



Physico-Chemical, Functional and Antioxidant Properties of Some Flours Types as Gluten-Free Ingredients Compared to Wheat Flour

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Preparation of gluten-free products is a big challenge to the manufacturers with the main challenge of finding suitable alternatives for gluten. Gluten-free products commercially available are poor sources of protein, fiber, minerals, and calories in the diet and poor sensory properties. Also, that GF products are not widely available and are both poor in quality and more expensive than gluten-containing products. The objective of this work was to investigate the chemical composition, functional properties, antioxidant activity, and total phenolic compound of some flours types as GF ingredients and compared to wheat flour containing gluten as a control. Among the GF ingredients used pseudocereals (quinoa and buckwheat), millet, rice, chickpea flours. The chemical composition of GF flours ranged between 10.34 – 11.71% moisture, 7.28 – 22.52% crude protein, 2.03 – 6.09% crude fat, 0.45 – 2.37% ash, 0.34 – 5.56% crude fiber, 61.89 – 88.91% starch, 66.82 – 89.90% Carbohydrates and 385.13 – 406.99 Kcal /100g on dry weight basis. While, wheat flour (extraction 72%) contained 11.30% moisture, 12.26% crude protein, 2.46% crude fat, 0.59% ash, 0.61% crude fiber, 82.57% starch, 84.08% carbohydrates and 407.50 Kcal /100g on dry weight basis. The total phenolic compound content and antioxidant activity were (279.89, 517.92, 163.99,

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50.67 and 232.19 mg/100g on dry weight basis) and (16.92, 43.83, 4.27, 2.75 and 8.20%) for quinoa, buckwheat, millet, rice, chickpea flours, respectively. Whereas, the total phenolic compound content and antioxidant activity for wheat flour was (147.56 mg/100g) and (4.26 %) on dry weight basis, respectively. On other hand, the results of water and oil holding capacity indicated that quinoa, buckwheat flours gave higher values than that observed for wheat flour. Also, it was found that quinoa, buckwheat, millet, rice, chickpea flours were higher soluble protein than the wheat flour. In addition, buckwheat, millet, rice, chickpea flours showed markedly higher foaming stability than of wheat flour.

Keywords: Chemical composition; functional properties; antioxidant activity; gluten-free flours; wheat flour.

1. INTRODUCTION

Celiac disease (CD) is caused by a lifelong intolerance to prolamines, which are found in wheat, rye, barley, and potentially oats. When these drugs are consumed, they induce injury to the intestinal mucosa and a reduction in nutrient absorption. CD is now recognized as a systemic disease that can affect people of any age, race, or ethnicity, is not always associated with the gut, and has a wide range of symptoms and problems [1].

There is no single test that can be used to diagnose CD. There is currently no cure for CD, but patients can control and eradicate symptoms by following a rigorous GF diet, which is easier said than done due to the difficulties in acquiring components to satisfy recommended daily nutritional intake [2].

Due to the growing number of gluten-intolerant and healthy individuals preferring to follow a GF diet, there has been a surge in demand for GF products recently [3]. The demand for GF products is continuously growing, with a global market valued at USD 21.61 billion in 2019 and is projected to reach almost USD 24 billion by 2027 [4].

In celiac patients, strict adherence to a gluten-free diet reduces consumption protein, fiber, minerals, phenols and micronutrient. Gluten-free products found to be high in carbs and lipids only because they are manufactured with refined gluten-free flour or starch that has not been enriched or fortified like their wheat-based counterparts [5]. Alternative nutrient-dense raw materials derived from non-gluten cereals, pseudocereals, legumes, seeds can be used to improve gluten-free products with improved physical and sensory properties as well as improved nutritional composition [6].

As the demand for GF products has grown, so has the research into various forms of GF flours.

These alternative flours were chosen based on unique qualities such as functional roles, cost, and nutritional and quality aspects of the finished goods. Rice, millet, corn, sorghum, chickpea, maize, and soybean flour, as well as pseudocereals like buckwheat, amaranth, and quinoa, have recently been employed as wheat flour substitutes, and they are high in phytochemicals that are beneficial to consumers' health [7].

