (C24:0)	nd	0.2	na
Nervonic (C24:1)	na	2.6	na
Unsaturated/saturated	3.3	4.9	3.9
Linoleic/α-linolenic	na	13.8	39.0

Data presented as g/100 g total fatty acids ± standard deviation. na, data not available; nd, not detected.

(ii) WC bread: baker's fat (40.9% palmitic acid, 31.5% oleic acid, 9.6% linoleic acid, 6.9% stearic acid and 4.8% total C18 trans-fatty acids).
(iii) 50% pseudobreads: vegetable oil (45.9% linoleic acid, 30.7% oleic acid, 10.2% palmitic acid, 6.2% α-linolenic acid, 3.4% stearic acid and 1.0 total C18 trans-fatty acids).
(iv) 100% pseudobreads: linseed oil (50.8% a-linolenic acid, 20.0% oleic acid, 14.9% linoleic acid, 6.5% plamitic acid, 5.9% stearic acid and 0.5 total C18 trans-fatty acids).

A high degree of unsaturation was observed in both 50% pseudobreads and GFC bread, and unsaturated/saturated ratios were 6.2, 4.7, 6.0 and 5.5 for GFC, A, Q and B breads, respectively; this ratio was significantly lower in WC breads (2.8). Similar to pseudocereal seeds, the most abundant fatty acids present in the pseudocereal-containing breads were linoleic, oleic and palmitic acids. The linoleic/α-linolenic ratio was lower ($P<0.01$) in the WC and P breads than in the GFC bread, in the order GFC (69.7), WC (9.9), A (7.8), B (5.8) and Q (5.6). When the breads were made with 100% pseudocereal flour and linseed oil, the fatty acid profile of the resultant breads improved significantly. In particular, the α-linolenic content of the breads increased dramatically (29.9% and 35.7%, respectively, for 100%Q and SpB breads). Total C18 *trans*-fatty acids were low for the gluten-free control, 50% pseudobreads and 100% pseudobreads (<1.0%), and significantly lower than for the wheat breads (1.1%).

Mineral content of the seeds and baked loaves

Seeds. The results of the mineral analysis are presented in Table VIII.

In general, the pseudocereals seeds contained higher levels of the minerals studied compared with wheat. Amaranth showed the highest content of calcium, magnesium and iron ($P<0.01$), while quinoa had the highest content of zinc ($P<0.01$) and the second highest level of iron. The calcium content of buckwheat was 60.9 mg/100 g, approximately twice that found in quinoa and wheat.

Table VII. Fatty acid composition of fat in gluten-free control, wheat control, amaranth, quinoa, buckwheat, 100% quinoa and sprouted buckwheat breads.

Fatty acid	GFC bread	WC bread	A bread	Q bread	B bread	100%Q bread	SpB bread
Palmitic (C16:0)	9.0 ± 0.1	22.5 ± 0.4	12.9 ± 0.1	10.4 ± 0.2	10.7 ± 0.1	8.1 ± 0.1	9.8 ± 0.3
Stearic (C18:0)	3.5 ± 0.1	2.7 ± 0.1	3.1 ± 0.0	2.3 ± 0.1	2.6 ± 0.1	3.2 ± 0.0	4.4 ± 0.1
Oleic (C18:1)	31.0 ± 0.1	26.0 ± 0.5	37.7 ± 0.1	37.5 ± 0.1	42.2 ± 0.2	23.9 ± 0.1	24.7 ± 0.2
Linoleic (C18:2)	53.5 ± 0.1	40.9 ± 0.3	38.8 ± 0.1	39.3 ± 0.2	34.8 ± 0.1	31.7 ± 0.0	22.3 ± 0.1
α-Linolenic (C18:3)	0.8 ± 0.0	4.1 ± 0.1	5.0 ± 0.0	7.0 ± 0.1	6.1 ± 0.1	29.9 ± 0.2	35.7 ± 0.1
Arachidonic (C20:0)	0.3 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	nd	nd
Cis-11,14-Eicosadienoic (C20:2)	0.3 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	0.9 ± 0.0	1.0 ± 0.0
Behenic (C22:0)	0.6 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.8 ± 0.0
Erucic (C22:1)	nd	nd	nd	0.5 ± 0.0	nd	0.7 ± 0.0	nd
Lignoceric (C24:0)	0.3 ± 0.0	nd	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
Saturated	13.9 ± 0.1	26.0 ± 0.5	17.5 ± 0.1	14.2 ± 0.3	15.3 ± 0.2	12.1 ± 0.1	15.9 ± 0.3
Monounsaturated	31.5 ± 0.1	26.4 ± 0.5	38.1 ± 0.1	38.4 ± 0.1	42.7 ± 0.2	24.9 ± 0.1	25.1 ± 0.2
Polyunsaturated	54.5 ± 0.1	45.1 ± 0.3	44.5 ± 0.1	47.3 ± 0.2	42.0 ± 0.1	62.4 ± 0.1	58.9 ± 0.1
Unsaturated/saturated	6.2 ± 0.1	2.8 ± 0.1	4.7 ± 0.0	6.0 ± 0.1	5.5 ± 0.1	7.2 ± 0.1	5.3 ± 0.1
Linoleic/α-linolenic	69.7 ± 0.5	9.9 ± 0.2	7.8 ± 0.0	5.6 ± 0.0	5.8 ± 0.1	1.1 ± 0.0	0.6 ± 0.0
Total C18 *trans*	0.4 ± 0.0	1.1 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.4 ± 0.0

