Nutritional and Functional Properties of Certain Gluten-Free Raw Materials

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Abstract

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Since the adherence to the gluten-free diet in celiac patients affects the consumption from the grain food group, questions have been raised about the effects of such diet on calcium, iron, and fiber intake, as well as total carbohydrate and grain food consumption. Therefore, in the framework of this research, eleven types of nutritionally valuable gluten-free raw materials were proposed for consumption in gluten-free diet. They were investigated considering their macronutritive composition, different starch classes, the contents of essential minerals, dietary fiber, and polyphenols, as well as their antioxidant activity. The results were compared to the values obtained with wheat flour that was used as the reference raw material indicating that, in many aspects, the gluten-free raw materials mentioned can be considered as nutritionally more valuable in comparison to wheat flour. Especially satisfying results were obtained regarding the contents of proteins, dietary fiber, polyphenols, iron, and calcium. Therefore, the raw materials investigated can be recommended as desirable components that may contribute to the diversity, functionality, and nutritional quality of gluten-free diet.

Keywords: antioxidant capacity; dietary fiber; gluten-free raw materials; minerals; polyphenols; starch

Celiac disease is a chronic disease causing inflammation of the proximal small intestine that occurs in genetically predisposed individuals consuming gluten, which is the storage protein in wheat, barley, and rye (Green & Cellier 2007). The disease injury usually resolves when gluten is excluded from the diet. Although the injury will heal, the reaction to gluten is permanent and will recur with the reintroduction of gluten. The condition is surprisingly common, affecting as many as 1% of European population, and is considered to be one of the most common life-long disorders in the western world (Rostom *et al*. 2006). The complete gluten withdrawal from the diet has been shown to lead to the normalisation of the mortality rate, improvement of osteoporosis and osteopenia, decrease of malignancy risk, etc.

Therefore, celiac patients have to follow a very strict diet and avoid any products containing wheat, rye, and barley (and sometimes oat), which leads to a relief from the symptoms and a significant improvement of the intestinal mucosa and its absorptive function. However, the avoidance of wheat is a formidable task. This is so because, apart from the obvious sources such as bread, cakes, pasta, and breakfast cereals, wheat is found as a thickener or extender in a wide range of foods, e.g. soups, sauces, sausages, and pâté. Both beer

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and malt have been shown to contain detectable levels of peptides coming from barley and wheat and so should also be avoided by truly intolerant patients. From a truly wheat-free diet, all these foods would have to be excluded, and many of these would be badly missed nutritionally, socially and in terms of palatability.

Due to the restricted selection of permitted foodstuffs, a wide range of problems occur linked to the nutritional status and nutritional quality of the diet in celiac disease patients. Average daily intake of energy is usually lower than the estimated average requirement and the patients consume a higher proportion of energy as fat and a lower proportion of energy as carbohydrates (Bardella *et al*. 2000). Magnesium and calcium deficiencies are very common in active celiac disease (Sabry & Okada 1992) because of the decreased intestinal absorption and because these minerals tend to bind with malabsorbed fat which passes through the system. Also, gluten-free diet is very often associated with inadequate dietary fiber intake since one of the most common high-fiber foods in the average western-type diet, whole wheat, contains gluten (Bardella *et al*. 2000; Thompson 1999, 2000). Additionally, another often consumed high fibre food, oats, can be tolerated by most but not all gluten intolerant people (Codex Stan 118-1979). According to the available literature data, the recommended amounts of fiber, iron, and calcium are consumed respectively, by only 46%, 44%, and 31% of women and 88%, 100%, and 63% of men on gluten-free diet (Thompson *et al*. 2005). In a recent study investigating the factors that influence the adherence to a gluten-free diet (Leffler *et al*. 2008), a large number of celiac patients reported concern regarding the accessibility and quality of gluten-free foods.

Until recently, nutritional therapy for celiac disease has centered around food allowed/not allowed on a gluten-free diet. Considering the nutritional deficiencies mentioned, emphasis should also be placed on the nutritional quality of the gluten-free diet, particularly as concerns the iron, calcium, and fiber consumption as well as total energy intake. A promising possibility is including other nutritionally rich gluten-free types of flour in everyday diet.

Therefore, in the frameworks of this research, nutritional quality of eleven gluten-free types of flour was estimated considering their macronutritive composition and the contribution of the macronutritive components to the total energy value: dietary fiber content; starch fractions, that can be associated with glycemic index of the raw material; and the contents of essential minerals Ca, Mg, Mn, Fe, and Cu. The functionality of the raw materials investigated was additionally assessed considering the total phenolic content, radical scavenging activity, and ferric reducing power. Although gluten containing components of usual diet include not only white wheat flour but also other cereal products and raw materials, the respective results obtained for gluten-free foods were compared to the nutritive characteristics of white wheat flour (T550) since it represents the gluten-containing raw material most commonly used in the usual diet.

