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Evaluation of Some Gluten-Free Biscuits Formulations Comparison to Wheat Flour Biscuits

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THIS study aimed to produce healthy, new, and alternative gluten-freebiscuits (GFB). Four formulations were designed from rice, quinoa, buckwheat, millet, and chickpea flours, in addition to cornstarch and xanthan gum. Wheat flour (72% extraction) was the first control sample, and gluten-free (GF) commercial formulawas the second control sample. The physico-chemical, nutritional, and sensory characteristics of the produced biscuits were evaluated. The GF composite formulas biscuits exhibited elevated levels of ash and fibre in comparison to the two control samples. The protein ratio ranged also in GF composite formulas biscuits samples from 7.03 to 7.58%, and the GF commercial formula biscuitssample recorded 3.63%. Additionally, there was an augmentation for macro- and micro-elements compared with the two control samples, as well as an increase in the total essential amino acid level, which ranged from 33.49 to 34.39% and was 26.11 and 31.94% for the wheat flour biscuits (WFB) and GF commercial formula biscuits, respectively. In the same vein, the biscuit samples prepared using GF composite flour also had a higher protein efficiency ratio, biological value, and in vitro protein digestibility (with both pepsin and pepsin followed by trypsin) compared with two control samples. On the other hand, the WFBsample recorded an increment in width and thickness compared with GFB samples; minimum width and thickness were observed in the GF commercial formula biscuits sample. Sensory evaluation showed all samples of GF composite formulas biscuits were acceptable, with no significant differences with the WFB, and better than GF commercial formula biscuits ($p \leq 0.05$).

Keywords: Gluten-free biscuits, Wheat flour, Composite flour and commercial formula.

Introduction

Celiac disease (CD), which affects about 1–2% of the world's population, is a chronic enteropathy brought on by intolerance to gluten or, more specifically, to certain proteins known as prolamines. It results in intestinal villi atrophy, malabsorption, and clinical symptoms that can develop in both childhood and adulthood (Calado and Verdelho, 2022). When persons with CD consume gluten-containing products, their immune systems react by harming or killing the intestinal villi, impairing nutritional absorption and negatively affecting many bodily systems (Cardoso-Silva et al., 2019). The only treatment for CD until now is a GF diet. A GF diet provides advantages, including restoring small intestinal villi and reducing the risk of malignant complications (Di Cairano et al., 2018).

The GF diet is frequently imbalanced and lacks many nutrients. Although there have been some developments recently, celiac patients still require access to higher-quality products (Simon et al., 2023). The commercially available GF products frequently presented inferior nutritional quality in comparison to their gluten-containing equivalents (Giuberti and Gallo, 2017). One of the most used strategies for ameliorating the nutritional profile of GF products is the partial substitution of

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commonly used GF flours (e.g., maize and rice) with novel nutrition-dense ingredients (Ciudad-Mulero et al., 2020). It has been reported that the use of more nutrient dense flours could improve the nutritional quality of GF products (Cannas et al., 2020; Martinez-Villaluenga et al., 2020). Among these, corn, sorghum, chickpea, and soybean flour, as well as pseudocereals such as buckwheat, amaranth, and quinoa, have been employed as substitute flours for wheat flour (Demirkesen and Ozkaya, 2022). These compounds have a number of advantages over cereals, including a higher protein and amino acid content as well as greater digestibility. Bioactive compounds such as fructooligosaccharides, antioxidants, resistant starch, fibres, mineral resources, and other nutrients are also present (Raungrusmee, 2023). The possibility of making biscuits with 10% buckwheat flour, 50% brown rice flour, 30% potato starch, and 10% millet flakes was looked. The biscuits had a high moisture content and a dark surface colour (Schober et al., 2003). The addition of buckwheat to the biscuits altered their physical and chemical characteristics, resulting in enhanced spread, hardness, and fracturability (Filipčev et al., 2011). According to Baljeet et al. (2010), adding buckwheat from 20% to 50% increases the biscuits sensory qualities, biofunctional qualities, protein, fibre, micronutrients, polyphenolic content, and antioxidant activity. GF bakery products also require polymeric substances that emulate the viscoelastic properties of gluten in doughs; the most normally utilized are hydrocolloids such as xanthan gum (Abdelmegiud et al., 2024).

Besides bread products, biscuits are a common food product that a wide spectrum of people eats; they have a variety of tastes, a long shelf life, and are relatively inexpensive (Abdelmegiud, 2024). Because they are a convenient food loved by all demographics, biscuits provide a wonderful vehicle to deliver nutrients to celiac patients (Di Cairano et al., 2018). Additionally, it is simpler to make GFB than GF bread since gluten has a far smaller impact on the processability and final product quality of biscuits than it does on bread, allowing for the use of alternative flours in a variety of combinations (Xu et al., 2020). Due to market competition and growing consumer demand, efforts are being made to adjust the nutritional composition of biscuits to raise their nutritive value and functionality (Ghoshal and Kaushik, 2020). Therefore, the aim of the present work was to use rice, quinoa, buckwheat, millet, and chickpea flour, in addition to cornstarch and xanthan gum, for the development of GFB with acceptable sensory and high nutritional value and compare this product to WFB and GF commercial formula biscuits.