Data presented as g/100 g total fatty acids ± standard deviation. nd, not detected.

Table VIII. Content of certain minerals in amaranth, quinoa, buckwheat and wheat seeds.

Seed	Calcium	Magnesium	Zinc	Iron
Amaranth	180.1 ± 6.1	279.2 ± 1.1	1.6 ± 0.0	9.2 ± 0.2
Quinoa	32.9 ± 3.3	206.8 ± 6.4	1.8 ± 0.0	5.5 ± 0.5
Buckwheat	60.9 ± 3.3	203.4 ± 8.8	1.0 ± 0.0	4.7 ± 0.1
Wheat	34.8 ± 0.0	96.4 ± 3.7	1.2 ± 0.1	3.3 ± 0.1

Data presented as mg/100 g dry-weight basis ± standard deviation.

Table IX presents some previously published mineral data for amaranth, quinoa and buckwheat seeds. The results presented in this study fall within the wide range of values found in the literature (Becker et al. 1981; Pedersen et al. 1987; Singhal and Kulkarni 1988; Chauhan et al. 1992; Koziol 1992; Ranhotra et al. 1992; Ruales and Nair 1993; Bressani 1994, 2003; Guzman-Maldonado and Paredes-Lopez 1998; Steadman et al. 2001b; Bonafaccia et al. 2003a). Calcium, zinc and iron contents in our amaranth and quinoa samples were lower than the average values presented in Table VIII. Variations in mineral content may be genetically controlled but environmental conditions during plant growth and seed set, especially soil mineral availability, are key factors (Ranhotra et al. 1992; Steadman et al. 2001b).

Breads. The results of the minerals analysis are presented in Table X. Each of the breads containing a pseudocereal flour had significantly higher levels of calcium, magnesium, zinc and iron contents compared with the GFC bread. Among the 50% pseudobreads, A bread had the highest calcium, magnesium and iron contents, and the second highest zinc content ($P < 0.01$). As expected, the mineral content increased further for the 100%Q and SpB breads compared with the 50% pseudobreads, and values were closer to that of the wheat bread. In particular, magnesium content was significantly higher in 100%Q and SpB breads than the wheat bread.

Discussion

Nutrient composition of amaranth, quinoa and buckwheat seeds

One of the more relevant characteristics of amaranth and quinoa is their high protein content. Their protein content in the present study was significantly higher than that of wheat. More importantly, amaranth, quinoa and buckwheat proteins are considered safe in the diets of CD (Kupper 2005). Contrary to most common grains, the proteins in amaranth, quinoa and buckwheat are composed mainly of globulins and albumins, which are primarily cytoplasmatic proteins, and contain very little or no storage proteins (prolamins and glutelins). The amino acid composition in primarily

Table IX. Published mineral content of amaranth, quinoa, and buckwheat seeds (Ruales and Nair 1993; Bressani 1994; Steadman et al. 2001b).

Seed	Calcium	Magnesium	Zinc	Iron
Amaranth	217	319	3.4	21.0
Quinoa	123	246	4.5	5.9
Buckwheat	30	253	2.9	3.0

Data presented as mg/100 g dry-weight basis.

Table X. Content of certain minerals in gluten-free control, wheat control, amaranth, quinoa, buckwheat, 100% quinoa and sprouted buckwheat breads.

