



Archives of Agriculture Sciences Journal

Print ISSN: 2535-1680

Online ISSN: 2535-1699

## Chemical composition and rheological properties of some gluten-free flour formulations

Abdelmeguid M. H.<sup>a\*</sup>, El-Soukkary F. A. H.<sup>b</sup>, El-Naggar E. A.<sup>a</sup>, Abdelsalam R. R.<sup>b</sup>

<sup>a</sup>Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University, Assiut 71524, Egypt

<sup>b</sup>Food Science Department, Faculty of Agriculture, Minia University, Minia 61519, Egypt

### Abstract

The increasing prevalence of celiac disease and gluten sensitivity has led to a growing demand for nutritious gluten-free products. This study aimed to develop gluten-free flour formulations using quinoa (F1), buckwheat (F2), millet (F3), and their combinations (F4), and to evaluate their chemical composition and rheological properties in comparison to wheat flour (WF) and commercial gluten-free flour (CGFF). The formulations were analyzed using the Mixolab to assess water absorption, dough development time, protein weakening, starch gelatinization, and retrogradation. Results showed that the gluten-free formulations exhibited higher protein, ash, and fiber contents than both WF and CGFF. F1 and F3 exhibited pronounced protein network weakening and starch gelatinization, while F2 and F4 demonstrated significant amyolytic activity. Principal component analysis revealed strong correlations between retrogradation, ash content, dough stability, and starch gelatinization. Based on these findings, F2 and F3 are recommended for producing gluten-free bread and biscuits due to their favorable rheological properties. The study suggests that these formulations can serve as promising alternatives for gluten-free bakery products, providing improved nutritional content and functional properties.

**Keywords:** Mixolab, gluten-free flour, rheological properties, starch retrogradation.

\*Corresponding author: Abdelmeguid M. H.,  
E-mail address: [dr.mahmoud88m@gmail.com](mailto:dr.mahmoud88m@gmail.com)

## 1. Introduction

Alternatives to wheat flour have several advantages, including the ability to improve health and reduce wheat imports (Noorfarahzilah *et al.*, 2014). Additionally, using non-wheat components instead of refined wheat flour can increase the availability of nutrients, the consumption of which is limited by a gluten-free diet (Unalp-Arida *et al.*, 2022). Grains, including rice, corn, sorghum, chickpea, maize, and soybean flour, as well as pseudocereals such as buckwheat, amaranth, and quinoa, have recently been utilized as wheat flour substitutes (Ilkem and Berrin, 2022). These grains are excellent sources of protein because they contain higher concentrations of lysine, arginine, histidine, methionine, and cysteine than wheat (Abdel-Aal and Hucl, 2002). Some are rich in phytochemicals that are beneficial for consumer health and have nutrient profiles that are equivalent to or even superior to those of common gluten-containing grains, such as wheat and barley (Dykes and Rooney, 2007). On the other hand, the complete substitution of wheat flour with other flours results in negative effects on the rheological properties of dough and the quality of end-bakery products. This is because non-wheat flour lacks gluten, which makes gluten-free dough unable to form the gluten network necessary to contain gas released during fermentation (Gallagher *et al.*, 2003). Therefore, the addition of hydrocolloids, such as starch and gums, that mimic the viscoelastic properties of gluten in doughs is essential for the production of gluten-free bakery products (Garcia *et al.*, 2005). The Inclusion of

gums affects the pasting temperature and viscosity of hot starch pastes, depending on the gum structure (Gallagher *et al.*, 2004). Synergistic interactions between gum and starch during pasting results in the formation of gum and starch complexes (Ribotta *et al.*, 2004). Changes in the mechanical properties of dough caused by heating and mixing can be recorded using Mixolab, which helps predict the mechanical and temperature conditions encountered during the baking process (Rosell *et al.*, 2007). Mixolab measures the torque (Nm) created by blending the mixture between two massaging arms in real-time (Anonymous, 2005). By enabling the user to obtain information on the characteristics of starch, proteins, and associated enzymes from a representative dough in a single test, this feature distinguishes Mixolab from the other testing instruments and techniques. In this study, nutritious gluten-free composite flour based on quinoa, buckwheat, and millet grains was tested for its functional and rheological characteristics using Mixolab in comparison to refined wheat flour (72% extraction) and commercial gluten-free flour as controls.

## 2. Materials and methods

### 2.1 Materials

Wheat flour (*Triticum aestivum*; 72% extraction rate) was obtained from Asyut Mills Co. (Asuit, Egypt). Quinoa (*Chenopodium quinoa*), buckwheat (*Fagopyrum esculentum*), millet (*Panicum miliaceum*), and chickpeas

seeds (*Cicer arietinum L.*), as well as white rice (*Oryza sativa L.*) were obtained from the Agronomy Institute, Agriculture Research Center, Egypt. The approximate chemical composition of these grains was as follows: the content of moisture, protein, fat, fiber, ash, and carbohydrates for quinoa flour were 10.34, 13.07, 6.09, 2.40, 3.63%, and 74.81%, for buckwheat flour were 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% for rice flour, and 11.71, 7.28, 2.03, 0.45, 0.34%, and 89.90%, respectively. A commercial gluten-free flour formulation (Sonbolat Elforat), composed of white rice, brown rice, quinoa, corn starch, and Arabic gum, was purchased from a local market in Assiut City, Egypt. Xanthan gum (XG) was purchased from Sigma Co. Ltd. (Germany) and obtained from El-Gamhoria Trading Chemicals and Drugs Co. (Assiut, Egypt).

## 2.2 Preparation of gluten-free flour

Quinoa, buckwheat, millet, and chickpea seeds were cleaned and freed of broken seeds, dust, and other foreign materials, and then ground using an electric mill (Quadrumat Junior flour mill or Model Type No: 279002, © Brabender® OHG, Duisburg 1979, Germany) to obtain a fine powder, which was passed through a 20-hole/inch linear sieve and stored in polyethylene bags in a refrigerator until use.

