



Physicochemical and microbiological stability of gluten-free cookies formulated with coconut and almond flours

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ABSTRACT

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Gluten-free bakery products have gained increasing popularity due to health concerns associated with gluten consumption, including conditions such as gluten intolerance. The absence of gluten in flour-based ingredients poses considerable technological challenges, particularly in maintaining desirable product quality. Therefore, our study aimed to develop gluten-free cookies using alternative ingredients and to evaluate their physicochemical and microbiological stability during storage. Gluten-free cookies were formulated using almond flour and coconut flour as wheat flour substitutes, while erythritol was used to replace sucrose at substitution levels ranging from 0 to 100%. The results showed that substituting wheat flour with almond flour had minimal effects on the physicochemical characteristics and microbiological quality of the cookies. The substitution of sucrose with erythritol at levels of 0–100% resulted in only slight changes in cookie quality. However, increasing the proportion of coconut flour as a substitute for almond flour affected product characteristics. Higher coconut flour levels led to an increase in lightness (L) and a decrease in redness (a*) and yellowness (b*). Moreover, moisture content and water activity (a_w) tended to increase with higher coconut flour proportions. No pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.*, were detected in any formulation throughout the 60-day storage period. These findings suggest that almond flour-based formulations provide greater storage stability, while coconut flour may increase moisture and water activity. The study demonstrates the potential application of almond and coconut flours in producing nutritious and microbiologically safe gluten-free cookies suitable for health-conscious consumers.

Contribution/Originality: This study contributes to the existing literature on gluten-free bakery products by examining coconut and almond flours as alternatives to wheat flour and erythritol as a substitute for sucrose. The findings indicate that coconut- and almond-based formulations demonstrate shelf-life stability and microbial safety.

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a member of the Arecaceae family and thrives in tropical climates. It is extensively grown in parts of Asia, Africa, and Central and South America. Coconuts and their by-products can be used in both food and non-food products, resulting in sustained global demand. The coconut industry generates increasing economic value from food-based coconut products each year, while non-food coconut derivatives, both processed and unprocessed, also contribute to income generation. Therefore, they play an important role in the agricultural sector

and exports of producing countries (FAO Regional Office for Asia and the Pacific, 2013; Ignacio & Miguel, 2021; Zainol et al., 2023). Coconut flour is a by-product obtained from the coconut milk production process. It is made by drying and grinding the residue from the production process into a fine coconut powder. This flour is characterized by a high proportion of dietary fiber, accompanied by appropriate amounts of protein and fat. Normally, the composition of coconut flour varies depending on the processing techniques used and the oil extraction methods applied. It is naturally gluten-free and is rich in dietary fiber, protein, and fat. These characteristics make coconut flour a popular alternative to traditional grain-based flours. Due to its compositional profile, coconut flour has gained increasing application as a functional ingredient for low-carbohydrate and gluten-free food products. Besides coconut flour, almond flour is another option for gluten-free food products. Likewise, almond flour has a favorable nutritional composition. It is rich in dietary fiber, as well as protein and fat, and is widely used in gluten-free food formulations as well. Collectively, these alternative flours offer promising potential for the development of special food products. Both coconut and almond flours are suitable for meeting the nutritional requirements of individuals following health-oriented diets. They provide valuable nutritional advantages for specific food products (Adeloye, Osho, & Idris, 2020; Hopkin, Broadbent, & Ahlborn, 2022; Makinde & Taibat, 2018).

From a market viewpoint, the gluten-free diet has expanded from a niche dietary option to a health-conscious consumer segment. This widespread trend appears to be strongly influenced by consumer attitudes that associate the gluten-free diet with a healthier lifestyle. Gluten-free bakery products are therefore one option within this food category, resulting in increasing demand for these products. This trend may be associated with the growing application of low-carbohydrate and adequate-protein dietary patterns for body weight control, as well as for the prevention and dietary control of diabetes and neurological disorders (Hopkin et al., 2022; Leone et al., 2019). In the past decade, studies on gluten-free bakery products have grown considerably. This is due to the increasing occurrence of celiac disease, non-celiac gluten sensitivity, and consumer interest in gluten-free diets for health-related reasons. Gluten is a protein complex found in wheat flour. In conventional baked products, wheat gluten plays an important role in dough formation, resulting in dough elasticity and gas-holding capacity. Therefore, the use of gluten-free ingredients presents challenges in bakery product development. Gluten-free flours are incorporated into bakery formulations to replace wheat flour and produce gluten-free products. Rice flour, maize flour, pseudocereals, legumes, and nut-based flours are used to enhance rheological behavior, textural properties, and overall product quality. Currently, research in gluten-free bakery products focuses on the optimization of both ingredient selection and processing methods. These efforts aim to improve the nutritional value, sensory acceptance, and shelf-life stability of gluten-free baked products, while also ensuring consumer acceptability and compliance with regulatory standards (Arendt & Dal Bello, 2008; Gallagher, Gormley, & Arendt, 2004; Melini & Melini, 2019). Bandara, Jayasooriya, and Senevirathne (2026) reported that tropical almond defatted press cake flour was a promising alternative protein source, and it can be incorporated at levels up to 50% in cookie production without significantly affecting cookie quality.