Rice flour is the most commonly used GF flour since it is natural, hypoallergenic, colorless, and mild tasting. It also has a low protein, salt, and fat content while being high in easily digestible carbs. Because of its mild flavor, it can be mixed with other GF flours such chestnut, chia, quinoa, corn, sorghum flour, and so on [8,9].

In the GF diet, the pseudocereals amaranth, quinoa, and buckwheat are emerging as nutritious alternatives to gluten-containing grains. They're not only naturally GF, but they're also high in a variety of nutrients [10].

Millet has a higher nutritional value than other cereals and deserves to be recognized for its possible health advantages. Millet has antioxidant, antibacterial, anti-inflammatory, antiviral, anticancer, antiplatelet aggregation, and cataract genesis inhibitory properties, so it may be employed as a source of nutraceutical and functional food ingredients in health promotion [11].

In addition, legumes are a good source of protein. In addition to their nutritional qualities, legume proteins have functional capabilities that are crucial in the formulation and processing of foods [12,13]. Because of the benefits connected with legumes, as well as the rising number of celiac sufferers, some have suggested that their research might be used as a substitute to typical flours in the preparation of GF products.

GF products with a greater micronutrient content are critical for improving the health of people who must follow a GF diet [14,15]. An increased availability of palatable gluten-free foods made from nutrient-rich grains such as the quinoa, buckwheat, millet and chickpeas would represent a significant step towards ensuring that celiac patients consume a nutritionally adequate diet.

The aim of this work was to evaluate physico-chemical, functional and antioxidant properties of some flours types as GF ingredients regarding their chemical composition in order to determine their contribution to the daily intake of nutrients. Special emphasis has been addressed to the protein, minerals, fiber content, and natural antioxidants such as phenolic compounds and compared with gluten-containing counterparts (wheat flour).

2. MATERIALS AND METHODS

2.1 Materials

Material Investigated samples are the most commonly used components included in the composition of GF products: WF (72% extraction), rice, quinoa, buckwheat, millet and chickpeas flours was purchased from Agricultural Research Center at Giza governorate, Egypt.

2.2 Analytical Methods

2.2.1 Gross chemical composition

The chemical composition of WF and GF formulas flours including moisture, protein, fat, ash, crude fiber and starch contents (on dry weight basis) was determined according to official methods as described in [16]. Carbohydrate was calculated by the difference (100- (protein + fat + ash) on the dry weight. All determinations performed in triplicates and the means and standard deviation was reported. The caloric value was calculated using value of 4 Kcal/g protein, carbohydrates and 9 Kcal/g fat according to [17].

2.2.2 Total Phenolic Compounds (TPC)

TPC of sample was determined using folin-ciocalteu reagent according to [18] with some modifications. A 0.1 ml of the sample extract was mixed with 0.9 ml Folin–Ciocalteu reagent (previously diluted 10 fold with distilled water) and allowed to stand for 5 min before the addition of 0.75 ml of 7% sodium bicarbonate. After 90 min, absorbance was measured at 725 nm using a UV–vis spectrophotometer. The

blank contains ethanol and water (1:1v/v) and the reagents. The calibration curve was prepared by measuring the absorbance of known concentrations of gallic acid. Total phenolic contents was expressed as gallic acid equivalent (mg/100g GAE) on dry weight basis [19].

2.2.3 Determination of antioxidant activity

Samples were extracted using methods described by Zielijski et al. [20]. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was carried out according to the method described by Lee et al. [21] with some modifications. The stock reagent solution (10^{-3} Mol) was prepared by dissolving 22 mg of (DPPH) in 50 ml of methanol and stored at 20°C until use. The working solution (6×10^{-5} Mol) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extract solution of tested samples (0.1 ml) was vortexed for 30 s with 3.9 ml of DPPH solution and left to react for 30 min, after which the absorbance was measured at 515 nm and recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Ab control} - \text{Ab sample}) / \text{Ab control}] \times 100}{1}$$

Where Ab is the absorbance at 515 nm.