Materials and Methods

Materials

Quinoa, buckwheat, millet, and chickpea, as well as white rice, were obtained from the Agronomy Institute, Agriculture Research Center, Giza, Egypt. The GF commercial formula (Sonbolat Elforat) composed of white rice flour, brown rice flour, quinoa flour, cornstarch, and Arabic gum was purchased from the local market in Assiut city, Egypt. Other ingredients (wheat flour 72% extraction, shortening, sugar, salt, sodium bicarbonate, ammonium bicarbonate, and baking powder) were purchased from the local market in Assiut city, Egypt (Table 1). The Xanthan gum, all chemicals and reagents used in the analytical methods (analytical grade), were purchased from El-Gamhoria Trading Chemicals and Drugs, Assiut city, Egypt.

Preparation of GF flour

Quinoa, buckwheat, millet, and chickpea cereals were cleaned and freed of broken seeds, dust, and other foreign materials and were subsequently ground using an electric mill (Quadrumat Junior, Model Type No. 279002, Brabender OHG, Duisburg 1979, Germany) to obtain a fine powder, the particles of which were passed through a 20-hole/inch linear sieve and stored in polyethylene bags in the refrigerator until use. The approximate chemical composition of these grains was as follows: The content of moisture, protein, fat, fibre, ash, and carbohydrates for quinoa flour was 10.34, 13.07, 6.09, 2.40, 3.63, and 74.81%; for buckwheat flour, 10.72, 16.23, 3.37, 2.37, 5.56, and 72.47%; for millet flour, 11.16, 12.05, 3.40, 1.27, 3.08, and 80.20%; and for rice flour, 11.71, 7.28, 2.03, 0.45, 0.34, and 89.90%, respectively.

Preparation of GF composite flour

Four formulations of GF cereal flours were prepared (F1, F2, F3, and F4), as shown in Table 1. The flour mixtures were blended and homogenized, packed in polyethylene bags, tightly closed, and stored at room temperature until used in the preparation of biscuits to be compared with WFB and the GF commercial formula biscuits.

Methods

Technological processes

Preparation of biscuits:

The method used to prepare the biscuits was the Sai Manoharm and Rao (1997) method, using the ingredients in Table 1 and shown in Fig 1.

TABLE 1. Ingredients used in preparation of biscuits.

Ingredients	Control 1	Control 2	F1	F2	F3	F4
Wheat flour (%)	100	-	-	-	-	-
GF commercial formula (%)	-	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
Cornstarch (%)	-	-	10	10	10	10
Xanthan gum (g)	-	-	2	2	2	2
Shortening (g)	20	20	20	20	20	20
Sugar (g)	30	30	30	30	30	30
Salt (g)	1	1	1	1	1	1
Sodium bicarbonate (g)	0.5	0.5	0.5	0.5	0.5	0.5
Ammonium bicarbonate (g)	1	1	1	1	1	1
Baking powder (g)	0.3	0.3	0.3	0.3	0.3	0.3
Water (ml)	16	16	16	16	16	16

- Control 1: 100% wheat flour 72% extraction, control 2: 100% gluten-free commercial formula, F1: 30% quinoa flour+50% rice flour+10% chickpeas flour+10% cornstarch,F2: 30% buckwheat flour+50% rice flour+10% chickpeas flour+10% cornstarch, F3: 30% millet flour+50% rice flour+10% chickpeas flour+10% cornstarch and F4: 10% quinoa flour+10% buckwheat flour+10% millet flour+50% rice flour+10% chickpeas flour+10% cornstarch.