Sucrose is the main sweetener in bakery formulations, appreciated not only for providing sweetness but also for its role in developing acceptable texture and sensory attributes, including color, crunchiness, and mouthfeel. As alternatives to sucrose in baked goods like cookies, polyols (sugar alcohols) offer several advantages. They provide roughly half the calories of sucrose, have a low glycemic index, are non-cariogenic, and possess bulk functionality that can replicate the textural role of sucrose. Maltitol and erythritol are used as replacements for sucrose and glucose syrup in oat cookies. Maltitol formulations showed characteristics similar to the control, although they exhibited greater adhesiveness. Overall, while erythritol led to notable texture changes, maltitol maintained a sensory profile more comparable to the control (De Vivo et al., 2026; Ding & Yang, 2021).

In the past few years, coconut flour and almond flour have been studied as supplementary ingredients in gluten-free bakery formulations. Promoting consumer awareness of the nutritional labeling of gluten-free bakery products could contribute to more informed and healthier food selection and consumption decisions. Our research describes

the materials and methods used to develop gluten-free cookies formulated with almond flour, coconut flour, and erythritol, including procedures for physicochemical and microbiological analyses during storage. This is followed by the presentation and discussion of the results, which focus on the effects of flour substitution and sweetener replacement on physicochemical properties and microbial stability. Finally, we summarize the key findings and highlight the practical implications for developing gluten-free bakery products. Therefore, this study aimed to examine the potential of coconut flour or almond flour as substitutes for wheat flour in gluten-free cookie formulation and to evaluate erythritol as a replacement for sucrose. The study further investigated the effects of these substitutions on the physicochemical characteristics and microbiological quality of the cookies, as well as their stability during a 60-day storage period.

2. MATERIALS AND METHODS

2.1. Preparation of Coconut Flour and Almond Flour

The husks and shells of fresh and mature coconuts were manually removed. After removing the shells, the coconut water was poured out. The coconut meat was grated into fine particles, and then coconut milk was obtained using a manual cold-press technique commonly applied in virgin coconut oil production. The residual cake was dried in a hot-air oven at $60 \pm 2^\circ\text{C}$ for 6–8 hours and cooled to room temperature ($25 \pm 2^\circ\text{C}$). Subsequently, the dried coconut cake was ground using a mechanical blender to produce coconut flour, which was then packed in a sealed foil bag. Almond flour was purchased from a commercial baking supply retailer in Surat Thani Province, Thailand. The prepared coconut flour and almond flour were stored at room temperature until used for further experiments.

2.2. Formulation of Basic Cookies Using Almond Flour

The ingredients and the basic cookie formulation used in the experiment were based on commercial formulations. However, as the research aimed to develop a healthier cookie formulation, gluten-free flour, low- or zero-calorie sugar, and butter were selected. The cookies were formulated with all-purpose wheat flour and a combination of almond flours, using the following main ingredients: flour (38%), sucrose (23%), unsalted butter (20%), whole egg (17.5%), vanilla extract (1.3%), baking powder (0.7%), and salt (0.5%). The four cookie formulations were designed based on the ratios of all-purpose flour to almond flour of 4:0, 2:2, 1:3, and 0:4 (w/w). All ingredients were weighed according to the specified formulation ratios. An electric kitchen mixer was used to cream unsalted butter and sugar for 9 minutes. After adding the vanilla and egg, the mixture was mixed for 2 minutes. Flour, baking powder, and salt were added and mixed at speed 1 for one minute. The cookie dough was rested at 0 to 5°C for 10 minutes. After that, it was shaped into small, round balls and further flattened to a thickness of approximately 0.5–1.0 cm. The cookies were placed on a baking tray lined with parchment paper and baked at 160°C for 15–18 minutes. The baked cookies were taken out of the oven, cooled to room temperature for an hour, then packaged in sealed polyethylene bags with low oxygen permeability and kept at room temperature for 24 hours for data analysis. Quality analyses were conducted for both physicochemical and microbiological parameters. Color, moisture content, and water activity were assessed as physicochemical parameters, whereas total viable count, yeast and mold counts, coliforms, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. were analyzed as microbiological quality indicators.