## 2.3 Preparation of flour formulations

Gluten-free flour formulations were prepared as shown in Table (1). The flour mixtures were blended and homogenized, packed in polyethylene bags, tightly closed, and stored at room temperature until they were utilized and compared with wheat flour and commercial composite gluten-free flour.

Table (1): Ingredients of composite gluten-free flour formulations.

Ingredient (%)	WF	CGFF	F1	F2	F3	F4
Wheat flour	100	-	-	-	-	-
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 (g)	-	-	2	2	2	2

WF: Wheat flour (100% wheat flour 72% extraction); CGFF: Commercial gluten-free flour; F1:30% quinoa flour + 50% rice flour + 10% chickpeas flour + 10% corn starch; F2:30% buckwheat flour + 50% rice flour + 10% chickpeas flour + 10% corn starch; F3:30% millet flour + 50% rice flour + 10% chickpeas flour + 10% corn starch; and F4:10% quinoa flour + 10% buckwheat flour + 10% millet flour + 50% rice flour + 10% chickpeas flour + 10% corn starch.

## 2.4 Chemical composition analysis

The moisture, protein, fat, ash, crude

fiber, and starch contents (on a dry weight basis) of WF and gluten-free flour formulations were determined according

to official methods (AOAC, 2010). Carbohydrates were calculated based on the difference (100 - (protein + fat + ash)) on a dry weight basis. All determinations were performed in triplicate, and the data are expressed as means  $\pm$  standard deviation. The energy value was calculated based on 2 kcal/g for crude fiber, 4 kcal/g for protein and carbohydrates, and 9 kcal/g for fat (FAO Food and Nutrition, 2003).

### 2.6 Mixolab dough analysis

The rheological properties of WF and gluten-free flour formulation doughs were analyzed using a Mixolab analyzer (Chopin, Tripette et Renaud, Paris, France) according to the ICC-Standard Methods No. 173 (ICC, 2010) by applying the standard Chopin + protocol, which simultaneously determines dough characteristics during the process of mixing at a constant temperature, as well as during the period of constant heating and cooling. The amount of flour required for analysis was calculated using Mixolab software according to the input values of flour moisture and water absorption. All measurements were performed using the standard Mixolab Chopin protocol, as follows: The starting temperature was 30 °C for 8 min, then increased to 90 °C for 15 min at a rate of 4°C/min, held at 90 °C for 7 min, decreased to 50 °C for 5 min at a rate of 4 °C/min, and finally held at 50 °C for 5 min. The mixing speed was kept constant at 80 rpm, dough weight was 75 g, and total analysis time was 45 min for

all samples. A typical Mixolab curve consists of five stages: stage 1, initial kneading; stage 2, protein weakening; stage 3, starch gelatinization; stage 4, cooking stability; and stage 5, starch gelation (Codina *et al.*, 2012).

### 2.7 Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and significant differences ( $p < 0.05$ ) were determined by Duncan's test using SPSS 25.0 software statistical package program (Chicago, IL, USA). Principal component analysis (PCA) was performed using SPSS version 25.0 (SPSS, 2011).

## 3. Results and Discussion

### 3.1 Chemical composition and energy value

The chemical compositions and energy values of wheat flour (WF) and gluten-free flour formulations (GFF) are listed in Table (2). The moisture contents of WF and the commercial gluten-free flour formulation (CGFF) were  $11.30 \pm 0.07\%$  and  $12.60 \pm 0.06\%$ , respectively. In contrast, the moisture content of the prepared gluten-free flour formulations ranged from  $12.23 \pm 0.08\%$  (F2) to  $12.90 \pm 0.01\%$  (F3). The F2 and F4 formulations had the highest protein content, with no significant difference ( $p < 0.05$ ) compared to WF. However, CGFF had the lowest protein content, approximately half the amount ( $5.07\% \pm 0.18$ ). On the other hand, F1 had

the highest fat content (2.96%±0.44), which can be attributed to the greater fat content of quinoa (Cotovanu *et al.*, 2020). Furthermore, no significant differences in fat content ( $p < 0.05$ ) were found between the other GFF formulations and controls. Compared to the controls, the F2 formulation showed higher ash and crude fiber content. The formulations significantly varied ( $p < 0.05$ ) in ash and crude fiber contents. The CGFF exhibited the lowest ash and crude fiber content (0.51%±0.08). Furthermore, there was no significant

difference in starch content ( $p < 0.05$ ) between WF and the F1 and F3 formulations, while the CGFF formulation showed the highest level of starch (90.02±1.17%). In addition, the CGFF formulation had the greatest carbohydrate content and energy value (91.88%±0.22 and 407.8±0.50 Kcal, respectively), while the F2 formulation had the lowest values (83.25%±0.12 and 401.7±0.43 Kcal, respectively). These results are consistent with those of previous studies (El-Sohaimy *et al.*, 2019; López, 2014).

Table (2): Chemical composition and energy value of WF and GFF formulations.

