Original Research Article

Physico-Chemical, Functional and Antioxidant Evaluation of Some Gluten-free Flours Formulas Compared with Available Commercial Formula

Abstract

Gluten-free (GF) products are made using commercial flours formulas and are poor in the protein, fiber, minerals and have weak physical properties that affect the quality of the final products. These factors are responsible for hampering adherence to the GF diet and for general dissatisfaction. The aim of this work was to physico-chemical, functional and antioxidant evaluation of some combinations of GF flours formulas that have been prepared compared with available GF commercial flour formula in the local market. The moisture content of GFF formula sold in the local market used in the research was 12.60%. On the other hand, the prepared formulas' moisture content ranged from 12.23% (F2) to 12.90% (F3). F2 and F4 formulas had the highest protein content, with no significant difference ($p<0.05$). GFF formula had the lowest protein content, about half the amount (5.07% on dry weight basis (dwb)). In comparison to control (GFF), F2 flour had more double the amount of ash and crude fiber. The ash and crude fiber contents of the various formulas differed significantly. The GFF had the lowest ash and crude fiber content (0.51 and 0.31%, respectively on dwb). The highest values of total phenolic compound and antioxidant activity was in F2 formula (313.15 mg/100g and 7.95%, respectively), followed by F4 formula (226.56 mg/100g and 7.22%, respectively), then F1 formula (223.57 mg/100g and 6.62%, respectively) on dwb. While, the lowest value was in the commercial formula sold in the local market (GFF) (75.10 mg/100g and 3.23%, respectively) on dwb. GF formulas flours exhibited high values for the water holding capacity in samples F2 (164.98%) and F1 (134.17%). While, GFF formula flour showed lower water binding capacity in comparison to other GF formulas flours. Significant differences in oil holding capacity of GF formulas flours were also observed. The mean values showed higher oil holding capacity for F2 (145.92%), followed by F4 (138.51%), F1 (130.11%) and F3 (126.64%), whereas, the lowest 75.43% was for GFF. The GF composite flour samples close values and non-significant variations at $p\leq0.05$

in the protein solubility. The increase in the values of emulsion stability and foam stability determined for GF flours formulas were significant at $p \leq 0.05$ as compared with those determined for GFF flour sample.

Key Words: Gluten-free, Products, Formulas, GF commercial flour, Local market

1. Introduction

In the last two decades, there has been an upsurge in the demand for GF products. The main origin of the GF trend can be traced to increased diagnosis of celiac disease (CD); a genetic enteropathy characterized by the inability to digest gluten proteins that are present in certain seeds such as wheat, barley, and rye, and in minors grains like oat, triticale and spelt **[1, 2]**.

The population of world suffering from celiac, gluten intolerance, and wheat allergy is 1-2 in every 100 people. Also, many are choosing GF diets nowadays because of the perception that it is a healthier option for them. Therefore, in the last decade, the GF market in the all over the world has seen significant growth. Globally, GF product sales reached 4.63 billion USD in 2017, and are expected to reach 6.47 billion USD by 2023 **[3]**.

Despite the growth of the GF market, individuals with CD still have trouble finding GF products because of not widely available and are poor in quality and more expensive than gluten-containing products, and they may lead to nutritional deficiencies in micronutrients and fiber **[4]**.

Studies have assessed the nutritional quality and adequacy of a GF diet the results reflected the fact that GF products are not nutritionally superior. Many GF foods were found to be deficient in several nutrients, contained significantly lower protein, fiber and minerals and higher fat and carbohydrates content **[5, 6]**. The findings also indicated that a GF diet was associated with higher energy **[7]**. It has been highlighted that the ideal GF diet should be nutrient-dense with naturally GF foods, balanced with macro- and micronutrients as well as reasonably priced, and easily accessible **[8]**. In another study, the sensory properties of GF food were found to be effective not only celiac patients but also non-celiac consumers' compliance with the GF diet **[9]**.

Between 20 and 38% of patients with CD have complications due to nutritional deficiencies, likely to be caused by GF foodstuffs having poor nutrition, or an imbalanced diet outside of GF foods **[10, 11]**.

The following of a GF diet is not simple for celiac patients since such a diet may cause nutrient deficiencies of some macro and micronutrients as well as lead to excess intake of saturated fats and carbohydrates. Consequently, most patients are more vulnerable to nutrient-related deficiencies such as osteoporosis, anemia, and failure to thrive **[12]**.