MATERIALS AND METHODS

Samples. Eleven types of gluten – free flours were analysed for their macronutritive compositions, the contents of essential minerals, extractable phenolic content, and different types of antioxidant activity with the aim of assessing their nutritive and functional properties. Gluten-free raw materials selected for the analysis were carob, amaranth, soy, red and orange sweet potato, red sweet potato flour mixed with 10% potato peel, red quinoa, buckwheat, corn, rice, and chickpea. Sweet potato and amaranth were donated by the growers from the Zagreb surrounding area and all other raw materials investigated were purchased from the local supplier. Wheat flour type 550 also purchased from the local supplier was analysed for comparison. The selected gluten-free raw materials are rarely used in the typical diet and were selected based on the available data on their nutritive quality, lack of gluten, and availability in the market.

Chemicals. All reagents used were of analytical grade. Folin-Ciocalteu reagent, guar gum, and TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) were purchased from Fluka (Buchs, Switzerland); Trolox (6-hydrox-tetramethylcroman-2-carboxylic acid), ABTS (2, 2'-azino-bis (3-ethylenbenzoline-6-sulfonic acid) radical cation), and total dietary fiber assay kit were purchased from Sigma-Aldrich (St. Louis, USA) as well as the enzymes used for the starch fractions determination: pancreatin (EC 232-468-9), pancreatic α-amylase (EC 232-565-6), heat stable α-amylase (EC 232-560-9), invertase (EC 3.2.1.665-7), and amyloglucosidase (EC232-877-2). Multielement standard stock solution for ICP AES was purchased from Merck (Darmstadt, Germany). GOD-PAP test was purchased from Herbos Dijagnostika (Sisak, Croatia). The other chemicals used were from Kemika (Zagreb, Croatia).

Macronutritive evaluation. The content of available carbohydrates was calculated as the sum of water soluble carbohydrates and starch; water soluble carbohydrates were determined according to Luff-Schorl (Acker 1967) and total starch according to the enzymatic method of Englyst *et al*. (1992). Total nitrogen was determined by the semi-automatic Kjeldahl method (AOAC 2000b) and the protein content was calculated by using the following specific factors: 5.51 for wheat flour, 4.74 for amaranth, 5.71 for soy flour, 5.16 for rice flour, 5.25 for buckwheat, 6.07 for corn, 5.39 for quinoa, and 6.25 for sweet potato flour and carob (MAFF 1975; Fujihara *et al*. 2008). The accuracy of the applied method was checked by investigating total nitrogen content of the following reference substances: L-phenylalanine, L-methionine, acetanilide, and nicotinamide (Table 1).

Fat content was determined gravimetrically by the Soxhlet method (AOAC 2000a) after 6-h extraction with petroleum ether as the extraction solvent.

The content of total dietary fiber was calculated as the sum of the soluble and insoluble dietary fiber fractions that had been determined in defatted samples using the official enzymatic-gravimetric method (AOAC 2000b). With the aim of assessing the energy value of the analysed samples, the amount of each macronutritive component was converted to food energy according to Food and Agriculture Organization (FAO) recommendations (FAO 2003) using an Atwater general factor system. Total energy value of the raw materials investigated was calculated as the sum of energy values of total carbohydrates, proteins, and fats. The moisture content was determined using the microwave oven procedure (Sharma & Hanna 1989).

Determination of mineral content. The macro- and trace elements investigated were determined by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) at the following wavelengths: 184.0 nm (Ca), 279.0 nm (Mg), 257.6 nm (Mn), 324.7 nm (Cu), and 238.2 nm (Fe). Detection limits were: 0.03 mg/l (Ca, Mg), 0.1 mg/l (Mn), and 0.5 mg/l (Cu, Fe). All tests were performed in an accredited laboratory according to ISO/IEC 17025:2005, ensuring the quality of the results obtained. The accuracy of the methods was assessed on flour T550 spiked with known amounts of the respective elements.

Three concentration levels of the working standard solutions were added to 600 mg of T550 flour in triplicates. Such samples (n = 9) were subjected to the overall analytical procedure parallel with the samples (n = 3) without added standards. Comparing the theoretical (expected) and true (found) element values, the mean recovery percentage of the methods was obtained. The repeatability of the sample preparation (intra-assay precision) was examined as well, and expressed as relative standard deviation (RSD) of nine determinations (three concentrations in three replicates) as shown in Table 2.