Constituents (%)*	WF	CGFF	F1	F2	F3	F4
Moisture	11.30±0.07 <sup>d</sup>	12.60±0.06 <sup>b</sup>	12.50±0.01 <sup>b</sup>	12.23±0.08 <sup>c</sup>	12.90±0.01 <sup>a</sup>	12.50±0.03 <sup>b</sup>
Protein	12.3±0.51 <sup>a</sup>	5.07±0.18 <sup>c</sup>	11.5±0.07 <sup>b</sup>	12.3±0.16 <sup>a</sup>	11.2±0.17 <sup>b</sup>	12.1±0.13 <sup>a</sup>
Fat	2.46±0.13 <sup>b</sup>	2.23±0.09 <sup>b</sup>	2.96±0.44 <sup>a</sup>	2.19±0.06 <sup>b</sup>	2.13±0.02 <sup>b</sup>	2.44±0.09 <sup>b</sup>
Ash	0.59±0.01 <sup>c</sup>	0.51±0.08 <sup>f</sup>	1.21±0.02 <sup>b</sup>	1.30±0.02 <sup>a</sup>	0.84±0.01 <sup>d</sup>	1.14±0.01 <sup>c</sup>
Crude fiber	0.61±0.08 <sup>c</sup>	0.31±0.09 <sup>d</sup>	0.83±0.06 <sup>b</sup>	1.01±0.10 <sup>a</sup>	0.76±0.08 <sup>bc</sup>	0.88±0.13 <sup>ab</sup>
Starch	82.8±1.70 <sup>bc</sup>	90.0±1.17 <sup>a</sup>	80.9±2.71 <sup>bcd</sup>	78.3±2.27 <sup>d</sup>	83.8±2.48 <sup>b</sup>	79.5±1.58 <sup>cd</sup>
Carbohydrates**	84.65±0.52 <sup>c</sup>	92.19±0.22 <sup>a</sup>	84.33±0.37 <sup>cd</sup>	84.21±0.12 <sup>d</sup>	85.83±0.27 <sup>b</sup>	84.32±0.22 <sup>d</sup>
Energy value (Kcal/100 g)*	408.72±0.82 <sup>a</sup>	408.52±0.50 <sup>a</sup>	408.26±2.36 <sup>a</sup>	403.72±0.43 <sup>c</sup>	405.72±0.24 <sup>b</sup>	405.86±0.84 <sup>b</sup>

\*Means of three determinations ± SD. \*\*Carbohydrates calculated by difference (100- (protein + fat + ash) on the dry weight; values are the mean of triplicate determinations with standard division. Different letters within the same row mean significant differences at ( $p \leq 0.05$ ).

### 3.2 Functional and rheological properties of dough

The rheological behavior of WF and GF flour formulations was investigated using the Mixolab Chopin protocol. The rheological characteristics of dough are presented in Table (3) and Figure (1). The first stage was determined for all samples using the following mixing parameters: water absorption (%), dough development time (min), and dough stability (min). In the following stages, the minimum torque C2 (Nm) and C1–2, related to protein reduction due to temperature rise peak

torque; C3 (Nm), related to starch gelatinization; minimum torque C4 (Nm), as the stability of hot-formed gel; and maximum torque C5 (Nm), as a starch retrogradation measure during dough cooling, were recorded. The differences between C3-2, C4-3, and C5-4 were also calculated.

#### 3.2.1 Water absorption

Water absorption (WA) plays an important role in dough properties and the baking process, affecting the volume efficiency of the baked quality. It is an indicator of the quantity of water required

for adequate consistency and the ability of protein molecules to absorb water to obtain a torque of  $C1=1.1\pm 0.05$  Nm using the standard Chopin + protocol (Liu *et al.*, 2019). The WA of flour formulations significantly varied ( $p<0.05$ ) and ranged from  $53.60\% \pm 0.60$  for F2 to  $60.60\% \pm 0.60$  for F3 (Table 3). However, no significant differences were found between

the F1 and control samples ( $p<0.05$ ). The lower water absorption of F2 dough influences its stability and development. Differences in WA can be attributed to factors such as grain hardness, damaged starch, protein content, pentosan or arabinoxylan content and composition, flour particle size, and differences in chemical composition (Sapirstein *et al.*, 2018).

Table (3): Functional and rheological properties of WF and gluten-free flour formulations.

Mixolab parameters	WF	CGFF	F1	F2	F3	F4
WA (%)	56.00±1.00 <sup>bc</sup>	57.50±1.50 <sup>b</sup>	56.00±0.50 <sup>bc</sup>	53.60±0.60 <sup>d</sup>	60.60±0.60 <sup>a</sup>	55.80±0.50 <sup>c</sup>
DDT (min)	2.92±0.01 <sup>c</sup>	1.35±0.15 <sup>c</sup>	7.28±0.05 <sup>a</sup>	1.10±0.05 <sup>f</sup>	5.83±0.06 <sup>b</sup>	1.53±0.03 <sup>d</sup>
ST (min)	6.05±1.00 <sup>d</sup>	7.17±1.13 <sup>cd</sup>	9.60±0.40 <sup>a</sup>	8.74±0.20 <sup>ab</sup>	7.85±0.15 <sup>bc</sup>	8.80±0.20 <sup>ab</sup>
Protein weakening (%)	61.95±1.98 <sup>a</sup>	53.64±6.72 <sup>b</sup>	44.55±4.14 <sup>c</sup>	57.41±1.99 <sup>ab</sup>	53.77±2.32 <sup>b</sup>	51.82±2.29 <sup>b</sup>
Retrogradation (%)	26.35±8.05 <sup>d</sup>	36.11±0.83 <sup>c</sup>	47.45±1.02 <sup>a</sup>	42.38±0.48 <sup>abc</sup>	38.70±1.80 <sup>bc</sup>	42.95±0.59 <sup>ab</sup>
C1 (Nm)	1.13±0.02 <sup>a</sup>	1.10±0.03 <sup>ab</sup>	1.10±0.01 <sup>ab</sup>	1.08±0.02 <sup>bc</sup>	1.06±0.01 <sup>c</sup>	1.10±0.01 <sup>ab</sup>
C2 (Nm)	0.43±0.03 <sup>c</sup>	0.51±0.06 <sup>b</sup>	0.61±0.04 <sup>a</sup>	0.46±0.03 <sup>bc</sup>	0.49±0.02 <sup>bc</sup>	0.53±0.03 <sup>b</sup>
C3 (Nm)	1.47±0.07 <sup>c</sup>	1.70±0.10 <sup>b</sup>	1.94±0.04 <sup>a</sup>	1.95±0.04 <sup>a</sup>	1.67±0.02 <sup>b</sup>	1.86±0.01 <sup>a</sup>
C4 (Nm)	1.09±0.06 <sup>c</sup>	1.84±0.04 <sup>b</sup>	1.75±0.05 <sup>c</sup>	2.23±0.03 <sup>a</sup>	1.60±0.05 <sup>d</sup>	1.78±0.03 <sup>bc</sup>
C5 (Nm)	1.48±0.08 <sup>f</sup>	2.88±0.10 <sup>d</sup>	3.33±0.03 <sup>b</sup>	3.87±0.02 <sup>a</sup>	2.61±0.01 <sup>e</sup>	3.12±0.02 <sup>e</sup>
$\alpha$ (Nm/min)	-0.048±0.01 <sup>b</sup>	-0.088±0.01 <sup>c</sup>	-0.018±0.01 <sup>a</sup>	-0.088±0.01 <sup>c</sup>	-0.062±0.01 <sup>b</sup>	-0.060±0.01 <sup>b</sup>
$\beta$ (Nm/min)	0.320±0.02 <sup>c</sup>	0.220±0.03 <sup>f</sup>	0.498±0.008 <sup>a</sup>	0.464±0.01 <sup>b</sup>	0.406±0.006 <sup>c</sup>	0.364±0.004 <sup>d</sup>
$\gamma$ (Nm/min)	-0.072±0.01 <sup>c</sup>	-0.010±0.005 <sup>b</sup>	0.022±0.002 <sup>a</sup>	-0.002±0.001 <sup>b</sup>	-0.026±0.003 <sup>c</sup>	-0.042±0.002 <sup>d</sup>