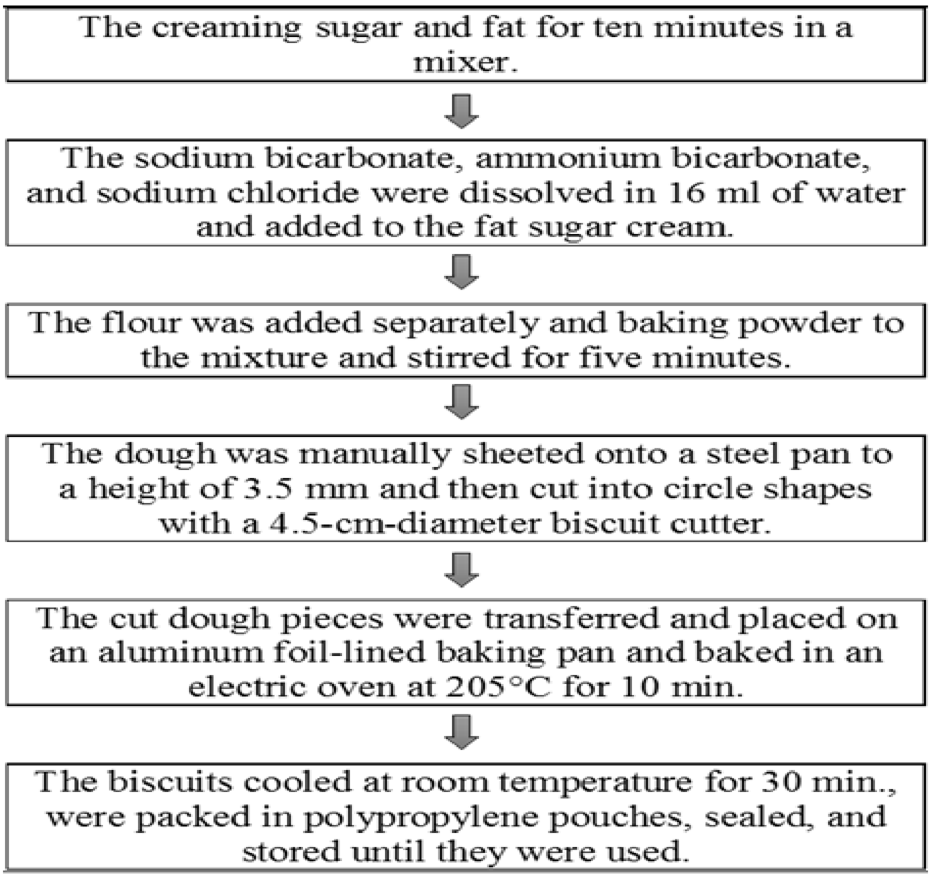

Fig. 1. The biscuits preparation process.

Analytical methods

Chemical composition:

Moisture, protein, fat, ash, crude fibre, and reducing and non-reducing sugars contents were determined according to official methods (AOAC, 2019). Carbohydrate content was determined by difference [% Carbohydrate = 100 - (moisture + protein + crude fat + ash + crude fibre]. The energy values were calculated using 2 kcal/g for fibre, 4 kcal/g for protein and carbohydrates, and 9 kcal/g for fat (Maclean et al., 2003).

Minerals composition:

Minerals composition was determined through extraction from the biscuits samples via the dry ashing method (Jackosn, 1973). Atomic absorption spectrophotometry (Perkin-Elmer Model 5000, Germany) was used to quantify iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), and magnesium (Mg). The sodium (Na) and potassium (K) were determined by flame photometric procedure (Corning instrument model 400) (Chapman et al., 1962), and phosphorus (P) was measured by ammonium molybdate method using a Philips PV 8650 spectrophotometer (AOAC, 2019).

Amino acids composition:

The amino acids content was determined according to the method described by Pellet and Young (1980) with some modifications and can be summarized as follows: 200 mg of dried sample was hydrolyzed with 5 ml of 6 N HCL in a sealed tube at 110°C for 24 hr, after which the hydrolysate was filtered. The residue was washed with distilled water, and the filtrate was evaporated on water at 50°C. The residue was dissolved in 5 ml of loading buffer (sodium citrate buffer, pH 2.2). Analysis was performed at the Central Service Unit, National Research Center, Egypt, using a Beckman Amino Acids Analyzer model 119 CL. Tryptophan was limited colorimetrically using the method described by Sastry (1985).

Protein efficiency ratio (PER)

The PER was calculated using the equation suggested by (Alsmeyer, 1974) as follows:

$$\boxed{\text{PER} = -0.468 + 0.454\,(\text{Leu}) - 0.105\,(\text{Tyr}).}$$

Biological value (BV)

The BV was calculated using the equation suggested by (Bender, 1960) as follow:

$$\boxed{\text{BV} = 49.9 + 10.53\,(\text{PER})}$$

Protein digestibility (in vitro)

The *in vitro* protein digestibility was assessed by employing pepsin and trypsin as described previously (Maliwal, 1983), with some modifications. The nitrogen content of the sample and supernatant after digestion was determined by the micro KJeledahl method. The results are expressed in terms of percent digestibility of protein as follows:

$$\text{Protein digestibility } \textit{in vitro} = \frac{\text{Digested protein in supernatant}}{\text{Protein content of sample}} \times 100$$

Physical properties

Width (cm), thickness (cm), spread ratio (%), and spread factor (%) were determined for six biscuits, and averages were recorded. The spread ratio, and spread factor were calculated according to Sai Manoharm and Rao (1997) as follows:

$$\text{Spread ratio} = \frac{\text{Width}}{\text{Thickness}}$$

$$\text{Spread factor} = \frac{\text{Spread ratio of sample}}{\text{Spread ratio of control}} \times 100$$

Sensory evaluation

The biscuits samples in pouches with different numbers were presented to 10 from staff of the Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University, Egypt, who were asked to rate biscuits samples by assigning a score from ten for colour, texture, appearance, odour, taste, and overall acceptability, using a hedonic scale as described by Sudha et al. (2007).