Prior to spectrometric analysis, the samples were wet ashed using 65% HNO_3 and 30% H_2O_2 in the microwave digestion unit that had been selected for the sample preparation since it was free from contamination risk and was not time-consuming. A detailed description of the sample pretreatment is reported elsewhere (Vitali *et al*. 2008)

Determination of nutritionally important starch fractions. With the aim of obtaining an insight into the ratios of nutritionally important starch fractions and roughly predicting the glycemic index of the investigated samples, the con-

Table 1. Accuracy of protein content determination

Standard substance	N theoretical (g/100 g)	N true (g/100 g)	Recovery (%)	Recovery $\bar{x}$
L-Phenylalanine	8.47	8.29 ± 0.43	97.87	98.13
L-Methionine	9.39	9.21 ± 0.55	98.08	
Acetanilide	10.36	10.22 ± 0.61	98.65	
Nicotinamide	22.94	22.46 ± 0.88	97.90	

Table 2. Accuracy and repeatability of Intra-assay precision (ICP-AES) measurements

Element	Mean recovery (%)	RSD (%)
Ca	102.3	1.2
Mg	99.1	0.9
Mn	102.5	2.8
Cu	101.8	3.1
Fe	98.6	2.2

tents of the rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and rapidly available glucose (RAG) were assessed using the method of Englyst *et al.* (1992). Briefly, the samples were subjected to successive enzymatic hydrolysis with invertase, pancreatic α-amylase, and amyloglucosidase, and the amounts of glucose released from the sample after 20 (G20) and 120 min (G120) were obtained spectrophotometrically using GOD-PAP test. Total glucose (TG) was obtained in the same way after additional gelatinisation of the starch in boiling water, treatment with KOH, and subsequent treatment with amyloglucosidase. Based on the obtained amounts of different glucose classes, RDS, SDS, RS, and RAG were calculated using the equations proposed by Englyst *et al.* (1992).

Determination of extractable phenolic content and antioxidant activity. Extractable phenols were determined according to Folin-Ciocalteu spectrophotometric method (Gao *et al.* 2002). The extraction solvent used was the mixture of HCl (36%), methanol, and water (1:80:10, v/v). The supernatants obtained after two successive extractions (2 h, 20°C, constant shaking) were combined and used for the determination of soluble polyphenols. The calibration curve was made using ferulic acid and therefore the obtained amounts of total phenolics were expressed as ferulic acid equivalents.

The extracts obtained using the previously described (methanol:HCl:water) extraction procedure were used for the determination of antioxidative capacity of the investigated flours using ferric reducing antioxidant power assay (FRAP) and ABTS radical cation assay.

FRAP assay was conducted according to the method of Pulido *et al.* (2000). The reducing potential of the raw materials investigated was expressed as the concentration (g/l) of the sample having the reducing ability equivalent to that of 1mM $FeSO_4 \cdot 7\,H_2O$ (that was determined using the corresponding regression equation). It is important to notice that in this case a lower value indicates a higher antioxidant activity of the sample. ABTS radical cation assay was conducted according to the procedure of Re *et al.* (1999). The calibration curve was prepared using Trolox as standard and the antioxidant activity of the extracts was expressed as Trolox equivalent antioxidant capacity (TEAC) by calculating the percentage of absorbance reduction and plotting it as a function of Trolox concentration.

Statistical analysis. The analyses were conducted in triplicates or quadruplicates depending on the method used and the data obtained were presented as means ± standard deviation of parallel investigations. Significant differences between the analysed samples were assessed using one way analysis of variance followed by Bonferroni's multiple comparisons test. The correlations were calculated for polyphenol content and antioxidant activity and from these values the mean value of Pearson's correlation coefficient was obtained. Values of $P \leq 0.05$ were considered as statistically significant and $P \leq 0.01$ as highly significant. The software used for all the above mentioned calculations was Graph Pad Prism, Version 3.02.

RESULTS AND DISCUSSION

Macronutritive composition and energy value

Macronutritive compositions of the raw materials investigated are presented in Table 3, containing the proportions of fats, proteins, dietary fiber, and available carbohydrates that were reported as the sum of each individual sugar and total starch.

Fat content in the analysed samples varied significantly ranging from 0.53 g/100 g dry mater (dm) in red sweet potato up to 24.19 g/100 g dm in soy flour. Relatively high fat contents were also found in red quinoa, amaranth, and chickpea (6.39, 6.28, and 5.84 g/100 g dm, respectively). Protein content of the investigated samples ranged from 5.38 g/100 g dm in carob up to 41.47 g/100 g dm in soy flour. In addition to soy, chickpea, amaranth, and orange sweet potato contained significantly higher proportions of proteins as compared to wheat flour T550.

