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Formulation of Gluten-Free Biscuits: Utilizing Alternative Flours with Post-Storage Rancidity Assessment

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ABSTRACT

The increasing prevalence of gluten-related disorders necessitates the development of gluten-free alternatives in the food industry. This study uses alternative flours from flaxseed, soybean, Moringa oleifera, and white quinoa seeds to formulate gluten-free biscuits. Additionally, the study assesses the impact of storage on rancidity parameters after 4 and 6 weeks, providing insights into product shelf-life and quality maintenance. Significant differences ($P < 0.05$) were observed among all sensory properties except hardness, colour, flavour, and odour in soybean biscuits ($P = 0.814, 0.519, 0.214$, and 0.198, respectively). Regarding rancidity parameters, the mean of iodine values of biscuits fortified with Quinoa increased over time, while flaxseed, Moringa Oleifera, and Soybean substitutions generally decreased iodine values. Furthermore, increased Flaxseed, Moringa Oleifera, and soybean substitutions resulted in reduced acid and peroxide values, indicating significantly improved oxidative stability. Conversely, quinoa substitutions showed mixed effects on acid and peroxide values. These findings underscore the potential of these alternative flours to influence the degree of rancidity of biscuits, with implications for their shelf-life and overall quality, and provide reassurance about the improved quality due to increased substitutions.

Keywords: Flaxseed, Quinoa, Soybean, Moringa oleifera.

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INTRODUCTION

Approximately ,one percent of the population of the world suffers from the celiac disease and the numbers are increasing continuously **(Kalra *et al.*, 2022)**.The only available treatment for this disease is life-long avoidance of a gluten-containing diet and hence dependency on a gluten-free food diet **(Nieto-Mazzocco *et al.*, 2020)**.

Biscuits are the most widely available baked goods, because of its combining nutrition and long shelf-life. Their shelf-life is extended due to their very low humidity content - which hinders microbial development and degradation, allowing the product to keep its optimal characteristics for longer, as long as appropriately kept dry **(David Wesley *et al.*, 2021).**

Among the gluten-free diet, biscuits are the most important cereal products which are preferred by many people besides bread. It is a snack with universal appeal, good nutritive properties, cheap, convenient, appetizing, and easily available **(Xu *et al.*, 2020).**

Flaxseed flour is renowned for its high content of omega-3 fatty acids, lignans, and dietary fiber. These components contribute to cardiovascular health, possess anti-inflammatory properties, and enhance digestive health. The mucilaginous nature of flaxseed aids in binding, making it a suitable replacement for gluten in baking applications **(Ganorkar and Jain, 2013; Kaushik *et al.*, 2016)**.

Soybean flour is a powerhouse of protein, essential amino acids, and isoflavones. Its high protein content and emulsifying properties can improve the texture and nutritional profile of gluten-free baked goods **(Kaushik *et al.*, 2016)**.

Moringa oleifera, often referred to as the "miracle tree," is rich in vitamins, minerals, and antioxidants. Its incorporation into biscuits can significantly boost their nutritional value. The bioactive compounds in Moringa can also provide health benefits such as improved immune function and anti-inflammatory effects **(Gopalakrishnan *et al.*, 2016)**.

Quinoa has a balanced amino acid profile, high protein content, and essential minerals like magnesium and phosphorus. Quinoa flour contributes to the structural integrity of gluten-free products while offering a mild flavor and superior nutritional benefits compared to traditional cereal grains **(Vega-Gálvez *et al.*, 2010)**

Formulating gluten-free biscuits with flaxseed, soybean, Moringa oleifera, and white quinoa flours offers an innovative solution for meeting the dietary needs of individuals with gluten intolerance. Utilizing the unique properties of these alternative flours makes it possible to produce both nutritious and enjoyable biscuits, enhancing the pleasure of a gluten-free diet. This research seeks to optimize the blend ratios and processing methods to achieve the best possible sensory and nutritional results, supporting the growing market for gluten-free foods and assessing the impact of storage on rancidity after 4 and 6 weeks.

MATERIALS AND METHODS

2.1. Biscuits preparation

The study was conducted at Nutrition Laboratory, High Institute of Public Health, Alexandria University. The ingredients used were prepared to get 13 different formulations of biscuits. Different three concentration of each flour including Moringa, Soybean, Flaxseed, and Quinoa compared to corn (100%) as control biscuits.