Statistical analysis

Data obtained from three replicates were analyzed by one-way analysis of variance (ANOVA) using SPSS 20.0 software statistical package program, and differences among the means were compared using Duncan's multiple range test (SPSS, 2011). A significance level of

0.05 was chosen, and continuous variables were described by the mean, and standard deviation.

Results and Discussion

Chemical composition and caloric values of biscuits

The mean values of chemical composition and caloric values of biscuits made from wheat flourand GF composite flourare given in Table 2. Results revealed that the moisture content of biscuits made from wheat flourwas 5.35±0.25%, while it ranged in GF composite formulas biscuits from 4.24±0.02 to 5.90±0.05%. Data shows that moisture content increased in biscuits samples F1, F2, and F3. This may be due to the higher content of moisture in the composition of the GF flours used in the preparation of biscuits from these samples compared with the moisture content of wheat flour.

From Table 2, it could be seen that the protein of the made biscuits samples GF composite flour was double that of the GF commercial formula biscuits. Where the protein content of biscuits prepared by GF composite flour was 7.03±0.22, 7.40±0.04, 7.55±0.20, and 7.58±0.22% ondry weight basis (dwb) for each of the samples F3, F1, F4, and F2, respectively, and in GF commercial formula biscuits (control 2) was 3.63±0.03%. While the protein of WFB (control 1) was 8.44±0.01%. On the other hand, crude fat content ranged from 13.18±0.05 to 14.28±0.05% on dwb for all GFB types, with no significant difference ($p>0.05$) between the control 2 and the F3 and F4 formulations biscuits. The crude fat content indicated that biscuits control made from wheat flour had the lowest content of fat (12.44±0.25%), while F1 formula biscuits had the highest crude fat content (14.28±0.05%) on dwb. This may be due to the higher fat content in quinoa flour that is added for the F1 formula (Abdelmegiud et al., 2022).

The ash and crude fibre contents of GF composite formulas biscuits were found to be higher than control samples by significantly different ($p\leq0.05$), which may be due to the incorporation of quinoa, buckwheat, millet, and chickpea flours, which had a high amount of content ash and fibre. Quinoa, buckwheat, millet, and chickpea flours have been considered functional food supplements because they are reckoned as a good source of dietary fibres, minerals, and vitamins (Abdelmegiud et al., 2021). On the other hand, incorporation of GF flours in biscuits resulted in a decrease in the carbohydrate content in samples F1, F2, and F4 as compared to the control 2 sample; this may be related to the lower content of carbohydrate in GF composite flour than in GF commercial formula flour. Besides, the lowest caloric values were found in the control 1 biscuits sample (453.48±1.69 kcal/100 g) due to their low fat content, while the F1 biscuits sample containing 30% quinoa flour had the highest caloric value (460.56±0.23 kcal/100 g). These results are in good agreement with those found by Ergin (2012) and Tamiru (2015).

Minerals content of biscuits

Minerals like calcium, magnesium, and iron are inadequate in GF products and diets. pseudocereals, millet, and legumes, which are high in these and other essential minerals, can help to alleviate this shortage (Alvarez-Jubete et al., 2010). Therefore, the study of minerals fortifications in the GF products is a major part of GF research. These methods are critical in supporting persons with CD and other gluten-related illnesses in consuming enough daily amounts of essential nutrients (Capriles et al., 2016). The minerals contents of WFB and GFB are presented from macro-elements (calcium, magnesium, sodium, potassium, and phosphorus) and micro-elements (iron, copper, and zinc) in Table 3 as mg/100 g sample on dwb.

TABLE 2. Chemical composition and caloric value of the prepared biscuits (g/100g on dwb).

Formula	Moisture	Protein	Crude fat	Ash	Crude fibre	Carbohydrates*	Caloric value (Kcal)
Control 1	5.35±0.25[c]	8.44±0.01[a]	12.44±0.25[d]	1.55±0.06[d]	0.63±0.05[c]	76.94±0.15[bc]	453.48±1.69[c]
Control 2	4.24±0.02[e]	3.63±0.03[d]	13.42±0.41[c]	1.26±0.05[e]	0.38±0.07[d]	81.31±0.36[a]	460.54±2.13[a]
F1	5.90±0.05[a]	7.40±0.04[b]	14.28±0.05[a]	1.95±0.00[b]	0.76±0.12[bc]	75.61±0.13[d]	460.56±0.23[a]
F2	5.66±0.01[b]	7.58±0.22[b]	13.84±0.09[b]	2.05±0.00[a]	1.04±0.09[a]	75.49±0.09[d]	456.84±0.09[b]
F3	4.77±0.01[d]	7.03±0.22[c]	13.29±0.01[c]	1.68±0.01[c]	0.69±0.11[bc]	77.31±0.31[b]	456.97±0.53[b]
F4	5.84±0.06[ab]	7.55±0.20[b]	13.18±0.05[c]	1.68±0.00[c]	0.81±0.08[b]	76.78±0.18[c]	455.94±0.57[b]

*Carbohydrates calculated by difference. -Abbreviations for symbols control 1, control 2, F1, F2, F3, and F4 see footnote of Table 1. -Values are the mean of triplicate determinations with standard division. - The different letters in the column mean significant differences at $p\leq0.05$, and the same letters mean no significant differences.