More recently however, GF cereals have been used to develop novel breads, pastas, breakfast cereals and puffed snacks. Many of these ingredients are grain-based. Some have a comparable or even better nutrient profile as traditional gluten grains like wheat and barley, and they are very rich in phytochemicals that are important to the health of consumers **[13]**. Alternative flours that are being researched include pseudocereals (quinoa, amaranth, buckwheat), cereals (millet, sorghum, teff, maize, rice), legumes (chickpea, soy, carob germ), among others **[14]**.

In order to compensate for the lack of gluten protein and hence to counteract the technological problems, several additives such as hydrocolloids, emulsifiers, enzymes, dairy proteins, etc. have been employed in GF formulations **[15]**. Hydrocolloids are long-chain polymers formed by polysaccharides and proteins. Their ability to modify rheological properties in the dough is what makes them valuable functional ingredients in making GF breads **[16]**.

GF products can also be produced with the combination of alternative flour and flour& starch types. Starches can be used in GF products. They provide better hydrolysis and improved gelatinization behavior with. Rice, corn, potato, cassava, sorghum, and tapioca have been widely used as starches types in GF formulations **[15]**.

The aim of this work was to physico-chemical, functional and antioxidant evaluation of some combinations of GF flours formulas that have been prepared compared with available GF commercial flour formula in the local market. For overcoming the drawbacks associated with GF commercial formals by finding alternative sources rich in various micronutrients. And that it has good functional properties that do not affect the quality of the final products, which helps to adhere to a GF diet.

2. Materials and Methods

2.1. Materials

Material Investigated samples are the most commonly used components included in the composition of GF products: rice, quinoa, buckwheat, millet, chickpeas flours and corn starch was purchased from Agricultural Research Center, Giza, Egypt. While, GF flour product (GFF) was purchased from local market at Assiut governorate, Egypt. Xanthan gum (XG) were obtained from Sigma Company, Germany.

GF flour formula sold in the local market consist from flour blend of brown rice, white rice, quinoa flours and corn starch, in addition to arabic gum was used in this research. While, GF flour formulas was made it from rice, quinoa, buckwheat, millet, chickpeas flours and corn starch in addition xanthan gum in various proportions **(Table 1)**.

Table 1: GF flours formulas and GF flour formula sold in the local market

Ingredients (%)	GFF	F1	F2	F3	F4
Gluten-free flour	100	-	-	-	-
Quinoa flour	-	30	-	-	10
Buckwheat flour	-	-	30	-	10
Millet flour	-	-	-	30	10
Rice flour	-	50	50	50	50
Chickpeas flour	-	10	10	10	10
Corn starch	-	10	10	10	10
Xanthan gum	-	2	2	2	2

GFF: gluten-free formula sold in the local market, F1: 30% quinoa flour + 50% rice flour + 10% chickpeas flour + 10% corn starch + 2% XG, F2: 30% buckwheat flour + 50% rice flour + 10% chickpeas flour + 10% corn starch + 2% XG, F3: 30% millet flour + 50% rice flour + 10% chickpeas flour + 10% corn starch + 2% XG, and F4: 10% quinoa flour + 10% buckwheat flour + 10% millet flour + 50% rice flour + 10% chickpeas flour + 10% corn starch + 2% XG.

2.2. Analytical Methods

2.2.1. Gross Chemical Composition

The chemical composition of GF flours formulas and GF flour formula sold in the local market including moisture, protein, fat, ash, crude fiber and starch contents (on dry weight basis) was determined according to official methods as described in **[17]**. 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 **[18]**.

2.2.2. Total Phenolic Compounds

Total phenolic compounds of sample was determined using folin- ciocalteu reagent according to **[19]** 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 **[20]**.

2.2.3. Determination of Antioxidant Activity

Samples were extracted using methods described by **[21]**. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was carried out according to the method described by **[22]** 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 vortexes 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:

DPPH radical scavenging activity (%) = [(Ab control - Ab sample) / Ab control] X 100. Where Ab is the absorbance at 515 nm.

2.2.4. Functional Properties Measurements

2.2.4.1. Water Holding Capacity

Method of **[23]** 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 **[24]**. 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 **[25]**. 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 **[26]**. 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 GF flour formula sold in the local market as control and GF flours formulas was measured according to the method described by **[27]**. 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 at1600 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 ºC 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) X 100.