2.2.4 Functional properties measurements

2.2.4.1 Water holding capacity

Method of [22] was implemented to determine water retention capacity of flour under a centrifugal force of 1000xg. Five grams of flour was mixed with an excess of water (25 ml) and then centrifuged at 1000xg for 15 min. The supernatant was decanted, the tube was weighed, and the absorbed water was calculated by difference (sediment weight minus sample weight).

2.2.4.2 Oil holding capacity

Oil holding capacity determination was carried out according the method described by Sosulski et al. [23]. 0.5 g of sample was mixed with corn oil (6 ml) in pre weighed centrifuge tubes and stirred for one minute to get a complete dispersion of the sample in the oil. After 30 min holding time, the sample was centrifuged at 3000 rpm for 25 min. The separated oil was then

removed with a pipette and the tubes was then allowed to stand for 25 min to remove the remained oil prior to reweight. The oil absorption capacity was expressed as grams of oil absorbed per gram of the sample.

2.2.4.3 Solubility

Solubility was determined according to the method proposed by Morr et al. [24]. The water-soluble fraction was obtained using a simple water extraction (flour to distilled water 1:10), with constant stirring (150 rpm). The extracts was centrifuged for 10 minutes at 5,000 rpm and the supernatant was separated and filtered through filter paper Whatman No. 1 in a 100 ml measuring flask and finally dilute with distilled water to the mark. Aliquots of extract was used for determination of soluble protein by semi-micro Kjeldahl method [25]. The determinations was carried out in triplicate. Soluble protein was calculated as percent of total protein of sample.

2.2.4.4 Emulsion Stability

Emulsion stability of wheat flour as control and GF formulas flours was measured according to the method described by Yasumatsu et al. [26]. The emulsion was prepared using 2 g of samples, 20 ml distilled water and 20 ml of olive oil. The solutions was blended for 120 s to form an emulsion in a Braun Blender at 1600 rpm. The emulsion was transferred to calibrated centrifuge tube and the total height of the liquid was measured (HT). The emulsion stability was estimated after heating the emulsion in a calibrated centrifuge tube at 80°C for 30 min in a water bath, cooled for 15 min under running tap water then centrifuged at 2000xg for 15 min and the height of the emulsified layer (H1) was recorded. Emulsion stability was calculated as $(\%) = (H1 / HT) \times 100$.

2.2.4.5 Foam stability

Foam stability was determined as described by Narayana et al. [27] with some modifications. 2 g of flour sample was mixed with 40 ml distilled water using a Braun Blender at 30°C in a 100 ml measuring cylinder. The suspension was stirred and shaken for 5 min at 1600 rpm to produce foam and the foam stability was expressed as the volume of foam over a time period from 0 to 60 min. The volume of foam was measured after 0 min (VT) and the volume of foam after 60 min (V1) was recorded. Foaming stability was expressed as $\% (V1 / VT) 100\%$.

2.2.5 Statistical analysis

The data was subjected to one-way analysis of variance (ANOVA) and significant difference ($p < 0.05$) was determined by Duncan's test using the (SPSS 25.0 software statistical package program, Inc., Chicago, IL, USA) [28].

3. RESULTS AND DISCUSSION

3.1 Gross Chemical Composition and Caloric Values

The chemical composition of some GF flours types and wheat flour are presented in Table 1. The moisture content of GF flours ranged between 10.34 – 11.71% and 11.30% for wheat flour (72% extraction). It could be demonstrated that, chickpeas flour (CF) contained the highest values in protein and ash (22.52 and 2.90% on dry weight basis), whereas it showed the lowest values in starch and total carbohydrates (61.89 and 66.82% on dry weight basis, respectively). Asif et al. [29] reported that chickpeas contain a moderately high amount of proteins (17 - 24%), have a low fat content and available carbohydrate (6.48 and 50%, respectively), and ash contents (2.95%).