2.1.1. Corn biscuits (control group) 100% corn flour

- Preparation of fortified **Moringa Oleifera** biscuits by using 10%, 20%, 30% moringa to 90%,80%,70% corn flour.
- Preparation of fortified **Quinoa** biscuits by using 30%,40%,50% quinoa to 70%,60%,50% corn flour.
- Preparation of fortified **flaxseed** biscuits by using 5%,7%,10% flaxseed to 95%,93%,90% corn flour.
- Preparation of fortified **Soybean** biscuits by using 5%,10%,15% soybean to 95%,90%,85% corn flour.

The preparation of biscuit dough, as per referenced studies, involves blending corn flour with different substitutions as mentioned above with flaxseed, soybean, Moringa oleifera, and white quinoa flours. To this mixture, 100g sugar, 100g butter, 1 egg, and 5 g baking powder gluten free product are added and thoroughly mixed to achieve a uniform consistency. The resulting dough is then rolled to a 5mm thickness, cut into desired shapes, and arranged on a baking sheet lined with parchment paper. Baking occurs in a preheated oven at 180°C (350°F) for 15-20 minutes, or until the biscuits achieve a golden brown color **(Kaur and Sandhu, 2010)**.

2.2. Sensory evaluation:

The prepared biscuits underwent sensory evaluation by 196 participants comprising teaching staff, students, and technicians from the Faculty of Physical Therapy at Pharos University. Participants, representing 98 male and 98 female. Before the sensory evaluation session, participants provided consent, and the researcher clarified all instructions. The evaluation utilized a 5-point Hedonic scale to assess parameters such as color, hardness, appearance, odor, flavor, sweetness, crunchiness, aftertaste, and overall acceptability. Participants assigned scores based on their preference, ranging from 5 for strong liking to 1 for strong disliking, with biscuits identified by unique codes. Sensory evaluation of the biscuits was performed according to (International Organization for Standardization [ISO]. 1998). A five-point hedonic scale was established separately for each product **(Klunklin and Savage, 2018)**.

2.3. Rancidity Measurement of different prepared biscuits before and after storage period (4-6) weeks

2.3.1. Samples preparation and extraction

The biscuit samples were crushed into powder using a small laboratory mill. Subsequently, solvent extraction of the oil from each biscuit sample was conducted using n-hexane. The samples were placed in glass containers, and n-hexane of analytical grade (boiling point 69ºC), procured from Sigma-Aldrich Company, Germany, was added in a ratio of 5:1 (v/w) relative to the sample. The mixture underwent mechanical stirring for 16 hours in a shaker at room temperature, followed by filtration twice using standard filter paper. The resultant clean filtration was concentrated using a rotating evaporator to eliminate the solvent from the oil. The distilled oil was left to stand in the open air at room temperature to ensure complete removal of any residual solvent. Sufficient quantity of oil was successfully extracted, which was then stored in a refrigerated bottle for subsequent analysis **(Ghendov-Mosanu *et al.*, 2023)**.

2.3.2. Acid value:

2.3.2.1. Principle:

The acid value of an oil or fat is defined as the number of mg of potassium hydroxide required to neutralize the free acidity in 1g of the sample. The result is often expressed as the percentage of free fatty acid.

2.3.2.2. Methods

The acid value of oil is determined through titration with a standardized potassium hydroxide (KOH) solution, employing phenolphthalein as an indicator. Initially, a precise amount of the oil sample (typically 2-5 grams) is weighed into a clean conical flask. A few drops of phenolphthalein are added to the sample, imparting a pink color. Then, a standardized KOH solution is titrated into the flask from a burette until the pink color fades, indicating neutralization of the free fatty acids in the oil. The volume of KOH solution used is recorded. The acid value (AV) is calculated using the formula: AV = (Volume of KOH solution used × Normality of KOH solution × 56.1) / Weight of oil sample. The result is expressed in milligrams of potassium hydroxide per gram of oil (mg KOH/g). Proper calibration of equipment and standardization of the KOH solution are essential before proceeding with the titration **(American Oil Chemists' Society [AOCS], 2009)**.