2.3. Effect of Replacing Sucrose with Erythritol on Almond Cookie Qualities

Almond cookies were produced in four formulations using different levels of sucrose, with erythritol used as a sucrose substitute. The proportion of the main ingredients was the same as those described in Section 2.2. The ratios of sucrose to erythritol were 4:0, 3:1, 2:2, and 0:4 (w/w). The cookie-making process was similar to the method described above. After baking, the almond cookies were kept at room temperature. Both physicochemical and microbiological parameters were analyzed to evaluate cookie quality.

2.4. Effect of Replacing Almond Flour with Coconut Flour in Gluten-Free Cookies

This experimental step aimed to investigate the effects of substituting almond flour with coconut flour in the formula of gluten-free cookies. Four different formulations were prepared based on varying proportions of almond flour to coconut flour of 4:0, 3:1, 2:2, and 0:4 (w/w). All ingredients were kept constant, except for the flour, in which the ratio of almond flour to coconut flour was adjusted. The cookie-making process was done as described in Section 2.2. After baking, the gluten-free cookies were packed and stored at room temperature. Physicochemical and microbiological parameters were tested to evaluate cookie quality.

2.5. Quality Changes During Storage of Gluten-Free Cookies

Three formulations of gluten-free cookies were produced using almond flour and coconut flour at ratios of 2:0, 1:1, and 0:2. After baking and cooling to room temperature, the cookies were packed in food-grade zip-lock bags. The gluten-free cookie samples were stored at room temperature and analyzed at storage periods of 0, 15, 30, 45, and 60 days. Color, moisture content, and water activity were analyzed as physicochemical parameters. Total viable count, yeast and mold counts, coliforms, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. were tested as microbiological quality indicators.

2.6. Quality Analysis Methods

2.6.1. Color values (L , a^* , b^*)

Color was measured using a Hunter system colorimeter (CHN, model CS-288). Cookie samples were placed on a non-reflective surface and measured at three uniform points to obtain average values for lightness (L), redness–greenness (a^*), and yellowness–blueness (b^*).

2.6.2. Water Activity (a_w)

a_w was measured using a benchtop water activity meter (Aqua Lab, model 4TE). Finely ground cookie samples were placed into the designated sample chamber, and readings were recorded once the system stabilized.

2.6.3. Moisture Content

Moisture content was determined by the oven drying method (AOAC International, 2000). Approximately 2–3 g of each sample was weighed into a pre-dried and pre-weighed metal dish and then dried in a hot-air oven at $105 \pm 2^\circ\text{C}$ for 4 hours or until constant weight was achieved. Moisture content was determined from the weight loss after drying.

2.6.4. Microbiological Qualities

Microbiological analyses were performed following the Bacteriological Analytical Manual protocols and included total viable count (TVC) (BAM, 2025), yeast and mold counts (BAM, 2001), coliforms (BAM, 2020), *E. coli* (BAM, 2020), *S. aureus* (BAM, 2016), and *Salmonella* spp. (BAM, 2024). Sample preparation methods were briefly described as follows: A volume of 450 mL of Butterfield's phosphate-buffered dilution water was added to a stomacher bag containing 50 g of sample and mixed for 2 min, resulting in a dilution of 10^{-1} . Serial dilutions of the original homogenate were promptly prepared using pipettes capable of delivering the required volume accurately. Each decimal dilution was prepared by adding 10 mL of the previous dilution to 90 mL of sterile diluent. For the Plate Count Method, 1 mL of each dilution was pipetted into duplicate, appropriately labeled Petri dishes. During yeast and mold analysis, 0.1 mL of each dilution was aseptically pipetted onto pre-poured, solidified agar plates, and the inoculum was spread evenly using a sterile bent glass rod. In the MPN method, using at least three consecutive dilutions, 1 mL aliquots from each dilution were inoculated into three tubes for a three-tube MPN analysis. The

preparation of foods for the detection and isolation of *Salmonella* spp., 25 g of sample was aseptically weighed into a sterile wide-mouth screw-cap jar (500 mL), and 225 mL of sterile lactose broth was added.