While, quinoa (QF) and buckwheat flours (BF) contained the highest values in fat and crude fiber (6.09 and 5.56% on dry weight basis, respectively). A growing number of studies, which were conducted on the intake of fiber in the dietary pattern of celiac patients have shown lower intake of dietary fiber [30,31]. Carbohydrate content in buckwheat flour is lower than wheat flour, but the fiber composition is contrary because, buckwheat is rich in fiber [32]. The lipid content of quinoa is between 2 and 3 times higher than in other cereals such as maize and wheat [33]. Quinoa fat content (ranges from 5.2 to 9.7 %) is higher than maize (4.7 %) and lower than soy (18.9 %) [34,35], quinoa has been considered an alternative oilseed crop [35].

On the contrary rice flour (RF) contained the lowest values of protein (7.28%), fat (2.03%), ash (0.45%) and crude fiber (0.34% on dry weight basis) compared to its counterparts in other flour samples, while it showed the highest values in moisture, starch and carbohydrates (11.71, 88.91 and 89.90% on dry weight basis). Rice flour is one of the best cereal flours for making GF items because of its natural, hypoallergenic, colorless, and bland taste. It also has a low protein,

sodium, fat, and fiber content, as well as a large number of easily digestible carbs [8,9].

Whereas wheat flour (72% extraction) showed highest value of caloric value (Kcal) (407.50 Kcal/100g on dry weight basis), but when comparing to most, it is not significant.

3.2 Total Phenolic Compound and Antioxidant Activity

The total phenolic compound and antioxidant activity of some GF flours types and wheat flour are shown in Table 2. Showing results of total phenolic compound, buckwheat flour present the highest value of total phenolic compound and antioxidant activity with 517.92 mg/100g and 43.83%, respectively; followed by quinoa flour (279.89 mg/100g and 16.92%) on dry weight basis.

Polyphenol compounds have been intensively explored for health-promoting qualities and their role in the prevention of degenerative diseases such as cancer and cardiovascular disease over the last two decades [36]. Buckwheat is one of the best grains for polyphenol compounds [37], with glycosides of the flavonol quercetin being the most abundant polyphenols, followed by glycosides of the flavones apigenin and luteolin [38]. Quinoa seeds are also high in flavonoids, which mostly consist of glycosides of the flavonols kaempferol and quercetin [39].

Whereas, the total phenolic compound content and antioxidant activity were (232.19 and 163.99 mg/100g on dry weight basis) and (8.20 and 4.27%) for chickpea and millet flours, respectively; on dry weight basis. Chickpeas and millet have high levels of polyphenols with good antioxidant properties [40,11].

On the other hand, samples rice and wheat flours had the lowest content of total phenolic compound (50.67 and 147.56 mg/100g on dry weight basis) and antioxidant activity (2.75 and 4.26%), respectively compared to the other samples, this may be due to the difference in the composition of the bean, the difference in the variety, degree of grinding, and other factors.

3.3 Functional Properties

The functional properties of some GF flours types and wheat flour are summarized in Table 3. Protein concentrates, in particular, have been demonstrated to exhibit a variety of physicochemical properties that help determine a

variety of functional features, including water hydration capacity, foaming and emulsion capacity [41]. Non-gluten proteins are so attractive to include in the creation of GF bread because of the nutritional and technological benefits they provide.

The most functional properties determined for flours samples exhibited somewhat close values with each other with some significant differences with each other at $p \leq 0.05$. Flours samples exhibited high values for the water holding capacity in samples BF (135.72%) and QF (118.62%). Water absorption and binding capacity are determined by the ability of protein in flours to physically bind water [42]. Because of their high dietary fiber content and natural antioxidants such as phenolic compounds, buckwheat and quinoa have promise as functional and bioactive components in food products [43].