WA: water absorption; DDT: dough development time; ST: stability time; C1: water absorption; C2: minimum torque during temperature increase; C3: peak viscosity; C4: cooking stability; C5: starch retrogradation;  $\alpha$ : speed of protein weakening;  $\beta$ : gelatinization rate;  $\gamma$ : cooking stability rate. \*Values are the mean of triplicate determinations with standard divisions. \*\* Different letters within the same row mean significant differences at ( $p\leq 0.05$ ).

### 3.2.2 Dough development time

Dough development time (DDT) was defined as the time from the beginning of water addition to the time of reaching the optimal consistency of dough (C1 torque =1.1 Nm). Dough development time varied significantly ( $p<0.05$ ) among the different flour formulations (Table 3). The dough of F1 and F3 formulations had a high DDT ( $7.28\pm 0.05$  and  $5.83\pm 0.06$  min, respectively). This indicates that these flour formulations require a longer time to hydrate than the other flour formulations. Some compounds, such as dietary fibers

and proteins, require a longer time to hydrate and increase DDT (Cotovanu and Mironeasa, 2021).

### 3.2.3 Dough stability (ST)

Dough stability (ST) is a measure of dough resistance to kneading. The F1 dough formulation had the highest stability (9.60 min), followed by the F4 formulation ( $8.80\pm 0.20$  min) and F2 formulation ( $8.74\pm 0.20$  min) formulations. Dough stability varied significantly ( $p<0.05$ ) among the three formulations and the control formulation. This may be

due to the interaction between polysaccharides (especially gums) and proteins in the composite flour, as reported by Rojas *et al.* (1999). In addition, dough stability is influenced by factors such as the protein content and particle size of flour as well as the addition of hydrocolloids such as XG (Garcia *et al.*, 2005). The F1, F4, and F2 formulations showed good stability, indicating that they can develop stronger and more elastic

dough for bread making compared with the CGFF flour, which is of medium quality. The WA, DDT, ST, and mechanical weakening are parameters that depict the batter behavior during blending at a constant temperature of 30 °C. Furthermore, during blending, the mixtures are hydrated and protein molecules are stretched and rearranged, resulting in the formation of a three-dimensional viscoelastic structure (Bonet *et al.*, 2006).