2.7. Data Analysis

All measurements were conducted in triplicate and analyzed using analysis of variance (ANOVA). Differences among means were further evaluated using Duncan's multiple range test (DMRT) at a significance level of $P \leq 0.05$.

3. RESULTS

3.1. Effect of Substitution of All-Purpose Flour with Almond Flour on Cookie Qualities

The study investigating the effects of replacing all-purpose flour with almond flour at varying ratios (4:0, 2:2, 1:3, and 0:4) on cookie qualities revealed significant changes in physicochemical and microbiological properties (Table 1). Lightness (L value) decreased from 71.11 ± 1.34 to 65.15 ± 1.20 for the 100% all-purpose flour formulation and the 100% almond flour formulation, respectively. The results showed that a higher almond flour proportion resulted in darker-colored cookies. However, increasing the level of almond flour substitution led to rises in both redness (a^*) and yellowness (b^*) values. The 100% almond flour formulation exhibited the highest a^* and b^* at 5.04 ± 0.61 and 10.47 ± 0.32 . Moisture content consistently declined with increasing almond flour proportion. The highest moisture content was found in the 100% all-purpose flour formulation, at $3.35 \pm 0.11\%$, while the lowest was in the 100% almond flour substitution, at $2.84 \pm 0.09\%$. A similar decreasing trend was observed for water activity (a_w), which declined from 0.62 in the 4:0 formulation to 0.54 in the 0:4 formulation. However, the differences among treatments were not statistically significant ($p > 0.05$).

For microbiological quality, both total viable count (TVC) and yeast and mold counts decreased as the almond flour ratio increased. Specifically, TVC declined from 5.0×10^2 CFU/g in the 4:0 formula to 1.2×10^2 CFU/g in the 0:4 formulation, while yeast and mold counts decreased from 2.5×10^2 CFU/g to 1.0×10^2 CFU/g. Furthermore, no pathogenic microorganisms, including coliforms, *E. coli*, *S. aureus*, and *Salmonella* spp., were detected in any of the cookie samples.

Table 1. Quality characteristics of gluten-free cookies formulated with almond flour as a substitute for all-purpose flour.

Parameters	All-purpose flour: Almond flour 4:0	All-purpose flour: Almond flour 2:2	All-purpose flour: Almond flour 1:3	All-purpose flour: Almond flour 0:4
Physical qualities				
Color				
- L	71.11 ± 1.34^a	68.65 ± 3.25^{ab}	66.58 ± 1.75^b	65.15 ± 1.20^b
- a^*	1.91 ± 0.20^c	2.01 ± 0.18^c	3.72 ± 0.29^b	5.04 ± 0.61^a
- b^*	5.50 ± 0.31^d	6.30 ± 0.36^c	8.81 ± 0.14^b	10.47 ± 0.32^a
a_w	0.62 ± 0.06^{ns}	0.58 ± 0.02	0.57 ± 0.03	0.54 ± 0.04
Chemical quality				
Moisture content (%)	3.35 ± 0.11^a	3.16 ± 0.07^b	2.99 ± 0.12^{bc}	2.84 ± 0.09^c
Microbiological qualities				
TVC (CFU/g)	5.0×10^2	4.5×10^2	2.2×10^2	1.2×10^2
Yeast and mold (CFU/g)	2.5×10^2	1.9×10^2	1.1×10^2	1.0×10^2
Coliforms (MPN/g)	<3.0	<3.0	<3.0	<3.0
<i>E. coli</i> (MPN/g)	<3.0	<3.0	<3.0	<3.0
<i>S. aureus</i> (MPN/g)	<3.0	<3.0	<3.0	<3.0
<i>Salmonella</i> spp.	ND	ND	ND	ND

Note: Data are expressed as means $\pm$ the standard deviations ($n = 3$).

^{ns} = The mean values within the row are not significantly different ($P > 0.05$).

Mean values within a row with different superscript letters (^{a,b,c,d}) are significantly different ($P < 0.05$).

3.2. Effect of Substitution of Sucrose with Erythritol on the Cookie Qualities

The substitution of sucrose with erythritol at varying levels (0%, 50%, 75%, and 100%) had minor effects on the physicochemical characteristics and microbiological properties of the cookies (Table 2). The lightness increased from

64.82 ± 1.95 to 66.29 ± 1.19 as the proportion of sucrose replaced by erythritol increased from 0% to 75%. However, when 100% erythritol was added, the lightness slightly decreased. Replacing sucrose with erythritol resulted in minor changes in the a^* and b^* values of the cookies. The highest moisture content was found in the sample using 75% erythritol at 3.25%, decreasing to the lowest at 2.87% when using 100% erythritol. The a_w value ranged from 0.55 to 0.57. Overall, adding different amounts of erythritol to substitute sucrose did not significantly affect the color, moisture content, and a_w values ($p > 0.05$).