Minor differences in oil holding capacity of raw flours were also observed. The mean values showed higher oil holding capacity for BF (123.29%), followed by QF (108.13%), WF (96.71%) and CF (93.68%), whereas, the lowest 90.54% was for RF. Kinsella et al. [44] explains the mechanism of fat/oil holding capacity as a physical entrapment of favor retention. Surface area and hydrophobicity, according to Chau et al. [45], improve oil holding capacity. Furthermore, dietary fiber has been shown to have functional roles in products such as increased water holding capacity, viscosity, oil holding capacity, and swelling capacity [46].

Some samples of flours showed higher values in the protein solubility, except the sample WF and BF (8.89 and 8.90, respectively), these was not a significant difference between them. The solubility of a protein is usually affected by its hydrophobicity or hydrophobic balance, depending on the amino acid composition, particularly at the protein surface [47]. Foaming functionalities and the solubility of the proteins in the flours significantly correlated with dough properties, which in turn effect the final bread quality [48]. In addition, the solubility of the proteins was found to have a similar or synergetic effect on the peak viscosity ($p < 0.05$), time and temperature. It is suggested that soluble proteins distribute more evenly in the liquid phase, creating a stronger network by linear aggregation when they denature, compared to the random aggregations formed by insoluble proteins [49].

Table 1. Gross chemical composition and caloric value of some GF flours types and wheat flour

Samples flours	Moisture (%)	Protein (%)*	Fat (%)*	Ash (%)*	Crude fiber (%)*	Starch (%)*	Carbohydrates (%)**	Caloric Value (Kcal/100g)*
WF	11.30±0.07 ^b	12.26±0.51 ^d	2.46±0.13 ^d	0.59±0.01 ^d	0.61±0.04 ^e	82.57±1.70 ^c	84.08±0.55 ^c	407.50±0.73 ^a
QF	10.34±0.07 ^d	13.07±0.47 ^c	6.09±0.12 ^a	2.40±0.03 ^b	3.63±0.44 ^b	71.09±1.42 ^e	74.81±0.80 ^e	406.33±2.42 ^a
BF	10.72±0.08 ^c	16.23±0.36 ^b	3.37±0.29 ^c	2.37±0.05 ^b	5.56±0.36 ^a	69.21±1.88 ^e	72.47±0.82 ^f	385.13±1.71 ^d
MF	11.16±0.04 ^b	12.05±0.31 ^d	3.40±0.12 ^c	1.27±0.02 ^c	3.08±0.24 ^c	77.78±1.87 ^d	80.20±0.48 ^d	399.60±0.46 ^c
RF	11.71±0.03 ^a	7.28±0.14 ^e	2.03±0.07 ^e	0.45±0.04 ^e	0.34±0.05 ^{et}	88.91±2.67 ^b	89.90±0.22 ^b	406.99±0.32 ^a
CF	11.43±0.36 ^b	22.52±0.16 ^a	5.34±0.27 ^b	2.90±0.08 ^a	2.42±0.13 ^d	61.89±1.06 ^f	66.82±0.52 ^g	405.42±0.49 ^a

*On dry weight basis **Carbohydrates calculated by difference. - **WF**: wheat flour (72% extraction); **QF**: quinoa flour; **BF**: buckwheat flour; **MF**: millet flour; **RF**: rice flour; **CF**: chickpeas flour. - Values are the mean of triplicate determinations with standard division.

- The different letters at the column mean significant differences at ($p \leq 0.05$), and the same letters mean no significant differences.

Table 2. Total phenolic compound and the antioxidant activity of some GF flours types and wheat flour

Samples flours	TPC (mg/100g GAE)*	Antioxidant activity (%)*
WF	147.56±13.42 ^d	4.26±0.34 ^d
QF	279.89±15.09 ^b	16.92±0.57 ^b
BF	517.92±33.87 ^a	43.83±5.16 ^a
MF	163.99±25.24 ^d	4.27±0.32 ^d
RF	50.67±5.50 ^e	2.75±0.44 ^d
CF	232.19±32.13 ^c	8.20±0.07 ^c

*On dry weight basis **TPC**: Total phenolic compounds

- Abbreviations for symbols WF, QF, BF, MF, RF and CF see footnote of Table (1).

- Values are the mean of triplicate determinations with standard division.