TABLE 3. Minerals content of the prepared biscuits (mg/100 g sample on dwb).

Formula	Macro-elements					Micro-elements		
	K	P	Na	Ca	Mg	Fe	Cu	Zn
Control 1	156.84^{e}	74.23^{d}	103.23^{a}	65.43^{e}	30.27^{e}	10.59^{c}	2.99^{e}	2.60^{d}
Control 2	102.90^{f}	42.06^{e}	98.40^{bc}	59.44^{f}	20.78^{f}	7.99^{d}	2.07^{f}	1.80^{e}
F1	256.62^{a}	103.25^{c}	105.34^{a}	78.32^{b}	46.63^{b}	10.68^{c}	4.94^{c}	4.29^{b}
F2	237.76^{b}	101.90^{c}	96.40^{c}	71.18^{d}	49.75^{a}	13.99^{a}	4.48^{d}	3.90^{c}
F3	181.82^{d}	129.07^{a}	105.23^{a}	78.67^{a}	39.66^{d}	9.99^{c}	5.24^{b}	4.29^{b}
F4	225.44^{c}	111.42^{b}	102.34^{ab}	76.07^{c}	45.35^{c}	11.56^{b}	5.55^{a}	4.83^{a}

-Abbreviations for symbols control 1, control 2, F1, F2, F3, and F4 see footnote of Table 1.

-Values are the mean of triplicate determinations with standard division. - The different letters in the column mean significant differences at $p\leq0.05$, and the same letters mean no significant differences.

It was evident from the data that the F3 biscuits sample was relatively higher in calcium (78.67 mg), phosphorus (129.07 mg), copper (5.24 mg), and zinc (4.29 mg/100 g) on dwb, while the F2biscuits sample was relatively higher in magnesium and iron (49.75 and 13.99 mg/100 g, respectively). Whereas the F1biscuits sample was relatively higher in potassium and sodium (256.62 and 105.34 mg/100 gon dwb, respectively). On the other hand, the control 2 had the lowest content in potassium, calcium, phosphorus, magnesium, iron, copper, and zinc (102.90, 59.44, 42.06, 20.78, 7.99, 2.07, and 1.80 mg/100 g, respectively). Finally, the studied GF composite flour formulations could be recommended as sources of various minerals and better than the GF commercial formula. As a result, their nutritional value in terms of macro- and micro-minerals contents can play a considerable role in enriching GFB with minerals.

Amino acids content of biscuits

The amino acids content of WFB and GFB are presented in Table 4 as g amino acid/ 100g protein. The protein content, its digestibility, and the number and amount of essential amino acids judge the food quality value. The nutritional value of protein is amounted by the amount of essential amino acids, which must be obtained from diet since they are not generated by the body (Vega-Galvez et al., 2010).

Most biscuits samples from GF composite flour were superior in their content of the essential amino acids isoleucine, leucine, lysine, threonine, and tryptophan compared to the control two samples of biscuits and was higher than the values recommended by WHO/FAO/UNU (2007). The control 2 biscuits sample had higher levels of valine, methionine, and phenylalanine than other biscuits samples. In the control 1 biscuits sample, the glutamine content was the highest in non-essential amino acids, followed by proline compared to non-essential amino acids observed for biscuits from GF composite flour. These results are in agreement with those reported by Vita Sterna et al. (2015).

PER, BV and in vitro protein digestibility of biscuits

The PER, BV, and *in vitro*protein digestibility of biscuits made from wheat flour and GF flourare presented in Table 5. Digestibility refers to the percentage of food components that are converted into potentially accessible material during digestion via physicochemical processes in the intestinal lumen, which can be measured *in vivo* or *in vitro*. *In vitro* digestion models provide a quick and low-cost way to investigate the digestibility and release of food components under simulated gastrointestinal conditions, as well as to screen samples for further in vivo research (Carbonell-Capella et al., 2014). Thus, *in vitro* studies could be a useful tool for scientific research, allowing researchers to gain a better understanding of GF products digestibility and bioavailability (Capriles et al., 2016).

The results presented in Table 5 indicated that the highest PER was recorded for the F3 biscuits sample (3.40±0.10), followed by the F4 biscuits sample (2.78±0.08), while the lowest value of PER was recorded in wheat biscuits as control 1 (2.28±0.08). The biscuits from GF composite flour also showed an increase in BV values than that observed for biscuits from wheat flour. On the other hand, the value of *in vitro* protein digestibility by pepsin for wheat biscuits was lower than the other values determined for biscuits made from GF composite flour. Furthermore, the protein digestibility by pepsin, followed by trypsin, gave higher values than that recorded for the treatment by pepsin alone in all biscuits samples.