TVC and yeast and mold counts slightly increased when sucrose was substituted with erythritol at levels of 50% and 75%, respectively. The highest TVC was observed in the 75% substitution group (3.0×10^2 CFU/g), while it declined in the 100% substitution formula (2.2×10^2 CFU/g). Similarly, yeast and mold counts peaked in the 75% group (1.4×10^2 CFU/g) and were lower in the 0% and 100% groups. Microorganisms, including coliforms, *E. coli*, *S. aureus*, and *Salmonella* spp., were not detected in any of the cookie samples.

Table 2. Quality characteristics of gluten-free cookies with sucrose substituted by erythritol.

Parameters	Erythritol 0%	Erythritol 50%	Erythritol 75%	Erythritol 100%
Physical qualities				
Color				
- L	64.82 ± 1.95 ^{ns}	65.08 ± 0.43	66.29 ± 1.19	64.75 ± 1.09
- a^*	4.98 ± 0.99 ^{ns}	5.24 ± 0.82	5.74 ± 0.28	5.24 ± 0.57
- b^*	10.25 ± 1.05 ^{ns}	9.85 ± 0.94	10.56 ± 1.02	10.05 ± 0.73
a_w	0.55 ± 0.01 ^{ns}	0.55 ± 0.05	0.57 ± 0.04	0.56 ± 0.03
Chemical quality				
Moisture content (%)	3.03 ± 0.20 ^{ns}	2.95 ± 0.34	3.25 ± 0.27	2.87 ± 0.26
Microbiological qualities				
TVC (CFU/g)	1.8×10^2	2.5×10^2	3.0×10^2	2.2×10^2
Yeast and mold (CFU/g)	1.0×10^2	1.2×10^2	1.4×10^2	1.2×10^2
Coliforms (MPN/g)	<3.0	<3.0	<3.0	<3.0
<i>E. coli</i> (MPN/g)	<3.0	<3.0	<3.0	<3.0
<i>S. aureus</i> (MPN/g)	<3.0	<3.0	<3.0	<3.0
<i>Salmonella</i> spp.	ND	ND	ND	ND

Note: Data are expressed as means ± the standard deviations (n = 3).
^{ns} = The mean values within the row are not significantly different ($P > 0.05$).

3.3. Effect of Substitution of Almond Flour with Coconut Flour on Gluten-Free Cookies Qualities

The study examining the replacement of almond flour with coconut flour in gluten-free cookie formulations at substitution levels of 0%, 50%, 75%, and 100% revealed effects on the physical, chemical, and microbiological qualities (Table 3). In terms of physical attributes, the lightness (L value) increased from 66.52 ± 2.78 in the 0% coconut flour formulation to 72.05 ± 1.67 in the 100% formulation; however, this change was not statistically significant ($p > 0.05$). The redness (a^*) value significantly decreased from 5.28 ± 0.69 to 2.26 ± 0.79 with increasing coconut flour substitution from 0% to 100% ($p < 0.05$). The results were similar to those of the b^* value, which was significantly reduced from 10.75 ± 2.31 in cookies formulated with 100% almond flour to 5.24 ± 0.44 in those with 0% almond flour ($p < 0.05$). The water activity significantly increased from 0.53 ± 0.03 in the 100% almond flour to 0.66 ± 0.07 in the 100% coconut flour substitution ($p > 0.05$). Moisture content (%) also increased with higher proportions of coconut flour, ranging from 2.80 ± 0.22 in the 0% formulation to 5.08 ± 0.17 in the 100% formulation.

Regarding microbiological quality, both total viable count (TVC) and yeast and mold counts increased with the coconut flour level. TVC rose from 1.5×10^2 CFU/g in the control to 5.8×10^2 CFU/g in the 100% formulation, while yeast and mold counts increased from 1.0×10^2 CFU/g to 2.3×10^2 CFU/g. However, no contamination with pathogenic microorganisms, including coliforms, *E. coli*, *S. aureus*, or *Salmonella* spp., was found in the cookie samples.