Bread type	Calcium	Magnesium	Zinc	Iron
GFC	23.7 ± 0.4	20.0 ± 0.0	0.3 ± 0.0	1.3 ± 0.2
WC	99.6 ± 0.1	74.7 ± 0.0	1.9 ± 0.1	5.2 ± 0.4
A	98.1 ± 1.4	149.7 ± 0.2	1.8 ± 0.0	4.3 ± 0.2
Q	28.7 ± 1.2	99.7 ± 0.1	2.1 ± 0.2	2.8 ± 0.4
B	41.3 ± 2.0	99.8 ± 0.1	1.5 ± 0.0	3.9 ± 0.1
100%Q	34.6 ± 2.2	163.6 ± 6.3	1.8 ± 0.0	5.5 ± 0.3
SpB	96.6 ± 2.0	168.6 ± 0.8	1.1 ± 0.0	4.7 ± 0.3

Data presented as mg/100 g dry-weight basis ± standard deviation.

cytoplasmatic proteins (globulins and albumins) and storage proteins (prolamins and glutelins) differs significantly. Storage proteins contain more proline and glutamic acid and smaller amounts of arginine, lysine, threonine and tryptophan, whereas cytoplasmatic proteins contain less glutamic acid and proline and more arginine and lysine (Koziol 1992). The amino acid profile of pseudocereal proteins, being high in lysine and low in glutamic acid and proline, is therefore significantly better than that of common grains, and amaranth, quinoa and buckwheat protein quality has been reported to be superior to that of conventional grains (Pomeranz and Robbins 1972; Pedersen et al. 1987; Koziol 1992; Ruales and Nair 1992; Bressani 1994; Li and Zhang 2001; Berghofer and Schoenlechner 2002). Moreover, this particular amino acid profile may also have implications in relation to cholesterol levels (Li and Zhang 2001) and buckwheat protein extract has showed strong cholesterol-lowering effects in rat feeding experiments (Kayashita et al. 1995). The same authors pointed to the low digestibility of buckwheat protein as another potential factor responsible for its cholesterol-lowering effect. The low protein digestibility of buckwheat protein can partly be explained on the basis of the abundance of thermally resistant trypsin inhibitors and tannins in buckwheat seeds (Wijngaard and Arendt 2006). In addition, protease inhibitors in buckwheat have shown allergenic reactivity with common symptoms including asthma, urticaria, wheezing and anaphylactic shock (Wijngaard and Arendt 2006). It has been suggested, therefore, that buckwheat potential allergenic reactivity needs to be taken into account when designing foods containing buckwheat (Li and Zhang 2001).

Another important feature of amaranth and quinoa nutrient composition is their fat content. In the present study, the fat content in amaranth and quinoa was 5.7% and 5.2%, respectively, more than twice that of wheat (2.5%). In the food industry, the fat content and its composition constitute important factors as fat deterioration can adversely affect food quality and shelf-life. Fat composition, mainly its fatty acid profile, is also responsible for the nutritional value of lipids. Results from epidemiological and experimental studies have shown an association between saturated fat intake and increased risk of chronic diseases, such as cardiovascular disease and cancer. However, consumption of diets high in monounsaturated fatty acids (mainly oleic acid) has been linked to a decreased incidence of cardiovascular disease (Field 2003). Amaranth, quinoa and buckwheat lipids are characterized by a high degree of unsaturation, which is desirable from a nutritional point of view. In the present study, amaranth, quinoa and buckwheat unsaturated/saturated ratios were 2.7, 6.2, and 2.6, respectively, mainly attributed to their high linoleic and oleic acid content. It is also worth noting the high level of α-linolenic acid (C18:3 n-3) found in quinoa samples, and its low linoleic:α-linolenic ratio. Linoleic acid (C18:2 n-6), α-linolenic acid (C18:3 n-3) and their long-chain derivatives have different metabolism and often opposite physiological functions, thus the balance of n-6/n-3 fatty acids in the diet has many health-related implications (Simopoulos 2001). According to Simopoulos (2001) a diet with a high n-6/n-3 ratio promotes the pathogenesis of many degenerative diseases such as cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune diseases, whereas an increased n-3 fatty acid intake reduces the biological markers associated with the above-mentioned diseases. The current n-6/n-3 ratio in western countries has been estimated to be in the range 14:1–20:1 (Field 2003), and is far from the recommended levels of 5:1–10:1 (FAO/WHO 1998). Among the four seeds analysed in this study, quinoa's n-6/n-3 ratio, at 6.2, falls within the recommended values.