Considering carbohydrate content, the investigated raw materials can be divided into three

Table 3. Macronutritive composition of investigated raw materials (g/100 g dm*)

	Carbohydrates		Total starch	Fats	Proteins	Dietary fibre
	glucose + fructose	sacharose				
Wheat flour (T550)	1.01 ± 0.00	1.24 ± 0.02	49.24 ± 3.37	1.19 ± 0.03	9.70 ± 0.02	4.59 ± 0.12
Carob	26.04 ± 0.33	14.33 ± 0.37	ND	0.56 ± 0.07	5.38 ± 0.03	43.45 ± 1.09
Soy flour	5.32 ± 0.00	3.71 ± 0.10	1.42 ± 0.05	24.19 ± 0.13	41.47 ± 0.64	26.07 ± 1.39
Amaranth	3.36 ± 0.08	0.59 ± 0.12	51.88 ± 1.78	6.28 ± 0.04	12.00 ± 0.04	17.08 ± 1.18
Orange sweet potato	28.63 ± 0.99	24.12 ± 1.00	34.07 ± 4.42	1.10 ± 0.16	12.25 ± 0.63	21.89 ± 0.26
Red sweet potato	37.37 ± 2.56	13.00 ± 3.78	41.76 ± 3.60	0.53 ± 0.05	7.79 ± 0.26	19.80 ± 0.6d
Red sweet potato+skin	28.14 ± 0.14	22.17 ± 0.21	33.77 ± 5.53	2.02 ± 0.06	7.60 ± 0.38	23.00 ± 1.00
Red quinoa	13.71 ± 0.07	1.58 ± 0.12	68.09 ± 2.93	6.39 ± 0.25	14.32 ±1.13	18.26 ± 0.90
Buckwheat	2.94 ± 0.25	0.41 ± 0.04	54.15 ± 1.81	2.25 ± 0.07	11.6 ± 0.80	23.42 ± 0.24
Corn	5.45 ± 0.07	1.52 ± 0.12	60.25 ± 3.45	3.83 ± 0.04	7.93 ± 0.01	19.07 ± 0.21
Rice flour	2.52 ± 0.07	1.13 ± 0.18	64.84 ± 1.12	2.44 ± 0.01	6.14 ± 0.35	13.19 ± 0.17
Chickpea	2.44 ± 0.14	5.3 ± 0.18	51.93 ± 1.44	5.84 ± 0.24	20.25 ± 0.63	22.05 ± 1.58

*values are presented as means of three parallel investigations ± standard deviation; same letters within the same column indicate no significant difference

groups – the samples with a low carbohydrate content (soy flour –10.64 g/100 g dm) the group of samples with moderate carbohydrate content ranging from 48.12 g/100 g dm to 68.56 g/100 g dm (wheat flour, carob, amaranth, buckwheat, corn, rice, and chickpea), and the samples with a very high carbohydrate content ranging from 83.46 g/100 g dm to 92.81 g/100 g dm (sweet potato flours and red quinoa). The estimated contents of macronutritive components were converted to food energy according to FAO recommendation using an Atwater general factor system (2 kcal/g for dietary fiber, 4 kcal/g for available carbohydrates and proteins, and 9 kcal/g for fat) and the contribution of each macronutrient group to the total energy value of the sample was calculated (Figure 1).

The calorific value of all gluten-free raw materials was significantly higher in comparison to that of wheat flour T550 (265 kcal/100 g) ranging from 275 kcal/100 g (carob) up to 485 kcal/100 g dm (red quinoa) which is desirable considering that more than 50% of coeliac patients have an inadequate energy intake.

Since the proper distribution of macronutrients in the diet is very important for maintaining optimal body functions, providing adequate intakes of other nutrients, and decreasing the risk of chronic diseases, acceptable macronutrient distribution ranges (AMDRs) have been developed by the FAO standing for the percentages of calories coming from protein, carbohydrate, and fat. Based on their role as the primary energy source for the brain and as a source of kilocalories to maintain the body weight, AMDR for carbohydrates has been set to 45–65%. AMDR for fat has been assessed to 25–35% bearing in mind their role as energy source, the source of essential fatty acids, and the vehicle necessary for the absorption of liposoluble vitamins while the upper end is based on decreasing the risk of chronic disease and providing adequate intake of other nutrients. AMDR for proteins as the major structural components of all cells in the body, due to their functions as enzymes, transport carriers, and sometimes hormones, was set to 10–35%. As obvious from Figure 1, in a majority of the raw materials investigated the contribution of available carbohydrates to the total energy value is higher than recommended by AMDR, ranging from 77.8% up to 91.9%.The exception is soy, but also amaranth and chickpea with which 19.7%, 70.8%, and 71.1% of energy comes from available carbohydrates. The contribution of proteins to the total energy value was satisfactory in some samples (wheat – 14.7%, soy – 34.7%, amaranth – 13.3%, orange sweet potato