Table 3. Quality characteristics of gluten-free cookies formulated with coconut flour as a substitute for almond flour.

Parameters	Almond flour: Coconut flour 4:0	Almond flour: Coconut flour 2:2	Almond flour: Coconut flour 1:3	Almond flour: Coconut flour 0:4
Physical qualities				
Color				
- L	66.52 ± 2.78 ^{ns}	68.82 ± 1.51	70.18 ± 2.12	72.05 ± 1.67
- a*	5.28 ± 0.69 ^a	3.81 ± 0.29 ^b	3.05 ± 0.23 ^{bc}	2.26 ± 0.79 ^c
- b*	10.75 ± 2.31 ^a	7.14 ± 0.74 ^b	6.89 ± 0.28 ^b	5.24 ± 0.44 ^b
a _w	0.53 ± 0.03 ^{ns}	0.59 ± 0.12	0.63 ± 0.05	0.66 ± 0.07
Chemical quality				
Moisture content (%)	2.80 ± 0.22 ^a	3.20 ± 0.26 ^a	3.90 ± 0.26 ^b	5.08 ± 0.17 ^c
Microbiological qualities				
TVC (CFU/g)	1.5 × 10 ²	1.9 × 10 ²	3.0 × 10 ²	5.8 × 10 ²
Yeast and mold (CFU/g)	1.0 × 10 ²	1.2 × 10 ²	1.7 × 10 ²	2.3 × 10 ²
Coliforms (MPN/g)	<3.0	<3.0	<3.0	<3.0
E. coli (MPN/g)	<3.0	<3.0	<3.0	<3.0
S. aureus (MPN/g)	<3.0	<3.0	<3.0	<3.0
Salmonella spp.	ND	ND	ND	ND

Note: Data are expressed as means ± the standard deviations (n = 3).

^{ns} = The mean values within the row are not significantly different (P > 0.05).

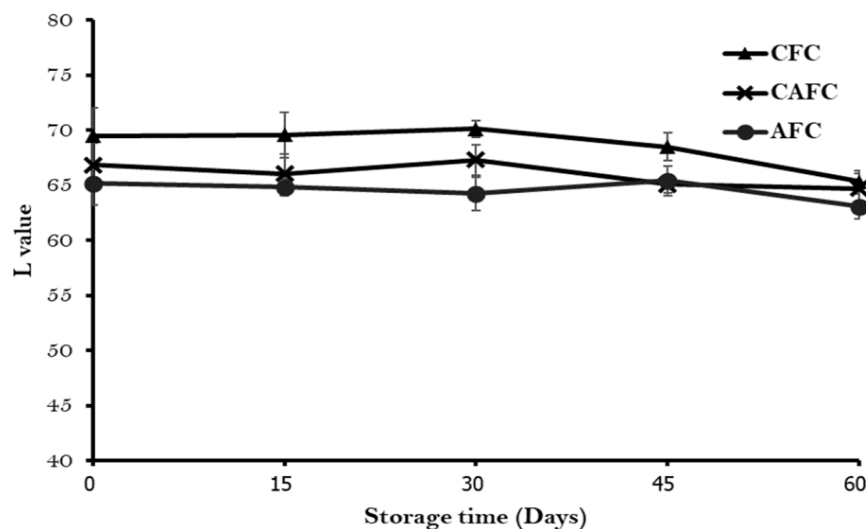
Mean values within a row with different superscript letters (^{a,b,c}) are significantly different (P < 0.05).

3.4. Quality Changes in Gluten-Free Cookie Products During Storage

3.4.1. Color

3.4.1.1. L Value

Figure 1 shows the changes in L value, representing the lightness of gluten-free cookies, over a 60-day storage period. Three cookie formulations were compared: coconut flour cookies (CFC), coconut flour and almond flour cookies (CAFC), and almond flour cookies (AFC). A higher L value of the food product indicates a lighter color compared to lower L values. On day 0, the L values of the three cookie formulations ranged from approximately 65-70, with the CFC showing the highest L value, followed by the CAFC and AFC, respectively. This result showed that the cookie surface had a light color at the beginning of storage. As the storage period increased to 30 days, the L values of each formulation changed only slightly, with the CFC still having the highest L value, while the CAFC and AFC exhibited lower values. However, the difference in L values between the formulations remained within a similar range. On day 45, the L values of all three formulations began to show a slight downward trend. After 60 days of storage, the L values of all formulations tended to decrease. The CFC and CAFC had similar L values at approximately 64-65, while the AFC showed the lowest L value at approximately 63.