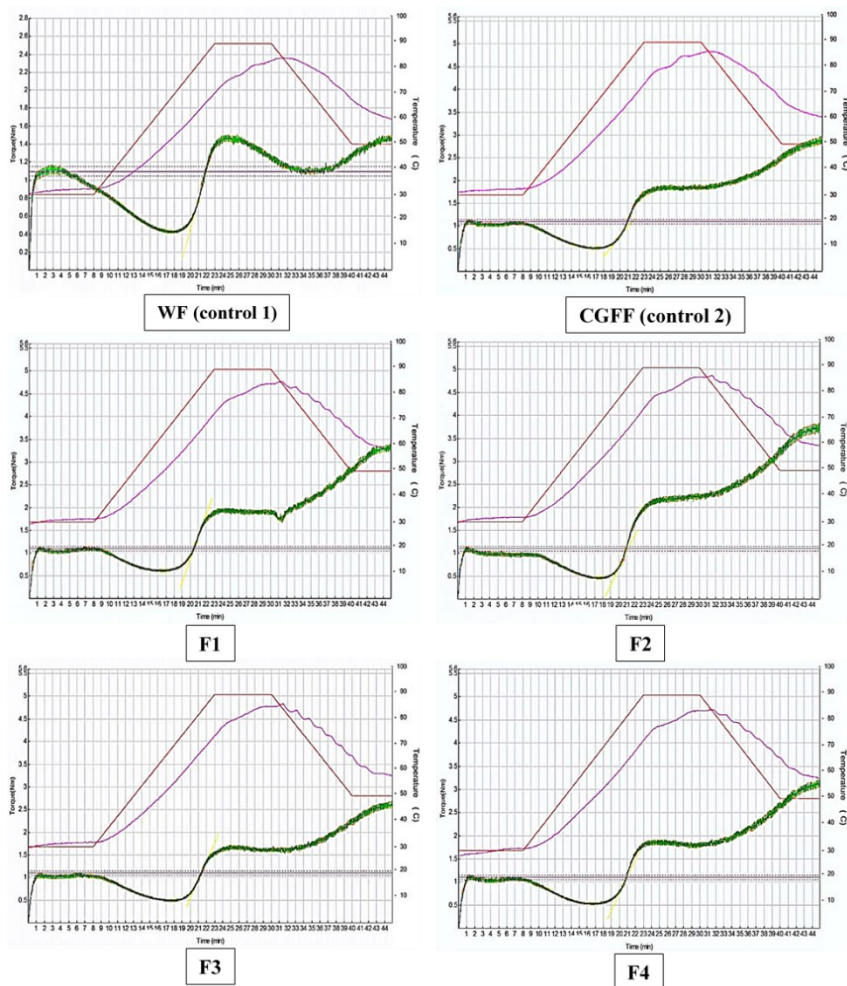


Figure (1): Rheological behavior for WF and GF flour formulas using Mixolab ‘Chopin protocol’.

### 3.2.4 Protein weakening

Protein aggregation and denaturation occurred, and the viscosity decreased as the dough temperature increased. The F1 formulation had a 60% higher protein network structure than that of the WF formulation (Table 3). The protein of the F1 formula ( $0.61\pm 0.04$  Nm) showed a lower weakening under mechanical and thermal constraints, indicating higher flour protein quality than the CGFF formulation ( $0.51\pm 0.06$ ), followed by the F4 formulation ( $0.53\pm 0.03$  Nm). Additionally, no significant differences ( $p < 0.05$ ) were found in protein weakening among F2, F3, and F4 and the control formulation. The difference between C1 and C2 torques (C1-2), which represents the rate of protein thermal weakening, was demonstrated by a difference in the values, with the highest value for the WF (0.70) and the lowest for the F1 (0.49) formulation. This variation can be explained by changes in the protein network structure due to kneading and temperature effects (Cotovanu and Mironeasa, 2021).

### 3.2.5 Starch gelatinization

Starch granules play a dominant role in the functional and rheological properties during the heat treatment of dough compared to proteins. The increase in C2 value is an indicator of starch gelatinization during the warming and cooking stages (Rosell *et al.*, 2007). Starch granules swell because of water

uptake, and amylose chains settling into the aqueous intergranular stage cause an increase in viscosity, which, in turn, causes an increase in torque. The formulations F2, F1, and F4 showed higher peak torques (C3) and gelatinization rates than the other formulations, indicating high dough viscosity during heating and high starch quality (Table 3). However, no significant differences ( $p < 0.05$ ) were found between the F3 and control formulations. It is well known that the starch gelatinization process can be influenced by amylase-lipid complex formation, the amount of amylose leaching, and competition for free water between leached amylose and ungelatinized granules (Qiu *et al.*, 2015). The decrease in C3 may be attributed to the low swelling of starch granules owing to the interaction of amylose with other compounds. Additionally, during the starch gelatinization process, ingredients other than starch in flour can compete with starch for water absorption, making starch more challenging to gelatinize (Codina *et al.*, 2019). The F2 formulation exhibited the largest difference between C3 and C2. This indicates a higher enzyme activity. In addition, starch–lipid and starch–protein interactions as well as water absorption limit starch swelling (Cotovanu *et al.*, 2020). The decrease in C3 could be due to the non-starch components (lipids, proteins, and dietary fibers) present in the flour, limiting the swelling and gelatinization of starch during cooking.



### 3.2.6 Cooking stability

The minimum torque C4 obtained because of the rupture of the swollen starch granules, which reduces the consistency of the hot-formed starch gel, was used to measure cooking stability. In the fourth stage, amylase activity and physical breakdown of starch granules were associated with a reduction in viscosity. The torque (C4) indicates the rate of enzymatic hydrolysis and the stability of hot gel formation. A lower C4 value indicates a low stability of the starch gel. Gluten-free flour formulations showed higher C4 torque values than the control, indicating lower amylase activity. The F2 formulation dough had the highest C4 value ( $2.23 \pm 0.03$  Nm, indicating the highest gel stability during the hot phase). In contrast, the peak value of C4 was significantly lower ( $p < 0.05$ ) in the WF and F3 formulations. The difference in torque between C4 and C3 (C4-3) was used to calculate amylase activity. The greater the difference between C4 and C3, the lower is the amylase activity. The difference was in the order  $WF (-0.38) < F1 (-0.19) < F4 (-0.08) < F3 (-0.07) < CGFF (0.14) < F2 (0.28)$ . The starch granules physically break down because of mechanical shear stress and high temperatures (Rosell *et al.*, 2007).

### 3.2.7 Starch retrogradation

The decrease in temperature to 50°C during the cooling stage resulted in a final viscosity associated with higher dough

resistance, and consequently with the C5 torque, which reflects starch retrogradation (Cotovanu *et al.*, 2020). During the cooling period, WF and F3 formulations showed low starch retrogradation and recrystallization (Table 3). The long shelf life of a product is correlated with its low retrogradation value (Wang *et al.*, 2015). The difference in torques between C5 and C4 indicates the starch retrogradation capacity. The retrogradation of the formulations was in the order  $F2 (1.64) > F1 (1.58) > F4 (1.34) > CGFF (1.04) > F3 (1.01) > WF (0.39)$ . Retrogradation is influenced by various factors, such as the botanical source of starch, amylose/amylopectin ratio, and the average chain length of amylose and amylopectin (Zheng *et al.*, 2019). The F1 formulation had the highest values of the  $\alpha$ ,  $\beta$ , and  $\gamma$  slopes (Table 3). However, the CGFF formulation exhibited the lowest values for the  $\alpha$  and  $\beta$  slopes. Moreover, WF had the lowest  $\gamma$  slope value, indicating high protein network weakness due to heating ( $\alpha$ ), gelatinization rate ( $\beta$ ), and cooking stability rate ( $\gamma$ ). Furthermore, flour with high enzymatic activity has a low absolute value of the  $\beta$  slope (Kahraman *et al.*, 2008; Tawfeuk and Gomaa, 2017).