2.2.4.5. Foam Stability

Foam stability was determined as described by **[28]** with some modifications. 2 g of flour sample was mixed with 40 ml distilled water using a Braun Blender at 30ºC 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) **[29]**.

3. Results and Discussion

3.1. Gross chemical composition and caloric values of GF flours formulas and GF flour formula sold in the local market

The chemical compositions and caloric values of GF flours formulas and GF flour formula sold in the local market are shown in **Table 2**. The moisture content of GFF formula sold in the local market used in the research was 12.60%. On the other hand, the prepared formulas' moisture content ranged from 12.23% (F2) to 12.90% (F3). The low moisture content discovered suggested that it had the potential for increased storage stability as well as a longer shelf life. This finding is line with the observations of **[30]**, who found that moisture flour with a moisture content of up to 12% had better storage stability.

F2 and F4 had the highest protein content, with no significant difference ($p<0.05$). GFF had the lowest protein content, about half the amount (5.07% on dwb). F1 formula had the highest fat content (2.96% on dwb). This result could be attributed to quinoa's fat content **[31]**. Furthermore, no significant differences in fat content ($p<0.05$) were found between other GF flours formulas and control.

In comparison to control, F2 flour had more double the amount of ash and crude fiber. The ash and crude fiber contents of the various formulas differed significantly. The GFF had the lowest ash and crude fiber content (0.51 and 0.31%, respectively on dwb). High-ash samples may increase the mineral content of newly formulated flour **[32]**. Furthermore, there was no significant difference in starch content ($p<0.05$) between the formulas F1 and F3, while the GFF formula had the highest level of starch (90.02%, on dwb).

Finally, the GFF formula had the highest carbohydrate content and caloric value (91.88% and 407.80 Kcal /100 g, respectively), while the F2 formula had the lowest values (83.25 and 401.70.43 Kcal /100 g, respectively) on dwb. Results obtained in the study were in close agreement with those previously reported **[33, 34, 35]**.

Table 2: GF flours formulas and GF flour formula sold in the local market

Formulas	Moisture (%)	Protein (%)*	Fat (%)*	Ash (%)*	Crude fiber (%)*	Starch (%)*	Carbohydrates (%)**	Caloric Value (Kcal/100 g)*
GFF	12.60±0.06^{b}	5.07±0.18^{c}	2.23±0.09^{b}	0.51±0.08^{e}	0.31±0.09^{c}	90.02±1.17^{a}	91.88±0.22^{a}	407.87±0.50^{a}
F_1	12.50±0.01^{b}	11.48±0.07^{b}	2.96±0.44^{a}	1.21±0.02^{b}	0.83±0.06^{b}	80.86±2.71^{bcd}	83.52±0.37^{cd}	406.64±2.36^{a}
F_2	12.23±0.08^{c}	12.25±0.16^{a}	2.19±0.06^{b}	1.30±0.02^{a}	1.01±0.10^{a}	78.34±2.27^{d}	83.25±0.12^{d}	401.71±0.43^{c}
F_3	12.90±0.01^{a}	11.18±0.17^{b}	2.13±0.02^{b}	0.84±0.01^{d}	0.76±0.08^{b}	83.87±2.48^{b}	85.09±0.27^{b}	404.25±0.24^{b}
F_4	12.50±0.03^{b}	12.13±0.13^{a}	2.44±0.09^{b}	1.14±0.01^{c}	0.88±0.13^{ab}	79.49±1.58^{cd}	83.41±0.22^{d}	404.12±0.84^{b}

*On dry weight basis. **Carbohydrates calculated by difference. - Abbreviations for symbols GFF, F1, F2, F3 and F4 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.

5.2.2. **Total phenolic compound and the antioxidant activity of GF flours formulas and GF flour formula sold in the local market**

The total phenolic compound and antioxidant activity of GF flours formulas and GF flour formula sold in the local market are shown in **Table 3**. The values of total phenolic compounds showed raise in GF formulas flours and the results indicated highly significant differences at P<0.05 between GFF and GF formulas flours. The highest values of total phenolic compound and antioxidant activity was in F2 formula (313.15 mg/100g and 7.95%, respectively), followed by F4 formula (226.56 mg/100g and 7.22%, respectively), then F1 formula (223.57 mg/100g and 6.62%, respectively) on dwb.