2.3.3. Iodine value:

2.3.3.1. Principle:

Iodine value is defined as the 1 gram of iodine absorbed per 100g sample. The higher the amount of unsaturation, the more iodine is absorbed, the higher the iodine value the greater degree of unsaturation.

2.3.4. Iodine value

Iodine value = (B-S) ×N×12.69 /weight of sample

Where: B = titration of blank, S = titration of sample, and N = normality of $Na_2S_2O_3$ solution.

2.3.5. Peroxide value (PV)

Peroxide value is defined as the milli-equivalent (mEq) of peroxide per kilogram of sample. It is a redox titrimetric determination. Peroxide values were determined according to AOAC Official Method 965.33, the peroxide value is determined by measuring the amount of iodine, which is formed by the reaction of peroxides (formed in fat or oil) with iodide ion. The excess of acetic acid present takes up the base produced in this reaction. The iodine liberated is titrated with sodium thiosulphate.

2.3.6. Energy value

Biscuits were analyzed for gross energy value according to the methods of **Krishna and Ranjhan (1981)**. The energy value of biscuits of each type of flour was calculated by multiplying the percentages of crude protein and carbohydrate with 4 and crude fat with 9.

Calculation

Energy value of food (Kcal/100g) = (% available carbohydrates×4) + (%protein×4) + (%fat×9)

2.3.7. Statistical analysis:

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percentage. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data was described using mean and standard deviation. The significance of the results obtained was judged at the 5% level.

RESULTS AND DISCUSSION

Part I

Sensory evaluation of gluten free biscuits:

Overall acceptability is highest for 50% quinoa (figure 1), with significant improvements in all sensory attributes. The acceptability decreases with lower quinoa content, but all quinoa-treated biscuits are preferred over 100% corn. The results of overall acceptability of quinoa flour align with **Cannas *et al*. (2020)**, who found that biscuits containing 25% and 50% quinoa flour were more favorably accepted compared to those with 75% and 100% quinoa flour. The color intensity of quinoa biscuits decreased with decreasing Quinoa Flour concentrations (figure 5), consistent with the findings of **Godse *et al*. (2020)**, who reported a linear relationship between quinoa flour levels and color scores.

According to figure 2, The overall acceptability scores increase significantly with the addition of 7% and 10% flaxseed compared to the 100% corn control. The 7% flaxseed biscuits have the highest overall acceptability score. The 5% flaxseed biscuits do not show a significant difference in overall acceptability compared to the control. However, our results differed regarding Flaxseed and quinoa, where no significant effect on hardness was observed. **Laelago *et al*. (2015)** reported that appearance decreased with increased concentration of sweet potato flour which gave a dark color to a biscuit which was not liked much by the panelist was agreement with our result concerning soybean but results concerning.

According to our study (figure 3), the sensory evaluation indicates that biscuits with higher concentrations of Moringa (10%, 20%, and 30%) have significantly lower scores in most sensory attributes compared to the 100% corn control (figure 3). Specifically, the 20% concentration shows some promise in terms of sweetness and overall acceptability, with no significant difference from the control. Moringa oleifera and quinoa whose mean appearance increase with increasing concentration of its flour in the same line with **Chopra *et al*. (2018)** which showed that mean appearance increases when measuring it in cookies contained 0, 10, 20, and 30 g quinoa flour.

Regarding soybean biscuits (figure 4), the overall acceptability of the 5% soybean biscuits is similar to the 100% corn control, with no significant difference. However, the overall acceptability of the 10% and 15% soybean biscuits is significantly lower, indicating that higher soybean concentration affects the overall acceptability negatively. Consumers' purchasing decisions and consumption behaviors are significantly influenced by the visual appeal of food products, particularly their color, which can shape perceptions of flavor (**Laignier *et al*., 2021**). Similarly, our study found that soybean flour substitutions in biscuits resulted in varying color acceptability scores, with scores of 4.50, 1.93, and 1.80 for concentrations of 5%, 10%, and 15% wheat flour substitutions, respectively. Our Study revealed that the hardness decreased with increasing soybean flour concentrations, in line with **Kaur *et al*. (2019)**, who reported decreased resilience and chewiness in cookies with higher levels of flaxseed flour and soy flour substitutions in biscuits, respectively. **Banureka and Mahendran (2011)** observed a decrease in the flavor of soybean biscuits as the concentration of soybean flour increased, consistent with our findings for soybean biscuits with 5%, 10%, and 15%, corn substitution, yielding flavor means of 4.29, 2.34, and 1.69 respectively.