### 3.3 Correlations and principal component analysis

The correlation heat map depicts the relationship between characteristics such as moisture, fat, protein, ash, and carbohydrate contents, and dough

rheological properties assessed by the Mixolab device (C2, C3, C4, and C5 torques; the difference between the C1 and C2 and peak values (C1-2); the difference between the C3 and C2 and peak values (C3-2); the difference between the C4 and C3 and peak values (C4-3); and the difference between C5 and C4 and peak values (C5-4)) for all flour formulations (Figures 2 and 3).

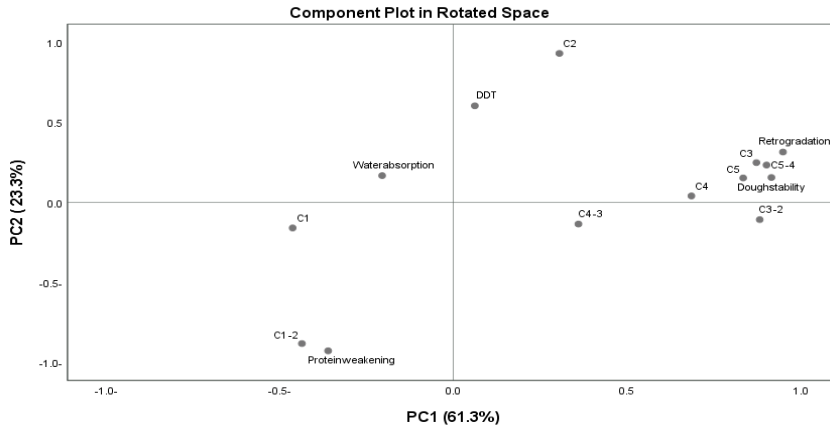


Figure (2): Loading plot of the first two principal components based on physicochemical and rheological properties of the composite flour samples: DDT, development time; ST, stability; C2, C3, C4, and C5-Mixolab torques, C1-2, C3-2, C4-3, and C5-4; difference between Mixolab peak values.

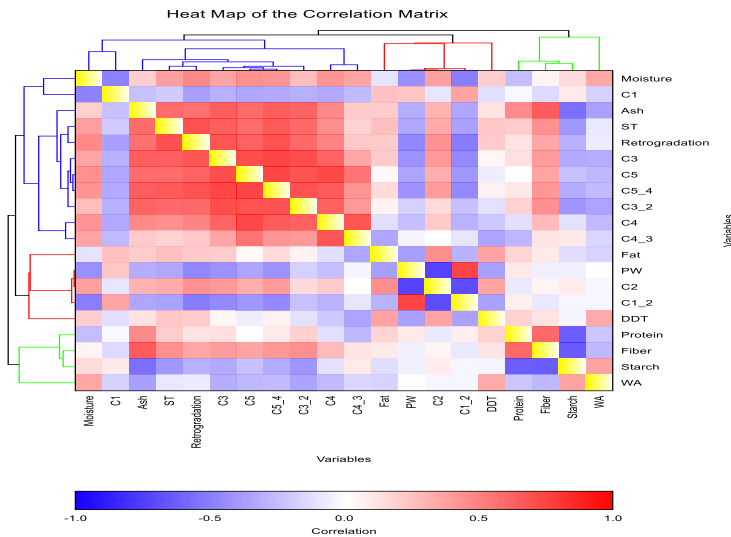


Figure (3): Heat map of the correlation matrix.

At  $p < 0.01$ , an inverse correlation was found between moisture content, retrogradation, and C1-2 ( $r = -0.67$  and  $-0.68$ ). The negative correlation between C1-2 and retrogradation could be attributed to the low  $\alpha$ -amylase and proteolytic activities of the flour formulations. Because both parameters are related to starch gelatinization, the negative correlation between starch and Mixolab values C3 and C3-2 can also be explained. There was a significant positive correlation between ash, ST, and retrogradation and C3 ( $r = 0.847$ ,  $0.839$ , and  $0.890$ , respectively). C4 measures amylase activity, and its value is inversely related to the amount of  $\alpha$ -amylase in WF (Kahraman *et al.*, 2008), which has a negative impact on starch gelatinization. Furthermore, the Mixolab values for dough pasting properties decreased due to an increase in the level of proteins in the dough system (Tawfeuk and Gomaa, 2017). Statistical principal component analysis (PCA) (Radziejewska-Kubzdela *et al.*, 2014) was performed to investigate the relationship between flour formulation physicochemical and Mixolab characteristics. The first two components (PC1 and PC2) accounted for approximately 84.6% of the overall variation. PCA factors with eigenvalues greater than one, which represent the total amount of variance that can be explained by a given principal component, were preserved because they provided more information than the starting variables (Hassan *et al.*, 2020). The PC1 vs. PC2 plot indicated a close link between starch quality (C3, C3-2, and

C4-3) and composite flour properties (protein, ash, fat, moisture, and carbohydrates) along the PC1 axis. PC2 contains Mixolab parameters for dough rheological properties during heating (C1-C2, C2, C3, C3-2, C4, and C4-3) as well as starch retrogradation values during cooling (C5 and C5-4). Because their PC1 loadings were close to zero, the effect of ST values was minimal. As observed in the PCA score map, the specimens collected inside different areas showed substantial variation.