Dietary fibre is another component of physiological relevance found in abundant amounts in the pseudocereal seeds, as previously presented in the results section. It is generally accepted that the consumption of food naturally rich in dietary fibre is beneficial to the maintenance of health (Champ et al. 2003). However, the intake of fibre in the gluten-free diet is considered to be inadequate and experts recommend a higher intake of whole grain cereals as opposed to refined grains (Thompson 2000; Kupper 2005; Thompson et al. 2005; Pagano 2006). Therefore, the incorporation of these seeds in the diets of CD patients should help alleviate, at least in part, the deficit in fibre intake in this sector of the population and also increase their overall consumption of whole grains.

Calcium, magnesium and iron are minerals that have been shown to be deficient in gluten-free products and in the gluten-free diet (Thompson 2000; Kupper 2005; Thompson et al. 2005). As discussed in the results section, the pseudocereals amaranth, quinoa and buckwheat are generally a good source of these and other important minerals. Various authors have also previously commented on the exceptional mineral content of these seeds. In amaranth seeds, the levels of phosphorus, calcium, potassium and magnesium are generally higher than those found in cereal grains (Bressani 1994). According to Koziol (1992), quinoa seeds are richer in calcium, iron, magnesium, copper, manganese and chloride than the other cereals. Buckwheat contains more nutritionally relevant minerals (with the exception of calcium) than many cereals such as rice, sorghum, millet and maize, and is a rich source of zinc, copper, manganese and magnesium (Steadman at al. 2001b). Therefore, their use as ingredients in gluten-free products could help improve the mineral profile of these speciality products and of the gluten-free diet in general

In summary, amaranth, quinoa and buckwheat seeds, besides being important energy sources due to their starch content, provide good quality protein, dietary fibre and lipids rich in unsaturated fats. In addition, they contain adequate levels of important micronutrients such as minerals and vitamins (Berghofer and Schoenlechner 2002; Taylor and Parker 2002; Bonafaccia et al. 2003b) and significant amounts of other bioactive components such as fagopyritols and flavonols in buckwheat (Steadman et al. 2000; Holasova et al. 2002), saponins in quinoa (Koziol, 1992), squalene in amaranth (Becker 1994; Bruni et al. 2001) and phytosterols in amaranth, quinoa and buckwheat (Koziol 1992; Bruni et al. 2001; Cai et al. 2004).

Nutritional value of the gluten-free pseudocereal containing breads versus the gluten-free control

Bread represents a staple food in most countries all over the world and its nutritional value is considered of great importance in human nutrition (Dewettinck et al. 2008). Ideal bread should have a lower glycaemic index, contain adequate amounts of protein and tolerated levels of dietary fibre, vitamins, magnesium, trace elements and antioxidants (Lopez et al. 2001). Gluten-free breads for coeliacs, as well as being safe, should also have a nutritional value equivalent to that of the gluten-containing breads they are intending to replace. However, the nutritional quality of gluten-free products currently in the market has been reported to be of concern and there seems to be a need for an improvement in their formulations (Thompson 2000). In the case of gluten-free breads, these findings are to be expected since gluten-free breads and baking mixes are made using refined flours and/or starches and, in contrast to their wheat-containing counterparts, gluten-free products are not normally fortified (Thompson 2000).

In the present work, we studied the impact of using the pseudocereals amaranth, quinoa and buckwheat on the nutritional quality of the gluten-free breads in comparison with a control that was based on refined flours and starches. The pseudocereals amaranth, quinoa and buckwheat not only are safe to the coeliac consumer but they are also naturally high in nutrients (Kupper 2005; Pagano 2006). As shown in the results section, amaranth, quinoa and buckwheat seeds, besides being important energy sources due to their starch content, provide good quality protein, dietary fibre and lipids rich in unsaturated fats.

When each of the pseudocereal flours replaced the potato starch in the gluten-free control formulation, the obtained breads were generally nutritionally superior to the control. The most relevant nutritional improvements brought about by the 50% pseudobreads in comparison with the gluten-free control were a more than double increase in protein and fibre contents and significant increases in fat and ash levels.