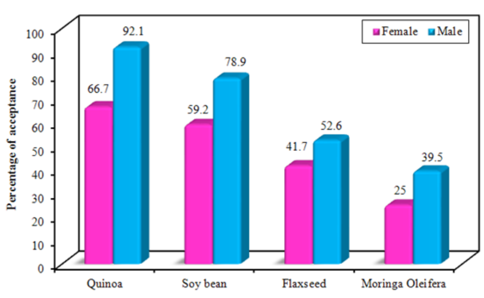

Figure 1. Percentage of acceptance of gluten free biscuits substituted with Quinoa, Soybean, Flaxseed and Moringa.

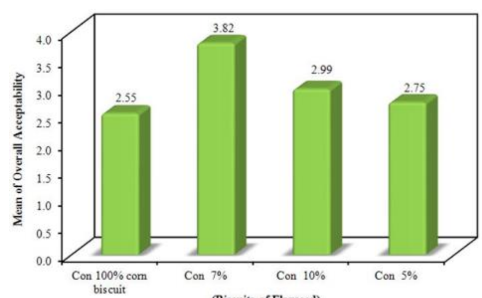

Figure 2. The Overall acceptability (mean ± SD) of gluten free biscuits substituted with Flaxseed different concentration 5%, 7%,10%.

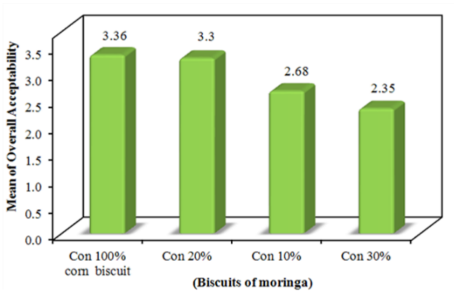

Figure 3. The Overall acceptability (mean ± SD) of gluten free biscuits substituted with Moringa oleifera of different concentration 10%, 20%, 30%.

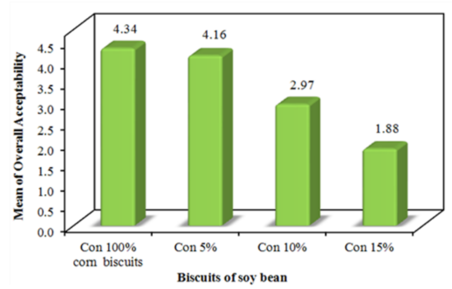

Figure 4. The Overall acceptability (mean ± SD) of gluten free biscuits substituted with Soybean of different concentration 5%, 10%, 15 %.

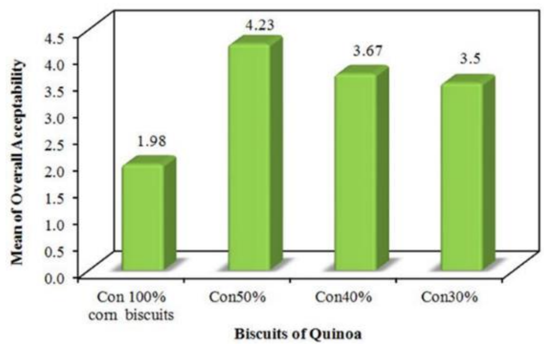

Figure 5. The Overall acceptability (mean ± SD) of gluten free biscuits substituted with Quinoa of different Concentration 30%, 40%, 50%.

Part II

Rancidity parameters after 4 and 6 weeks:

Rancidity poses a significant challenge in biscuit production due to the fat content, leading to undesirable off-flavors. The extent of oxidative rancidity in biscuits was assessed by monitoring changes in acid, peroxide, and iodine values over 4 and 6 weeks.