#### 4. Conclusion

The results of this study revealed that the optimal values for WA, DDT, ST, C2, C3, C4, and C5 torques were significantly higher for the developed gluten-free flour formulations than for the control samples. Moisture influenced the C1 and C2 parameters and was linked to dough softening due to higher amounts of unbound water, resulting in lower values with increasing hydration rates and higher C5 values. In terms of C4, F2 formula dough had the highest value of 2.23 Nm, indicating that it was the most stable gel during the hot phase. The F3 formula had a low starch retrogradation value, which indicates the potentially long shelf life of the end product. Therefore, peak data can be used to forecast rheological behavior during the manufacturing process of gluten-free bakery products. Based on its chemical composition and rheological properties, the developed gluten-free flour formulations can be used as convenient

flour formulations for producing gluten-free bread or biscuits of good quality.

## References

- AOAC (2010), *Official Methods of Analysis* (15th ed.), Association of Official Analytical Chemists, Washington, D.C., USA, Analytical Chemists International, Arlington, Virginia, USA. Available at: [http://sutlib2.sut.ac.th/sut\\_contents/H125800.pdf](http://sutlib2.sut.ac.th/sut_contents/H125800.pdf) (Accessed: 10 April 2024).
- Abdel-Aal, E. S. M. and Hucl, P. (2002), "Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products", *Journal of Food Composition and Analysis*, Vol. 15 No. 6, pp. 737–747. <https://doi.org/10.1006/jfca.2002.1094>.
- Anonymous (2005), *Mixolab User's Manual*, Chopin Technologies, Villeneuve-la-Garenne, France. Available at: <https://www.kpmanalytics.com/product/s/mixolab-2> (Accessed: 10 April 2024).
- Bonet, A., Blaszczyk, W. and Rosell, C. M. (2006), "Formation of homopolymers and heteropolymers between wheat flour and several protein sources by transglutaminase-catalyzed cross-linking", *Cereal Chemistry Journal*, Vol. 83 No. 6, pp. 655–662. <https://doi.org/10.1094/CC-83-0655>.
- Codina, G. G., Istrate, A. M., Gontariu, I. and Mironeasa, S. (2019), "Rheological properties of wheat–flaxseed composite flours assessed by Mixolab and their relation to quality features", *Foods*, Vol. 8 No. 8, Article No. 333. <https://doi.org/10.3390/foods8080333>.
- Codina, G. G., Mironeasa, S., Mironeasa, C., Popa, C. N. and Tamba-Berehoiu, R. (2012), "Wheat flour dough Alveograph characteristics predicted by Mixolab regression models", *Journal of the Science of Food and Agriculture*, Vol. 92 No. 3, pp. 638–644. <https://doi.org/10.1002/jsfa.4623>.
- Cotovanu, I., Batariuc, A. and Mironeasa, S. (2020), "Characterization of quinoa seeds milling fractions and their effect on the rheological properties of wheat flour dough", *Applied Sciences*, Vol. 10 No. 20, Article No. 7225. <https://doi.org/10.3390/app10207225>.
- Cotovanu, I. and Mironeasa, S. (2021), "Buckwheat seeds: Impact of milling fractions and addition level on wheat bread dough rheology", *Applied Sciences*, Vol. 11 No. 4, Article No. 1731. <https://doi.org/10.3390/app11041731>.
- Dykes, L. and Rooney, L.W. (2007), "Phenolic compounds in cereal grains and their health benefits", *Cereal Foods World*, Vol. 52 No. 3, pp. 105–111. <https://doi.org/10.1094/CFW-52-3-0105>.
- El-Sohaimy, S. A., Shehata, M. G., Taha, M. and Zeitoun, M. A. (2019), "Nutritional, physicochemical, and sensorial evaluation of flatbread

- supplemented with quinoa flour", *International Journal of Food Science*, pp. 1–8. <https://doi.org/10.1155/2019/4686727>.
- FAO (2003), "Food energy – methods of analysis and conversion factors", *FAO Food and Nutrition Paper 77*, Food and Agriculture Organization of the United Nations, Rome, Italy. Available at: <https://www.ars.usda.gov/research/publications/publication/?seqNo115=172524> (Accessed: 10 April 2024).
- Gallagher, E., Gormley, T.R. and Arendt, E.K. (2003), "Crust and crumb characteristics of gluten-free breads", *Journal of Food Engineering*, Vol. 56 No. 2, pp. 153–161. [https://doi.org/10.1016/S0260-8774\(02\)00244-3](https://doi.org/10.1016/S0260-8774(02)00244-3).
- Gallagher, E., Gormley, T. R. and Arendt, E. K. (2004), "Recent advances in the formulation of gluten-free cereal-based products", *Trends in Food Science and Technology*, Vol. 15 No. 3, pp. 143–152. <https://doi.org/10.1016/j.tifs.2003.09.012>.
- Garcia, M. E., Zaritzky, N. E. and Califano, A. N. (2005), "Effect of composition on rheological properties of gluten-free dough disks for 'empanadas'", *X Congreso Argentino de Ciencia y Tecnología de Alimentos*, Asociación Argentina de Tecnología de Alimentos. Available at: [https://www.academia.edu/download/75904248/effect\\_of\\_composition\\_on\\_rheological\\_pro20211207-12939-1eerxlb.pdf](https://www.academia.edu/download/75904248/effect_of_composition_on_rheological_pro20211207-12939-1eerxlb.pdf) (Accessed: 10 April 2024).
- Hassan, E. M., Fahmy, H. A., Magdy, S. and Hassan, M. I. (2020), "Chemical composition, rheological, organoleptical and quality attributes of gluten-free Fino bread", *Egyptian Journal of Chemistry*, Vol. 63 No. 11, pp. 4547–4563. <https://doi.org/10.21608/ejchem.2020.28290.2597>.
- Ikem, D. and Berrin, O. (2022), "Recent strategies for tackling the problems in gluten-free diet and products", *Critical Reviews in Food Science and Nutrition*, Vol. 62 No. 3, pp. 571–597. <https://doi.org/10.1080/10408398.2020.1823814>.
- Kahraman, K., Sakiyan, O., Ozturk, S., Koksel, H., Sumnu, G. and Dubat, A. (2008), "Utilization of Mixolab to predict the suitability of flours in terms of cake quality", *European Food Research and Technology*, Vol. 227 No. 2, pp. 565–570. <https://doi.org/10.1007/s00217-007-0757-y>.
- Liu, Y., Cai, C., Yao, Y. and Xu, B. (2019), "Alteration of phenolic profiles and antioxidant capacities of common buckwheat and tartary buckwheat produced in China upon thermal processing", *Journal of the Science of Food and Agriculture*, Vol. 99 No. 13, pp. 5565–5576. <https://doi.org/10.1002/jsfa.9825>.
- López, E. P. (2014), "Influence of the addition of lupine protein isolate on the protein and technological characteristics of dough and fresh bread with added Brea gum", *Food Science and Technology*, Vol. 34 No.