**Figure 1.** Changes in L value during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies

3.4.1.2. a^* Value

The changes in a^* values, representing the redness of the gluten-free cookie surface over the 60-day storage period, were presented in Figure 2. A positive a^* value indicates an increasing tendency toward redness in the product. The results showed that the a^* values of the three cookie formulations during 60 days of storage indicated a low to moderate redness. At the beginning of storage, the AFC formulation had the highest a^* value at approximately 5.0, followed by the CAFC formulation at approximately 3.5, and the CFC formulation with the lowest a^* value at approximately 2.7–2.8. During days 15–45 of storage, the a^* values of the three cookie formulations changed only slightly. This indicated that the redness of the three cookie formulations tended to remain relatively constant during this period, although there were slight increases or decreases between storage times. At the 60-day storage period, the a^* values of all formulations changed only slightly.

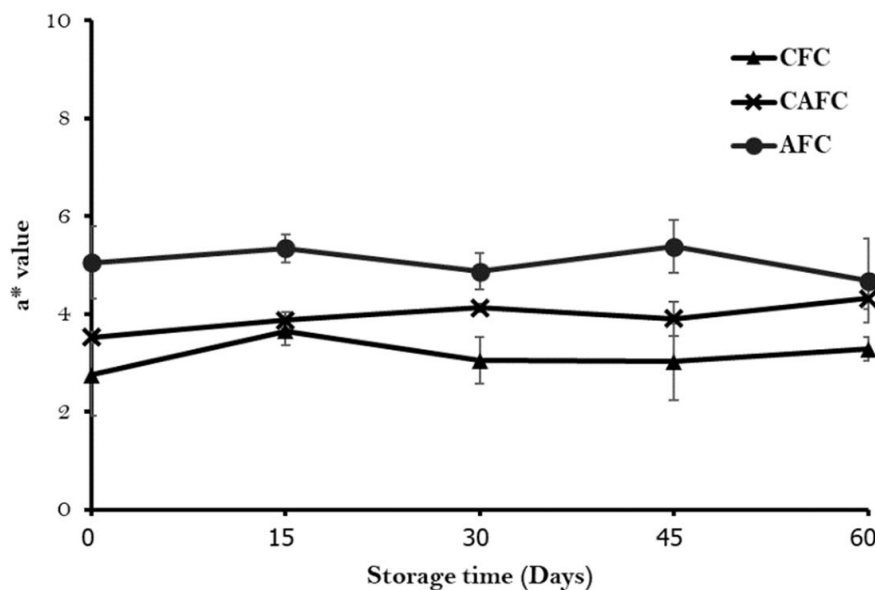


Figure 2. Changes in a^* value during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies.

3.4.1.3. b^* Value

Changes in b^* values during storage of gluten-free cookies are shown in Figure 3. The results revealed that the b^* values of the three cookie formulations ranged from approximately 4 to 10 throughout the 60-day storage period, indicating low to moderate yellowness. The AFC had the highest b^* value throughout the storage period, at approximately 9.5–10.2, showing a greater yellowness than the other formulations. The CAFC revealed a moderate b^* value, ranging from approximately 6.5–7.8, which increased slightly during Days 15–45 before decreasing gradually on Day 60. Considering the changes over storage time, the AFC had a b^* value of approximately 9.8 at the start of storage, then slightly increased to near 10 during Days 15–45 before slightly decreasing on Day 60. The CAFC had a b value of approximately 6.5 at Day 0. The b^* value increased to approximately 7.5–7.8 during Days 15–30, after which it tended to decrease slightly to around 7.2 on Day 60. For the CFC, the initial b^* value was approximately 4.5, increasing to around 5.8 on Day 15, decreasing slightly on Day 30, and increasing again to around 5.2 on Day 60.