TABLE 4. Amino acid content of the prepared biscuits (g amino acid/ 100g protein).

Amino acid		Control 1	Control 2	F1	F2	F3	F4	WHO/FAO (2007)
		Formula						
		Essential amino acids						
Valine	**VAL**	4.06^{d}	5.56^{a}	5.09^{c}	5.12^{c}	5.39^{b}	5.13^{c}	3.9
Methionine	**MET**	1.48^{d}	2.08^{a}	1.93^{b}	1.82^{c}	1.96^{b}	1.92^{b}	1.6
Isoleucine	**ILE**	3.57^{d}	3.82^{c}	4.04^{a}	3.80^{c}	3.92^{b}	3.85^{bc}	3.0
Leucine	**LEU**	6.77^{e}	7.64^{c}	7.54^{c}	7.43^{d}	9.31^{a}	8.01^{b}	5.9
Phenylalanine	**PHE**	4.93^{e}	5.56^{a}	5.26^{d}	5.45^{ab}	5.39^{bc}	5.29^{cd}	3
Lysine	**LYS**	1.97^{f}	3.13^{e}	5.09^{a}	4.79^{b}	3.43^{d}	4.17^{c}	4.5
Threonine	**THR**	2.71^{d}	3.82^{a}	3.86^{a}	3.80^{a}	3.59^{c}	3.69^{b}	2.3
Tryptophan	**TRY**	0.62^{e}	0.35^{f}	1.58^{b}	1.98^{a}	0.98^{d}	1.44^{c}	0.6
Total essential amino acids		26.11^{d}	31.94^{c}	34.39^{a}	34.16^{a}	33.99^{a}	33.49^{b}	-
		Non-essential amino acids						
Aspartic	**ASP**	4.68^{f}	9.03^{d}	10.53^{a}	10.23^{b}	8.66^{e}	9.46^{c}	-
Serine	**SER**	3.82^{e}	4.86^{b}	4.04^{d}	4.29^{c}	5.39^{a}	4.81^{b}	-
Glutamic	**GLU**	34.85^{a}	18.40^{bc}	17.37^{c}	18.65^{bc}	19.61^{b}	19.07^{bc}	-
Proline	**PRO**	10.96^{a}	4.86^{c}	4.39^{d}	4.46^{d}	5.39^{b}	4.97^{c}	-
Glycine	**GLY**	3.69^{d}	4.51^{c}	5.09^{a}	4.95^{b}	3.43^{e}	4.49^{c}	-
Histidine	**HIS**	2.22^{c}	2.43^{b}	2.81^{a}	2.48^{b}	2.45^{b}	2.40^{b}	1.5
Alanine	**ALA**	2.96^{f}	5.56^{c}	5.09^{d}	4.79^{e}	8.01^{a}	6.57^{b}	-
Tyrosin	**TYR**	3.08^{f}	4.51^{a}	3.86^{c}	3.96^{b}	3.43^{e}	3.69^{d}	-
Arginine	**ARG**	3.69^{e}	7.64^{c}	8.95^{a}	8.75^{b}	6.37^{d}	7.69^{c}	-
Cysteine	**CYS**	2.96^{a}	2.08^{d}	2.46^{b}	2.31^{c}	2.29^{c}	2.40^{bc}	0.6
Total non-essential amino acids		72.91^{a}	63.89^{b}	64.56^{b}	64.85^{b}	65.03^{b}	65.54^{b}	-

-Abbreviations for symbols control 1, control 2, F1, F2, F3, and F4 see footnote of Table 1. - The different letters in the row mean significant differences at p≤0.05, and the same letters mean no significant differences.

TABLE 5. PER, BV and *in vitro* protein digestibility of the prepared biscuits.

Formula	PER	BV	*In vitro* protein digestibility (%)	
			Pepsin	Pepsin + trypsin
Control 1	2.28±0.08^{d}	73.91±2.00^{d}	67.21±1.05^{d}	71.87±0.52^{c}
Control 2	2.53±0.03^{c}	76.54±1.50^{bcd}	71.93±1.21^{c}	76.76±1.20^{b}
F1	2.55±0.05^{c}	76.75±0.75^{bc}	75.23±0.59^{b}	77.59±0.59^{b}
F2	2.49±0.09^{c}	76.12±0.50^{cd}	79.04±0.57^{a}	80.67±0.61^{a}
F3	3.40±0.10^{a}	85.70±2.00^{a}	79.43±0.62^{a}	81.35±0.58^{a}
F4	2.78±0.08^{b}	79.17±1.00^{b}	77.25±2.32^{ab}	80.74± 0.00^{a}

- PER: Protein efficiency ratio BV: Biological value -Abbreviations for symbols control 1, control 2, F1, F2, F3, and F4 see footnote of Table 1. -Values are the mean of triplicate determinations with standard division. - The different letters in the column mean significant differences at p≤0.05, and the same letters mean no significant differences.