As table 1 illustrated results, a noticeable, statistically significant decrease in iodine value is observed with increasing quinoa content. The significant pairwise comparisons indicate that corn (100%) has a higher iodine value compared to quinoa blends. Similar to iodine values, acid values decrease with increasing quinoa content. The reduction in acid values with higher quinoa content might indicate a lower level of free fatty acids, which can be associated with better shelf stability and reduced rancidity over time. Peroxide values show minimal variation across storage periods. The stable peroxide values across all samples indicate that there is no significant lipid oxidation occurring over the storage period, suggesting good preservation of the oil quality in both corn and quinoa blends.

Table 1. Mean Iodine value (mg/g), Acid value (mg KOH/g) and peroxide value (mEq/Kg) of biscuits substituted with Quinoa after 4 and 6 weeks of storage

Sample	After 4 weeks of store Mean ± SD.	After 6 weeks of store Mean ± SD.
Iodine value (mg/g)		
Corn 100%	0.56 ± 0.02	0.57 ± 0.05
Quinoa 50%	0.39^{a**} ± 0.04	0.42 ± 0.03
Quinoa 40%	0.28^{ab**} ± 0.09	0.31^{a**} ± 0.03
Quinoa 30%	0.21^{a**} ± 0.01	0.25^{a**} ± 0.17
F(p)	**27.373*(<0.001*)**	**7.142*(0.012*)**
Acid value (mg KOH/g)		
Corn100%	1.92 ± 0.04	1.91 ± 0.06
Quinoa 50%	1.78^{a*} ± 0.09	1.83 ± 0.02
Quinoa 40%	1.64^{a**b*} ± 0.03	1.68^{a**b**} ± 0.05
Quinoa 30 %	$1.24^{a**b**c**}$ ± 0.08	$1.25^{a**b**c**}$ ± 0.06
F(p)	**60.682*(<0.001*)**	**102.842*(<0.001*)**
Peroxide value (mEq/Kg)		
Corn100%	2.1	2.2
Quinoa 50%	1.96	1.99
Quinoa 40%	1.93	2.00
Quinoa 30%	1.9	1.94

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
a: significant with group Corn 100%
b: significant with group Quinoa 50%
c: significant with group Quinoa 40%
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

Rancidity Parameters after 3 and 6 weeks of storage period of fortified biscuits with soybean

Table 2 presents the iodine values, acid values, and peroxide values of biscuits substituted with varying percentages of Soybean (5%, 10%, and 15%) derived from soybean flour, alongside Plain biscuits (100% corn), after 4 and 6 weeks of storage. No significant differences in iodine values among the groups at 4 weeks (F = 3.520, p = 0.069), but significant differences were observed in 6 weeks (F = 5.917, p = 0.020). The iodine value is higher in the corn sample compared to the soy-substituted samples. The significant differences at 6 weeks suggest that the type of sample (corn vs. soy) has a considerable impact on the iodine value after longer storage periods. Regarding the acid value there was a significant difference in acid values among the groups at both 4 and 6 weeks. The decrease in acid values for Soya 15% and Soya 10% indicates better shelf stability, likely due to the antioxidative properties of soy components that slow down lipid oxidation. However, the increase in Soya 5% suggests possible differences in the lipid composition or oxidative stability among the formulations, potentially influenced by lower concentrations of soy. The increase in peroxide values over time suggests that lipid oxidation is occurring, but the values remain relatively low, indicating good oil quality.

Table 2. Mean of Iodine value (mg/g), Acid value (mg KOH/g) and peroxide value (mEq/Kg) of biscuits substituted with Soybean after 4 and 6 weeks of storage.

Sample	After 4 weeks of store Mean ± SD.	After 6 weeks of store Mean ± SD.
Iodine analysis (mg/g)		
Corn100%	0.56 ± 0.02	0.57 ± 0.05
Soybean 10%	0.39 ± 0.01	0.35^{a**} ± 0.09
Soybean 15%	0.37^{a*} ± 0.18	0.32^{a**} ± 0.11
Soybean 5%	0.34^{a*} ± 0.02	0.41^{a*} ± 0.05
F(p)	**3.520(0.069)**	**5.917*(0.020*)**
Acid value (mgKOH/g)		
Corn 100%	1.92 ± 0.06	1.91 ± 0.06
Soybean 15%	1.25^{a**} ± 0.03	1.14^{a**} ± 0.03
Soybean 10%	1.19^{a**} ± 0.01	1.12^{a**} ± 0.04
Soybean 5%	1.14^{a**b*} ± 0.06	$1.32^{a**b**c**}$ ± 0.02
F(p)	**196.146*(<0.001*)**	**251.985*(<0.001*)**
Peroxide value (mEq/Kg)		
Corn100%	2.1	2.2
Soybean 15%	1.8	1.88
Soybean 10%	1.77	1.8
Soybean 5%	1.62	1.65