- 1, pp. 195–203. <https://doi.org/10.1590/S0101-20612014005000016>.
- Noorfarahzilah, M., Lee, J. S., Sharifudin, M. S., Fadzelly, M. A. and Hasmadi, M. (2014), "Applications of composite flour in development of food products", *International Food Research Journal*, Vol. 21 No. 6, pp. 2061–2074. Available at: <http://www.ifrj.upm.edu.my> (Accessed: 10 April 2024).
- Qiu, S., Yadav, M. P., Chen, H., Liu, Y., Tatsumi, E. and Yin, L. (2015), "Effects of corn fiber gums on the pasting and thermal behaviours of maize starch", *Carbohydrate Polymers*, Vol. 115, pp. 246–252. <https://doi.org/10.1016/j.carbpol.2014.08.071>.
- Radziejewska-Kubzdela, E., Czapski, J., Czaczyk, K. and Biegańska-Marecik, R. (2014), "The effect of pretreatment and modified atmosphere packaging on contents of phenolic compounds and sensory and microbiological quality of shredded celeriac", *Journal of the Science of Food and Agriculture*, Vol. 94 No. 6, pp. 1140–1148. <https://doi.org/10.1002/jsfa.6381>.
- Ribotta, P. D., Ausar, S. F., Morcillo, M. H., Perez, G.T., Beltramo, D. M. and Leon, A. E. (2004), "Production of gluten-free bread using soybean flour", *Journal of the Science of Food and Agriculture*, Vol. 84 No. 14, pp. 1969–1974. <https://doi.org/10.1002/jsfa.1915>.
- Rojas, J. A., Rosell, C. M. and Benedito de Barber, C. (1999), "Pasting properties of different wheat flour-hydrocolloid systems", *Food Hydrocolloids*, Vol. 13 No. 1, pp. 27–33. [https://doi.org/10.1016/S0268-005X\(98\)00066-6](https://doi.org/10.1016/S0268-005X(98)00066-6).
- Rosell, M. C., Collar, C. and Haros, M. (2007), "Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab", *Food Hydrocolloids*, Vol. 21 No. 3, pp. 452–462. <https://doi.org/10.1016/j.foodhyd.2006.05.004>.
- Sapirstein, H., Wu, Y., Koxsel, F. and Graf, R. (2018), "A study of factors influencing the water absorption capacity of Canadian hard red winter wheat flour", *Journal of Cereal Science*, Vol. 81, pp. 52–59. <https://doi.org/10.1016/j.jcs.2018.01.012>.
- SPSS (2011), *SPSS for Windows*, Release 25.0, Standard Version, IBM Corp., Armonk, NY. Available at: <https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25> (Accessed: 10 April 2024).
- Tawfeuk, H. Z. and Gomaa, R. A. (2017), "Physico-chemical, rheological and technological evaluation of new bread wheat varieties grown in Toshka, Aswan (Egypt) by Mixolab", *Journal of Food and Dairy Sciences*, Vol. 8 No. 3, pp. 163–168. Available at: [https://journals.ekb.eg/article\\_37150\\_5f7168bb005d63126ad1fbdd1cb5ac7b.pdf](https://journals.ekb.eg/article_37150_5f7168bb005d63126ad1fbdd1cb5ac7b.pdf) (Accessed: 10 April 2024).
- Unalp-Arida, A., Liu, R. and Ruhl, C. E.

- (2022), "Nutrient intake differs among persons with celiac disease and gluten-related disorders in the United States", *Scientific Reports*, Vol. 12 No. 1, pp. 1–12. <https://doi.org/10.1038/s41598-022-09346-y>.
- Wang, S., Li, C., Copeland, L., Niu, Q. and Wang, S. (2015), "Starch retrogradation: A comprehensive review", *Comprehensive Reviews in Food Science and Food Safety*, Vol. 14 No. 5, pp. 568–585. <https://doi.org/10.1111/1541-4337.12143>.
- Zheng, M., Su, H., You, Q., Zeng, S., Zheng, B., Zhang, Y. and Zeng, H. (2019), "An insight into the retrogradation behaviors and molecular structures of lotus seed starch-hydrocolloid blends", *Food Chemistry*, Vol. 295, pp. 548–555. <https://doi.org/10.1016/j.foodchem.2019.05.166>.