Physical characteristics of biscuits

The mean values of physical characteristics of WFB and the GF composite formulas biscuits are shown in Table 6. Data recorded an increment of width and thickness (4.80±0.04 and 0.77±0.02 cm, respectively) for the control 1 biscuits sample in comparison with the GFB samples. The reason for the decrease in width may be due to the biscuits cell structure being unable to retain gas during proofing and baking (Hooda and Jood, 2005). While maximum width and thickness were observed in GFB samples in the F3 biscuits sample (4.74±0.02 and 0.67±0.05 cm, respectively), whereas minimum width and thickness were observed in the control 2 biscuits sample (4.56±0.03 and 0.37±0.08 cm, respectively).

On the other hand, the changes in width and thickness are reflected in the spread ratio, which was calculated by dividing the width by the thickness of biscuits. Table 6 showed that spread ratio and spread factor increased slightly in F3 and F2 biscuits samples compared with the control 1 biscuits sample; however, there is no significant difference between them (p>0.05). In the same direction, spread ratio and spread factor increased by a higher percent in the control 2 biscuits sample compared to other biscuits samples with a significant difference (p≤0.05). These results agree with Rabou (2017) and Korus et al. (2017).

TABLE6. Physical properties of the prepared biscuits.

Formula	Width (cm)	Thickness (cm)	Spread ratio (%)	Spread factor (%)
Control 1	4.80±0.04[a]	0.77±0.02[a]	6.24±0.15[c]	100±0.00[c]
Control 2	4.56±0.03[c]	0.37±0.08[d]	12.74±2.56[a]	204.46±22.56[a]
F1	4.67±0.06[b]	0.53±0.02[c]	8.82±0.28[b]	141.40±3.86[b]
F2	4.66±0.04[b]	0.63±0.06[b]	7.45±0.75[bc]	119.51±12.45[bc]
F3	4.74±0.02[a]	0.67±0.05[b]	7.11±0.56[bc]	114.12±10.89[bc]
F4	4.61±0.02[bc]	0.54±0.02[c]	8.55±0.36[b]	137.13±7.49[b]

-Abbreviations for symbols control 1, control 2, F1, F2, F3, and F4 see footnote of Table 1. -Values are the mean of triplicate determinations with standard division. - The different letters in the column mean significant differences at $p\leq0.05$, and the same letters mean no significant differences.

The big increase in spread ratio may be in the GF commercial formula biscuits sample (control 2) due to the formula used in biscuits manufacturing being GF, firstly, and the same goes for other GFB samples; secondly, the biscuits sample is low in protein and fibre content compared to other biscuits samples. The increase in biscuits content of protein and dietary fibre reduces the spread ratio, as they are known for having more water-binding capacity, thus reducing the amount of water that is available to dissolve sugars in the formula and making the biscuits spread less during baking (Arshad et al., 2007; Ganorkar, 2014).

Sensory evaluation of biscuits

It could be observed sensory evaluation of WFB and the GF composite formulas biscuits in Fig. 2 and 3. For the appearance, colour, odour, and taste attributes, there were no significant differences ($p\leq0.05$) observed between the control 1 biscuits sample and between biscuits samples F1, F2, F3, and F4. It is reported that the taste and odour of the samples are positively correlated with the biscuits content of protein, ash, fat, and crude fibre and negatively correlated with moisture and total carbohydrate (Man et al., 2021). Whereas the texture score of biscuits was increased, the F3 biscuits sample had the best texture score according to judges score, while the lowest score was observed in the control 2 biscuits sample.

Besides, Fig. 2 indicated that both the control 1 biscuits sample and the F3 biscuits sample had the best taste score according to the judges' score; the lowest score was observed in the control 2 biscuits sample. As for the overall acceptability, all samples of GFB were acceptable from the sensory point of view, with no significant difference ($p>0.05$) between them and the control 1 biscuits sample (wheat biscuits). The inclusion of pseudocereals flours in biscuits formulations had a significant impact on the physicochemical and sensory qualities of the biscuits; it could be concluded that pseudocereals flours can be successfully mixed up to 30% to produce biscuits with improved nutritional quality and acceptable sensory qualities. The obtained results of this study agree with those reported by Ergin (2012) and Abozeid et al. (2023).

Conclusion

The use of more complex formulations of rice, quinoa, buckwheat, millet, chickpea flours, and cornstarch in GFB could be beneficial for nutritional reasons, as it enriches products with protein, minerals, fibre, and amino acids, as well as for economic reasons, as it lowers cost than from available GF commercial products. The majority of these formulations were acceptable and had better sensorial characteristics than commercially available combinations (control 2), and some were even comparable to their wheat-based counterparts (control 1). Therefore, it should be to using these formulations in commercial products to address nutritional deficiencies and poor sensory characteristics that are often present in GF commercial products. In addition, they assist individuals in sticking to a strict GF diet, promoting social inclusion, and improving quality of life. Future research directions involve further research on improving GF products, shelf-life studies, large-scale production trials, and consumer testing to ensure more diverse and accessible dietary options.

Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, writing and/or publishing of this paper.

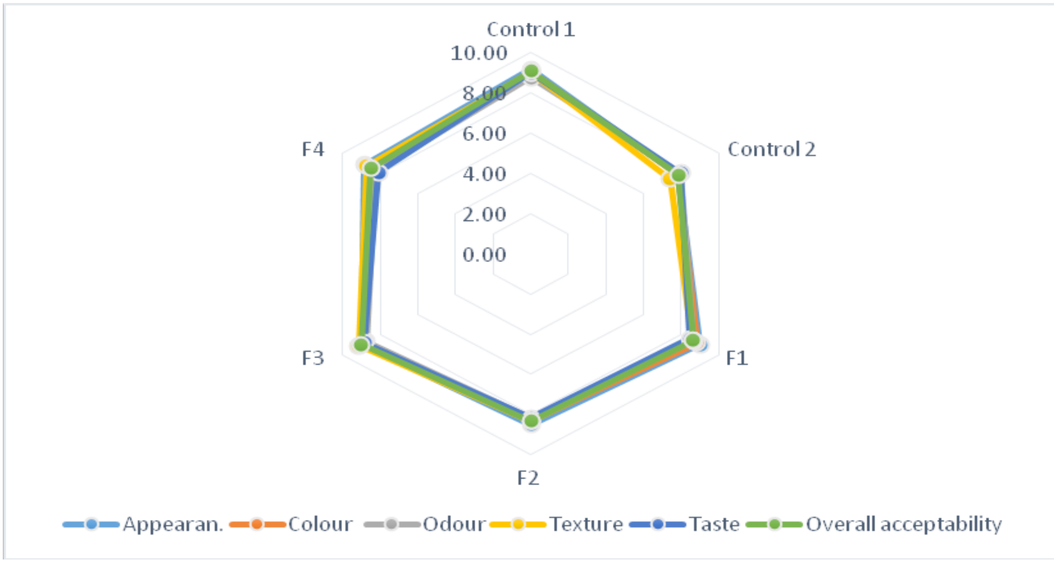

Fig. 2. Sensory evaluation of the prepared biscuits.

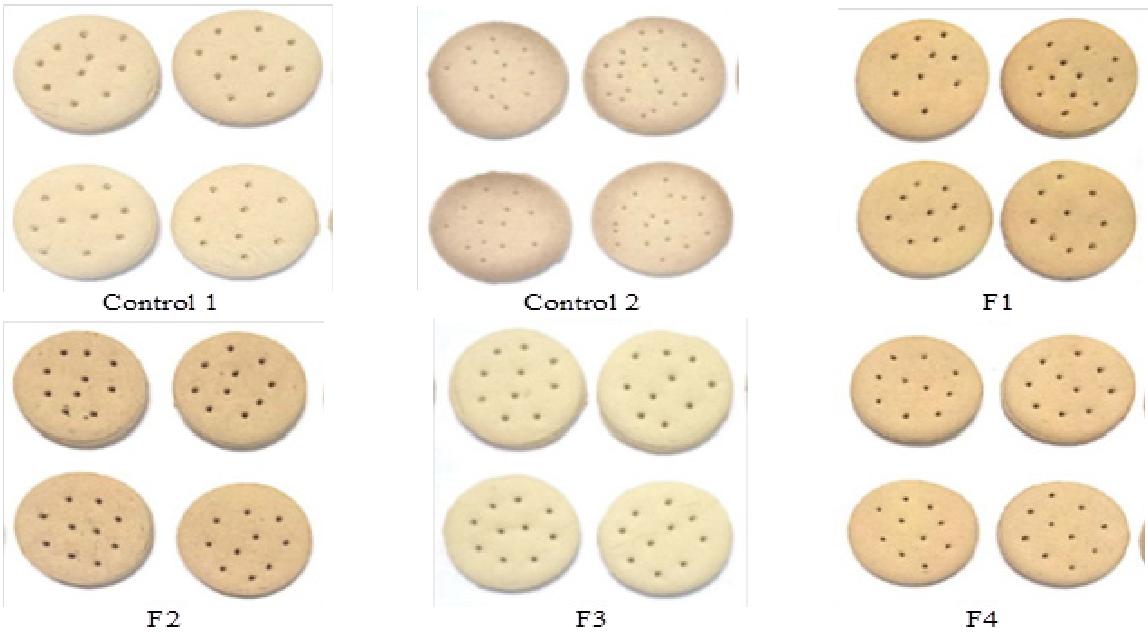

Fig. 3. The biscuits of wheat flour and GFflour.

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