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
a: significant with group Corn 100%
b: significant with group Quinoa 50%
c: significant with group Quinoa 40%
*: Statistically significant at p ≤ 0.05
**: Statistically significant at p ≤ 0.01

Rancidity Parameters after 3 and 6 weeks of storage period of fortified biscuits with Moringa

Table 3 indicates that Moringa substitution in biscuits influences iodine and acid values significantly, with the highest concentration (30%) showing stability in iodine retention and acid values. Lower Moringa concentrations (20% and 10%) also show stable acid values with minor decreases in iodine values. Peroxide values remain relatively stable across all samples, indicating good preservation of oil quality.

Table 3. Mean of Iodine value (mg/g), Acid value (mg KOH/g) and peroxide value (mEq/Kg) of biscuits substituted with Moringa after 4 and 6 weeks of storage.

Sample	After 4 weeks of store Mean ± SD.	After 6 weeks of store Mean ± SD.
	Iodine analysis (mg/g)	
Corn100	0.56 ± 0.02	0.57 ± 0.05
Moringa 30%	1.1^{a**} ± 0.09	1.1^{a**} ± 0.07
Moringa 20%	0.72^{a*b**} ± 0.08	$0.69^{a*\,b**}$ ± 0.05
Moringa 10%	0.64^{b**} ± 0.05	0.62^{b**} ± 0.01
F(p)	**39.425*(<0.001*)**	**70.120*(<0.001*)**
	Acid value (mgKOH/g)	
Corn100 %	1.92 ± 0.04	1.91 ± 0.06
Moringa 30%	2.3^{a**} ± 0.08	2.24^{a**} ± 0.07
Moringa 20%	2.1^{a**b**} ± 0.03	1.96^{b**} ± 0.05
Moringa 10%	1.93^{b**c**} ± 0.02	1.90^{b**} ± 0.06
F(p)	**41.151*(<0.001*)**	**21.171*(<0.001*)**
	Peroxide value (mEq/Kg)	
Corn100 %	2.1	2.2
Moringa 30%	2.3	2.31
Moringa 20%	2.17	2.15
Moringa 10%	2.14	2.15

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
a: significant with group Corn 100%
b: significant with group Quinoa 50%
c: significant with group Quinoa 40%
*: Statistically significant at $p \le 0.05$
**: Statistically significant at $p \le 0.01$

Rancidity Parameters after 3 and 6 weeks of storage period of fortified biscuits with Flaxseed

Flaxseed substitution significantly increases the iodine value compared to 100% corn (table 4). The higher concentrations of flaxseed (10% and 7%) maintain higher iodine values over time. Regarding acid value there was a significant difference among the groups observed at both 4 weeks ($F = 48.818$, $p < 0.001$) and 6 weeks ($F = 84.252$, $p < 0.001$). The significant differences among the groups highlight the impact of flaxseed content on acid values. The higher flaxseed content (10%) shows a significant decrease and better stability. The lower acid values in flaxseed-substitute samples indicate reduced free fatty acids and improved shelf stability. The peroxide values in flaxseed-substituted biscuits are similar to those in 100% corn biscuits, suggesting that flaxseed has minimal impact on lipid oxidation. The slight increases in peroxide values over time are consistent with normal storage conditions, indicating good preservation of oil quality and shelf stability

Table 4. Mean of Iodine value (mg/g), Acid value (mg KOH/g) and peroxide value (mEq/Kg) of biscuits substituted with Flaxseed after 4 and 6 weeks of storage.