With regards to protein, not only are the 50% pseudobreads high in protein but also their protein is of a high nutritional quality, as previously discussed, with a high content of the amino acid lysine, otherwise typically deficient in common cereals. The increase in fibre levels is also desirable from a nutritional point of view, particularly among coeliac patients, as research has shown that intake of fibre among this sector of the population is inadequate (Thompson 2000; Kupper 2005; Thompson et al. 2005; Pagano 2006). The significantly higher fat content of the 50% pseudobreads compared with the GFC can also be considered as beneficial as fat in all bread samples showed a high degree of unsaturation, with linoleic, oleic and palmitic acid as the main fatty acids present. Also *trans*-fatty acid content in all bread samples was measured to be $<1.1\%$. Intake of *trans*-fatty acids should ideally be kept to a minimum as research has shown a correlation between their intake and the risk of cardiovascular disease (Stender and Dyenberg 2004). The higher ash content in the 50% pseudobreads compared with the GFC bread is reflective of the higher mineral content of the former compared with the latter. Each of the 50% pseudobreads had significantly higher levels of calcium, magnesium, zinc and iron content compared with the gluten-free control bread. Once again, this characteristic matches the recommendations for the coeliac population to increase their intake of calcium, magnesium and iron (Thompson 2000; Kupper 2005; Thompson et al. 2005).

Breads containing 100% quinoa and sprouted buckwheat were also produced in this study (100%Q and SpB). Sprouted buckwheat flour was used as an ingredient in SpB bread with the aim of further improving the nutritional value of the final bread. During sprouting of seeds, metabolic changes such as improved protein and starch digestion, increased sugar and B vitamin content and decreased levels of phytases and protein inhibitors take place, which results in an improved nutritional quality of the seeds (Chavan and Kadam 1989). In these formulations, linseed oil was used, due to its high α-linolenic acid content. Linseed oil has also been previously successfully used in bakery products to improve the α-linolenic acid content of these products (Pohjanheimo et al. 2006). As expected, 100%Q and SpB breads represented an increase in the protein, fibre and ash content ($P<0.01$) in comparison with Q and B breads. A further improvement was observed in the fatty acid composition of the lipids in the 100% pseudobreads compared with the 50% pseudobreads due to the use of linseed oil in their formulation. Linolenic acid (C18:3 n-3) content in these breads increased, and consequently the n-6/n-3 ratio decreased. When considering the current n-6/n-3 ratio in western countries (14:1-20-20:1) (Field 2003) versus the recommended (5:1-10:1) (FAO/WHO 1998), the intake of foods with a high content of linolenic acid (C18:3 n-3), such as the 100% pseudobreads made with linseed oil, could help to lower the overall dietary n-6/n-3 ratio and bring it closer to the general recommendations. Total C18 *trans*-fatty acids were also lower in the 100% pseudobreads compared with the 50% pseudobreads.

In summary, the application of amaranth, quinoa and buckwheat flours in gluten-free baking leads to breads that follow the recommendations established for coeliacs to increase consumption of whole grains (Pagano 2006), and to help rectify their inadequate intake of fibre, iron and calcium (Ciacci et al. 2002; Thompson et al. 2005). These breads are also an important source of good quality protein and lipids rich in unsaturated fats. In addition, the manufacture of gluten-free breads using quinoa and buckwheat, both of which have been suggested as potential ingredients in the design of foods with low glycaemic index (Skrabanja et al. 2001; Berti et al. 2004), could potentially results in products with a low glycaemic index, a characteristic which is highly desirable in CD diets (Berti et al. 2004). Finally, the presence of vitamins and other bioactive components—such as fagopyritols and flavonols in buckwheat, saponins in quinoa, squalene in amaranth and phytosterols in amaranth, quinoa and buckwheat—further add to the nutritional quality of gluten-free breads containing pseudocereals.

Conclusions

Amaranth, quinoa and buckwheat are safe seeds for CD patients. When used in gluten-free baking as a replacement for commonly used ingredients such as refined gluten-free flours and starches, the resulting gluten-free breads have an enhanced nutrient profile. In particular, the gluten-free breads containing pseudocereals had significantly higher levels of protein, fat, fibre and minerals. The nutritional value of these breads is also in line with the existing nutritional recommendations for CD diets and CD products. The results suggest that the pseudocereals amaranth, quinoa and buckwheat are feasible in the manufacture of nutrient-rich gluten-free products.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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