While, the lowest value was in the commercial formula sold in the local market GFF (75.10 mg/100g and 3.23%, respectively) on dwb. Polyphenols have been traditionally considered undesirable components in food products because they may cause darkening due to oxidation of phenols, leading to formation of dark pigments.

Table 3: Total phenolic compound and the antioxidant activity of GF flours formulas and GF flour formula sold in the local market

Formulas	TPC (mg/100g GAE)*	Antioxidant activity (%)*
GFF	75.10 ± 3.61^{d}	3.23 ± 0.44^{d}
F1	223.57 ± 20.38^{b}	6.62 ± 0.26^{b}
F2	313.15 ± 32.38^{a}	7.95 ± 0.19^{a}
F3	151.22 ± 9.65^{c}	4.27 ± 0.92^{c}
F4	226.56 ± 29.22^{b}	7.22 ± 0.19^{ab}

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

- Abbreviations for symbols GFF, F1, F2, F3 and F4 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≤0.05), and the same letters mean no significant differences.

In addition, they have been considered anti-nutritional components because they can react with certain essential amino acids, limiting their availability **[36]**. Nevertheless, in more recent years, polyphenols in general, and flavonoids in particular, have been recognized as food components with health-promoting properties, including antioxidant and anti-proliferative activities in cells **[37, 38]**.

5.2.3. Functional properties of GF flours formulas and GF flour formula sold in the local market

The functional properties of GF flours formulas and GF flour formula sold in the local market are summarized in **Table 3**. The most functional properties determined for GF formulas flours exhibited higher values than that observed for GFF (except for the soluble protein as % of total sample protein) and showed significant variations at $p \leq 0.05$. GF formulas flours exhibited high values for the water holding capacity in samples F2 (164.98%) and F1 (134.17%), which may be due to the high protein content (12.25%) in the sample F2. The ability of protein in flours to bind water physically is a determinant of its water absorption and binding capacity **[39]**. GFF formula flour showed lower water binding capacity in comparison to other GF formulas flours **(Table 3)**.

Significant differences in oil holding capacity of GF formulas flours were also observed. The mean values showed higher oil holding capacity for F2 (145.92%), followed by F4 (138.51%), F1 (130.11%) and F3 (126.64%), whereas, the lowest 75.43% was for GFF. The mechanism of fat/oil holding capacity explained by **[40]** as a physical entrapment of favor retention. **[41]** reported that surface area and hydrophobicity improve oil holding capacity. The GF composite flour samples close values and non-significant variations at $p \leq 0.05$ in the protein solubility. 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 **[42]**.

Table 4: Functional properties of GF flours formulas and GF flour formula sold in the local market

Formulas	WHC (%)	OHC (%)	Soluble protein as % of total sample protein	Emulsion stability (%)	Foam stability (%)*
GFF	97.54±3.55[c]	75.43±2.55[d]	11.84±1.19[a]	42.49±0.66[c]	87.78±3.19[c]
F1	134.17±6.95[b]	130.11±6.06[bc]	11.67±0.43[a]	57.50±1.75[b]	105.09±2.66[b]
F2	164.98±13.85[a]	145.92±5.04[a]	9.33±0.42[b]	62.50±2.25[a]	114.23±5.48[a]
F3	127.56±2.04[b]	126.64±7.03[c]	8.19±0.50[b]	55.03±2.13[b]	100.52±3.40[b]
F4	139.88±4.15[b]	138.51±3.07[ab]	7.57±0.15[b]	65.21±1.94[a]	118.79±3.36[a]

- **WHC:** Water holding capacity; **OHC:** Oil holding capacity. *Foaming stability (%) after 30 min.
- Abbreviations for symbols GFF, F1, F2, F3 and F4 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 increase in the values of emulsion stability and foam stability determined for GF composite flours were significant at $p \leq 0.05$ as compared with those determined for GFF flour samples. The results obtained in this study indicated that composite flours from pseudocereals, millet and chickpea flours had good functional properties.

4. Conclusions

GF flours formulas made with rice, quinoa, buckwheat, millet, chickpeas flour and corn starch in addition to xanthan gum showed improvement in nutritional value by: increase in protein, fiber, minerals and antioxidants, as well as improved the physical properties of the formulas compared by the GF commercial formula available in the local market, which is distinguished by its high content of starch and carbohydrates. The findings imply that F2, F4 and F1, respectively the best formulas in this study for make nutrient-dense GF foods.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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