Sample	After 4 weeks of store Mean ± SD.	After 6 weeks of store Mean ± SD.
	Iodine analysis (mg/g)	
Corn 100%	0.56 ± 0.02	0.57 ± 0.05
Flaxseed 10%	0.84^{a**} ± 0.01	0.81^{a**} ± 0.03
Flaxseed 7%	0.61^{b**} ± 0.05	0.55^{b**} ± 0.06
Flaxseed 5%	$0.42^{a**b**c**}$ ± 0.05	$0.39^{a** b**c*}$ ± 0.09
F(p)	**66.527*(<0.001*)**	**23.841*(<0.001*)**
	Acid value (mgKOH/g)	
Corn100%	1.92 ± 0.04	1.91 ± 0.06
Flaxseed 10%	1.65^{a**} ± 0.08	1.48^{a**} ± 0.01
Flaxseed 7%	1.58^{a**} ± 0.03	$1.61^{a** b*}$ ± 0.07
Flaxseed 5%	$1.28^{a**b**c***}$ ± 0.09	$1.24^{a**b**c**}$ ± 0.05
F(p)	**48.818*(<0.001*)**	**84.252*(<0.001*)**
	Peroxide value (mEq/Kg)	
Corn100%	2.1	2.2
Flaxseed 10%	1.86	1.89
Flaxseed 7%	1.8	1.81
Flaxseed 5%	1.77	1.8

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
a: significant with group Corn 100%
b: significant with group Quinoa 50%
c: significant with group Quinoa 40%
*: Statistically significant at $p \le 0.05$
**: Statistically significant at $p \le 0.01$

Part III

Energy value

The impact of various types of highly accepted concentrations of gluten-free flour in biscuits on the mean calorific value is illustrated in table 5. Flaxseed biscuits have the highest energy content (578.06 kcal/100g), and this substantial increase in energy content can be attributed to the high-fat content in flaxseed, followed by soybean biscuits (402.93 kcal/100g), quinoa biscuits (361.56 kcal/100g), and moringa biscuits (47.05 kcal/100g), indicating that Moringa flour contributes significantly less energy compared to other flours used in the study. The exceptionally low calorific value suggests that moringa adds very little energy content, possibly due to its high fiber and low-fat content.

Table 5. Effect of different types of gluten free flour of biscuits on the means of calorific value (kcal\100g).

Energy value	Corn biscuits (plain)	Flaxseed biscuits	Soybean biscuits	Quinoa Biscuits	Moringa biscuits	F(p)
Minimum	377.38	586.12	401.86	361.12	44.1	7150.9* (<0.001*)
Maximum	380.44	570	404	362	50	
Mean	378.91	578.06^{a**}	402.93^{a**b**}	$361.56^{a**b**c**}$	$47.05^{a**b**c**d**}$	

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
a: significant with Corn biscuits
b: significant with Flaxseed biscuits
c: significant with Soybean biscuits
d: significant with group Quinoa biscuits
*: Statistically significant at $p \le 0.05$
**: Statistically significant at $p \le 0.01$

CONCLUSION

Regarding sensory evaluation of gluten free biscuits in our study, the highest acceptability and sensory scores are observed at 50% quinoa concentration, showing it is the most preferred formulation. The significant differences ($p < 0.001$) highlight the impact of higher soybean content on the sensory properties of the biscuits, suggesting that lower concentrations of soybean are preferable for maintaining sensory quality. The 20% moringa concentration shows similar sensory attributes to the 100% corn control, suggesting it may be a better option for maintaining sensory quality while incorporating moringa. The significant differences ($p < 0.001$) highlight the positive impact of 7% and 10% flaxseed on the sensory properties of gluten-free biscuits, suggesting that a higher concentration of flaxseed is needed to achieve noticeable improvements in sensory quality

The stable peroxide values across all samples of quinoa concentrations indicate that there is no significant lipid oxidation occurring over the storage period, suggesting good preservation of the oil quality. Slightly higher peroxide values in moringa-substituted samples compared to 100% corn suggest that moringa might contribute to mild lipid oxidation. However, the values remain within acceptable limits, indicating good oil quality and stability over time. Higher soy content (10% and 15%) shows better stability in acid values, while the impact on iodine values varies. Peroxide values remain relatively stable across all samples, indicating good preservation of oil quality. Flaxseed substitution can enhance the nutritional value of biscuits by reducing free fatty acids while maintaining good oil quality and shelf stability.

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