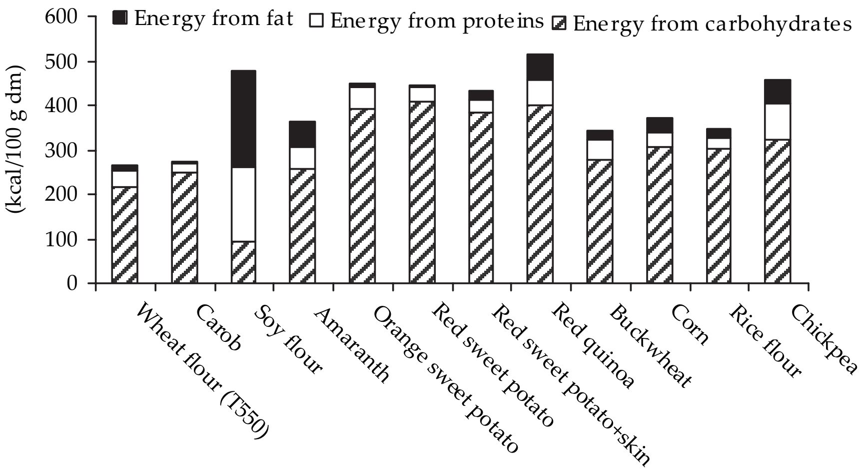

Figure 1. Contribution of macronutrients to the total energy value of raw materials

(10.9%), red quinoa – 11.1%, buckwheat – 13.5% and chickpea – 17.7%) and, in the rest of them, lower than recommended. The contribution of fat to the total energy was generally too low (except for soy) and the contribution of carbohydrates was too high and therefore, according to FAO recommendations, the raw materials investigated cannot be considered as balanced energy sources. However, having in mind the already mentioned too high a contribution of fat to the total energy intake by an average celiac patient, the raw materials investigated might contribute to balancing their daily diet and energy intake.

Amaranth, chickpea, and especially soy differ from the other raw materials investigated since the contributions of protein and fat to the total energy value are significantly higher in their case. Regarding the acceptable macronutrient distribution ranges, amaranth and chickpea are considered to be the most balanced sources of energy among the raw materials investigated.

Considering the often inadequate total dietary fibre intake by celiac patients, the raw materials investigated were also evaluated as the sources of soluble and insoluble fibers and compared with wheat flour. The obtained values ranged from 13.19 g/100 g dm (rice) to 43.45 g/100 g dm (carob), being significantly higher in comparison to only 4.59 g/100 g dm in wheat flour. Considering DRI for fibres, the consumption of 100 g of the raw materials investigated can cover from 44% to 145% of the recommended intake which makes them valuable fibre sources in everyday nutrition. Since their physiological and health benefits depend not only on their contents but also on their chemical characteristics, the soluble/insoluble fiber ratio has been assessed and is presented in Figure 2.

In a majority of the samples, the content of insoluble fiber was significantly higher in comparison to the soluble fiber fraction which was rather low. However, the investigated sweet potato flours, in addition to a satisfactory insoluble fiber content

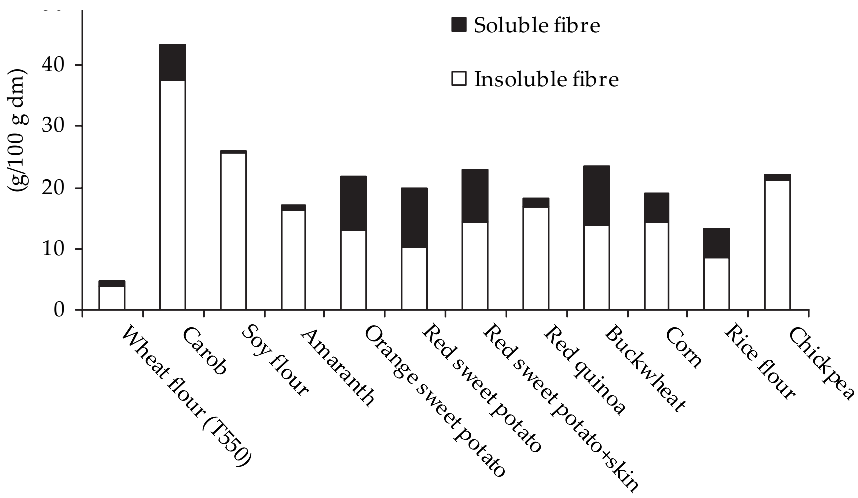

Figure 2. The content of soluble and insoluble dietary fiber fraction

(10.31–14.45 g/100 g dm), contained also significant amounts of soluble dietary fiber (8.56 g/100 g dm to 9.50 g/100 g dm). Total dietary fiber content of the investigated sweet potato flours was consistent with the data published by Yadav *et al.* (2006). Additionally, our results reveal a significant impact of sweet potato sort on the dietary fiber content (21.9 g/100 g vs 19.8 g/100 g) as well as the possibilities of increasing the fiber content by enriching the flour with a dose of potato peel (19.8 g/100 g vs 23.0 g/100 g). The content of soluble fibre was lower in buckwheat, carob, corn, and rice (9.67, 5.98, 4.69, and 4.59 g/100 g dm, respectively) but still significantly higher in comparison to that in wheat flour (0.68 g/100 g dm).