- The different letters at the column mean significant differences at ($p \leq 0.05$), and the same letters mean no significant differences.

Table 3. Functional properties of some GF flours types and wheat flour

Samples flours	WHC (%)	OHC (%)	Soluble protein as % of total sample protein	Emulsion stability (%)	Foam stability (%)*
WF	100.38±5.95 ^c	96.71±3.93 ^c	8.89±1.79 ^b	47.97±0.68 ^b	77.71±1.12 ^b
QF	118.62±2.97 ^b	108.13±1.88 ^b	11.40±0.76 ^b	34.09±2.27 ^d	62.31±2.17 ^c
BF	135.72±2.88 ^a	123.29±3.85 ^a	8.90±0.34 ^b	46.78±3.62 ^{bc}	78.31±1.42 ^b
MF	95.01±3.06 ^c	91.73±3.60 ^c	10.75±0.87 ^b	43.17±2.26 ^c	78.91±2.18 ^b
RF	92.51±4.84 ^c	90.54±4.33 ^c	13.39±1.44 ^b	45.45±2.27 ^{bc}	83.06±2.03 ^b
CF	97.68±9.98 ^c	93.68±2.03 ^c	9.06±1.42 ^b	55.33±2.02 ^a	102.81±7.66 ^a

- **WHC**: Water holding capacity; **OHC**: Oil holding capacity. *Foaming stability (%) after 30 min.

- Abbreviations for symbols WF, QF, BF, MF, RF and CF see footnote of Table (1).

- Values are the mean of triplicate determinations with standard division.

- The different letters at the column mean significant differences at ($p \leq 0.05$), and the same letters mean no significant differences.

The values of emulsion stability and foam stability higher in the CF sample (55.33 and 102.81, respectively) compared to other types of raw flour. The ability of proteins to contribute stability to an emulsion for resistance to stress and changes is generally reflected in emulsion stability, which is related to the consistency of the interfacial area throughout time [50].

Foam formation and stability are largely determined by the interfacial coating generated by proteins, which keeps air bubbles suspended and prevents coalescence. Foaming properties are influenced by proteins as well as other ingredients in the flour, such as carbohydrates [51]. Proteins with high foaming properties lead to a higher dough viscosity [48].

Legume proteins, such as chickpea flour, exhibit a variety of functional properties, including water holding capacity, fat binding, foaming, and gelation, making them a suitable raw material for a number of food products [52]. Also, Chickpea liquid, known as "aquafaba" is used as an egg

replacer because of its foaming properties. On other hand, the amino acid content, protein structure, and conformation, as well as processing variables like pH and temperature, and interactions between proteins and other food components like salts, fats, carbohydrates, and phenolics, all influence the functional properties of food proteins.

When gluten is removed, GF products perform more like gluten containing one when added an alternative rich in nutrients and functional properties such as chickpea, buckwheat, quinoa, and millet flours.

4. CONCLUSIONS

The need for research to generate novel products from GF ingredients such grains that suit the nutritional, aesthetic, and organoleptic criteria of end consumers is expanding, as is the demand for GF food. Some are high in phytochemicals, which boost the nutritional worth of their final products.

Because of their high protein content and quality, pseudocereals (buckwheat and quinoa) are considered as promising dietary sources. They also contain bioactive and useful elements including dietary fiber and natural antioxidants like phenolic compounds. Bread, biscuits, cakes, and pasta are the most commonly consumed and thus appropriate carriers for protein enrichment due to their diverse applicability in diets. Protein, fat, fiber, and minerals were significantly higher in GF products containing pseudocereals. These pseudocereals can be considered GF products and can be included in the diet of CD patients.

Rice, millet, and chickpeas are all healthy cereals to eat if you have CD. When used as a substitute for typical GF ingredients like refined GF flours and starches in GF products. The nutritional value of these products is in line with current dietary and product recommendations for CD diets. The findings imply that buckwheat, quinoa and chickpeas the best samples in this study for make nutrient-dense GF foods.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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