Starch fractions and prediction of glycemic index of investigated raw materials

A detailed investigation of the carbohydrate profile in the analysed samples was conducted bearing in mind the fact that a satisfactory representation of raw materials rich in complex carbohydrates is imperative in healthy and balanced diet. Starch is the main carbohydrate in human nutrition and its nutritional quality strongly depends on its chemical characteristics. Starch digestibility in human small intestine can be modified from a rapid digestion to indigestibility as in the case of resistant starch. In spite of the lack of well designed clinical studies, it is believed nowadays that less digestible starch fractions have a moderate impact on GI and offer a range of health benefits due to their stabilising effect on postprandial glucose levels. Therefore, the values for free glucose (FG), rapidly available glucose (RAG = G20 – glucose released from food after 20 min), glucose released after 120 min incubation (G120), and total glucose (TG-obtained by gelatinisation of the starch) were determined and are presented in Table 4.

Based on the data presented, the proportions of different starch fractions were calculated using the equations suggested by Englyst *et al.* (1992) and are presented in Figure 3.

The rate and extent of starch digestion in vitro can be used for the prediction of glycemic index (GI) in foodstuff. Namely, highly significant positive correlations ($P < 0.001$) were observed between GI and both RDS and RAG (Englyst *et al.* 1996) since the measurement of RAG *in vitro* provides values for direct calculation of the amount of glucose likely to be readily absorbed in the small intestine. Therefore, considering the obtained RAG values, it can be assumed that out of the raw materials investigated, only soy is expected to have lower GI compared to T550 wheat flour. In terms of functionality, it is of great importance to com-

Table 4. Free glucose, glucose available after incubation (20 min and 120 min) and total glucose in investigated samples (g/100 g dm*)

	FG	RAG	G120	TG
Wheat flour (T550)	0.46 ± 0.03	10.15 ± 0.97	33.73 ± 1.70	62.50 ± 3.37
Carob	20.55 ± 0.41	20.31 ± 0.25	20.46 ± 0.26	22.26 ± 0.55
Soy flour	4.50 ± 0.05	4.40 ± 0.01	5.12 ± 0.01	6.08 ± 0.01
Amaranth	2.41 ± 0.02	32.47 ± 3.18	58.62 ± 1.26	69.11 ± 1.78
Orange sweet potato	18.85 ± 0.21	21.13 ± 1.07	35.97 ± 0.87	56.71 ± 4.43
Red sweet potato	17.72 ± 0.59	18.63 ± 1.72	34.29 ± 2.64	64.11 ± 3.65
Red sweet potato+skin	19.33 ± 0.73	20.26 ± 1.55	39.91 ± 2.55	56.85 ± 5.58
Red quinoa	4.82 ± 0.00	19.79 ± 0.51	62.32 ±3.07	80.47 ± 0.62
Buckwheat	0.58 ± 0.03	8.90 ± 0.31	58.91 ± 0.23	60.75 ± 5.08
Corn	1.02 ± 0.08	8.73 ± 0.76	32.35 ± 0.44	67.96 ± 0.11
Rice flour	1.17 ± 0.03	13.58 ± 0.96	49.08 ± 0.48	73.22 ± 4.79
Chickpea	1.21 ± 0.11	2.76 ± 0.12	18.05 ± 0.21	58.91 ± 0.21

*Values are presented as means of four parallel investigations ± standard deviation

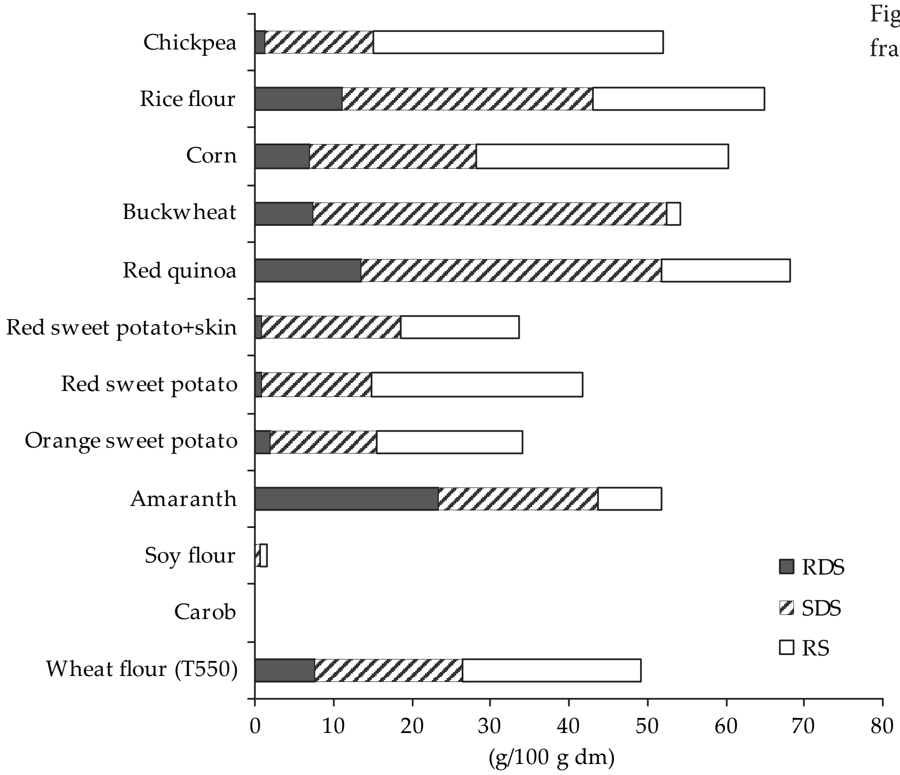

Figure 3. Content of different starch fractions in investigated samples

ment on RS content of the investigated samples due to its reduced calorific content and several physiological and metabolic functions of dietary fiber (Delcour & Eerlinger 1996). It is obvious from the results obtained that chickpea, (red) sweet potato, corn, and wheat can be considered as valuable sources of RS in nutrition.

Mineral content

Since the adherence to a gluten-free diet has been shown to cause nutritional deficiencies of different micronutrients including essential minerals (especially Ca, Fe, and Mg) (Schwarzenberg & Brunzell 2002), the contents of Fe, Mn, Cu, Ca,

Table 5. The content of Fe, Mn, Cu, Ca, and Mg in investigated samples (mg/100 g dm*)

	Fe	Mn	Cu	Ca	Mg
Wheat flour (T550)	1.31 ± 0.01	0.87 ± 0.01	0.19 ± 0.00	15.8 ± 1.5	27.7 ± 1.5
Carob	1.73 ± 0.06	3.75 ± 0.22	0.76 ± 0.01	153.8 ± 8.4	285.8 ± 11.1
Soy flour	6.81 ± 0.09	2.85 ± 0.01	1.32 ± 0.01	209.6 ± 12.8	245.9 ± 18.2
Amaranth	9.85 ± 0.09	1.02 ± 0.05	0.52 ± 0.00	325.9 ± 14.2	68.5 ± 2.3
Orange sweet potato	4.50 ± 0.02	0.42 ± 0.00	1.12 ± 0.01	152.7 ± 0.5	97.9 ± 1.3
Red sweet potato	3.92 ± 0.05	0.33 ± 0.00	0.72 ± 0.01	146.5 ± 0.6	80.3 ± 0.3
Red sweet potato+skin	13.83 ± 0.03	0.69 ± 0.01	0.78 ± 0.02	153.9 ± 1.2	102.4 ± 0.9
Red quinoa	5.13 ± 0.09	1.40 ± 0.02	0.61 ± 0.03	66.1 ± 2.0	209.3 ± 12.7
Buckwheat	2.15 ± 0.09	0.99 ± 0.02	1.21 ± 0.05	22.2 ± 2.0	204.8 ± 11.2
Corn	1.02 ± 0.05	0.14 ± 0.01	0.11 ± 0.00	5.6 ± 0.1	39.4 ± 1.7
Rice flour	0.55 ± 0.02	1.35 ± 0.09	0.19 ± 0.01	15.5 ± 0.9	44.7 ± 2.6
Chickpea	5.51 ± 0.09	1.77 ± 0.01	0.88 ± 0.03	51.2 ± 1.4	171.2 ± 11.0

*values are presented as means of four parallel investigations ± standard deviation and Mg were determined in raw materials suggested for the enrichment of the gluten-free diet and are presented in Table 5.

Iron content in the investigated samples ranged from 0.55 mg/100 g dm in rice flour up to 13.83 mg/100 g dm in peel-enriched sweet potato flour. It is important to emphasise that most of the gluten-free raw materials investigated (with the exception of corn and rice flour) contained significantly higher amounts of iron as compared to T550 flour, and that the daily consumption of 100 g of the investigated raw materials can cover from 10% up to 77% of the estimated RDA value for women, which is important in view of the often inadequate iron intake (and status) of celiac patients (Thompson *et al*. 2005). Calcium contents in corn rice and wheat flour were about equal but they were significantly higher in all other raw materials ranging from 22.2 mg/100 g dm in buckwheat up to 326 mg/100 g dm in amaranth. Since the consumption of 100 g of sweet potato, carob, soy, and amaranth can cover from 12% up to 25% of the estimated RDA, those gluten-free raw materials can be considered as valuable substitutes for wheat as well as important nutritive sources of calcium in gluten-free diet.

Wheat flour was the poorest source of magnesium among the investigated samples while chickpea, buckwheat, red quinoa, soy, and amaranth stand out as the richest sources of this nutritionally important element. The poorest sources of manganese were two investigated varieties of sweet potato flour; somewhat higher concentrations were found in T550 wheat- and peel-enriched potato flour while amaranth, soy and carob contained the highest amounts of manganese among the investigated samples (1.02–3.75 mg/100 g dm, respectively). The content of copper was about equal in all investigated samples with the exception of wheat, rice, and corn that contained significantly lower copper amounts in comparison to the other raw materials investigated.

Extractable phenolic content and *in vitro* antioxidant activity

Although the positive physiological effects of whole-grain products have been mainly ascribed to dietary fibre, it is now known that phenolic compounds significantly contribute to these beneficial effects as well. Due to their antioxidant activity, a balanced diet containing sufficient amounts of polyphenol-rich whole grain foods can enhance the naturally occurring endogenous system and may protect the body against oxidative stress. Therefore, polyphenolic content, radical scavenging activity, and ferric reducing power of gluten-free

Table 6. Polyphenolic content and antioxidant activity of investigated samples

	Total phenols[a]	ABTS[b]	FRAP[c]
Wheat flour (T550)	108.09 ± 1.57	1.60 ± 0.17	452.16 ± 15.03
Carob	2382.03 ± 53.33	292.70 ± 3.16	1.45 ± 0.03
Soy flour	361.28 ± 8.69	15.30 ± 1.44	35.24 ± 0.45
Amaranth	186.13 ± 6.24	6.01 ± 0.84	147.14 ± 15.20
Orange sweet potato	418.88 ± 6.56	84.94 ± 3.66	23.89 ± 0.38
Red sweet potato	429.24 ± 15.45	82.57 ± 0.16	20.82 ± 0.66
Red sweet potato+skin	746.56 ± 11.49	103.75 ± 2.29	11.03 ± 0.65
Red quinoa	427.65 ± 2.76	8.95 ± 0.09	26.35 ± 0.10
Buckwheat	799.89 ± 25.0	111.7 ± 0.24	9.76 ± 0.01
Corn	164.97 ± 8.43	2.09 ± 0.44	67.31 ± 3.71
Rice flour	115.19 ± 4.49	3.25 ± 0.01	76.88 ± 0.19
Chickpea	164.63 ± 3.44	1.90 ± 0.02	135.34 ± 2.47

[a] ferrulic acid equivalents; means ± standard deviations of three parallel investigations; [b]Trolox equivalents; means ± standard deviations four parallel investigation; [c] Fe^{2+} equivalents; means ± standard deviations four parallel investigation

raw materials were investigated and the results obtained are presented in Table 6.

Polyphenolic content in all investigated gluten-free samples (with the exception of rice flour) was significantly higher compared to that in T550 wheat flour, whereas carob stands out as by far the richest source of polyphenols. A high polyphenolic content was also found is buckwheat, sweet potato, and soy. It is also important to emphasise the significant enrichment of red sweet potato flour with phenolic compounds (74%) that was achieved by mixing it with a dose of sweet potato peel. Radical scavenging activity of the investigated samples ranged from 1.60 (T550 wheat flour) up to 292.70 TE (carob). In addition to carob, buckwheat and peel-enriched red sweet potato flour also stand out as the raw materials with a significant antiradical activity (111.77 TE and 103.75 TE, respectively). By correlating the obtained radical scavenging activity with polyphenolic content, a highly significant correlation coefficient was obtained ($r = 0.956$; $P < 0.01$) indicating that polyphenols can be considered as the most important bearers of the antiradical activity measured. The lowest ferric reducing power was again found in T550 wheat flour (452.16 Fe^{2+} eq.) while the values obtained with gluten-free raw materials ranged from 147.14 (amaranth) to 1.45 Fe^{2+} eq. (carob). In addition to carob, buckwheat and sweet potato can be considered as raw materials with a significant reductive potential (especially when enriched by the addition of a dose of peel). There was no significant correlation between ferric reducing power and polyphenolic content thus indicating that other plant components, aside from polyphenols, significantly contribute to the samples reductive potential.

CONCLUSION

Essential mineral and dietary fibre contents of the investigated gluten-free raw materials were significantly higher in comparison to those in wheat flour, and therefore their introduction to celiac patients diet might significantly improve and balance the intake of these limiting nutrients. Chickpea, rice flour, corn, quinoa, and different types of sweet potato flour have all been shown to be excellent sources of resistant starch which is related to several metabolic functions of dietary fibre, while soy, chickpea, amaranth, and orange sweet potato flour must be pointed out as excellent protein sources. Additionally, a higher energy value of gluten-free raw materials might contribute to making up for the inadequate energy intake by celiac patients. The consumption of the raw materials investigated, and especially of carob, soy, buckwheat, and sweet potato, might also significantly increase the daily intake of food antioxidants which has often been correlated with numerous health benefits in humans. Based on the compositional data of the studied gluten-free raw materials, it may be stated that these raw materials or their combinations are suitable for the realisation of a nutritionally adequate gluten-free diet.

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