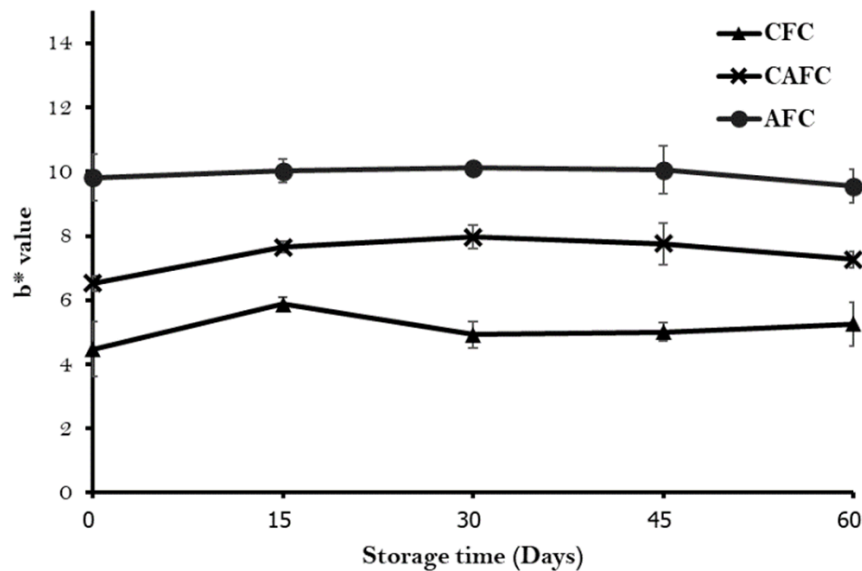


Figure 3. Changes in b^* value during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies.

3.4.2. Water Activity

Figure 4 shows the a_w values of all gluten-free cookie formulations stored at room temperature during the 60-day storage period. The results revealed that the a_w values of the three cookie formulations (CFC, CAFC, and AFC) were similar from the start of storage. On Day 0, the a_w values of all formulations were approximately 0.50–0.52, indicating low water activity from the beginning. The a_w values of all cookie formulations changed only slightly throughout the storage period. From Days 15–30, the a_w values of each formulation increased slightly from the initial value and ranged from approximately 0.51–0.53. After that, from Days 45–60, the a_w values continued to increase slightly. The a_w values ranged from approximately 0.53 to 0.55. The CAFC had slightly higher a_w values than the other formulations, while CFC and AFC remained similar throughout the experiment.

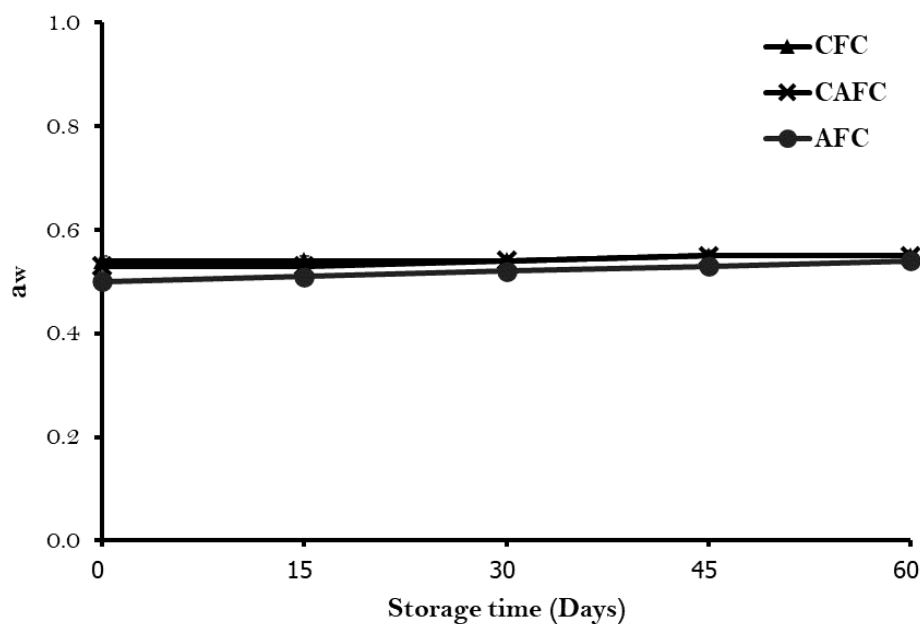


Figure 4. Changes in a_w during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies.

3.4.3. Moisture Content

Changes in the moisture content of the three gluten-free cookie formulations during the 60-day storage period at room temperature are shown in Figure 5. On Day 0, all three formulations had relatively low and similar moisture content, ranging from approximately 4.0-5.0%. This indicated that the resulting cookies were low-moisture baked products, consistent with the general characteristics of cookie-type bakery products. As a result, the AFC had the lowest initial moisture content, followed by the CAFC and CFC, respectively. During storage, the moisture content of all three cookie formulations exhibited a gradual increase throughout the experiment, from Day 15 to Day 60. This increase was continuous across all formulations. At the end of storage, the CFC had the highest moisture content, while the AFC maintained the lowest moisture content throughout the storage period.

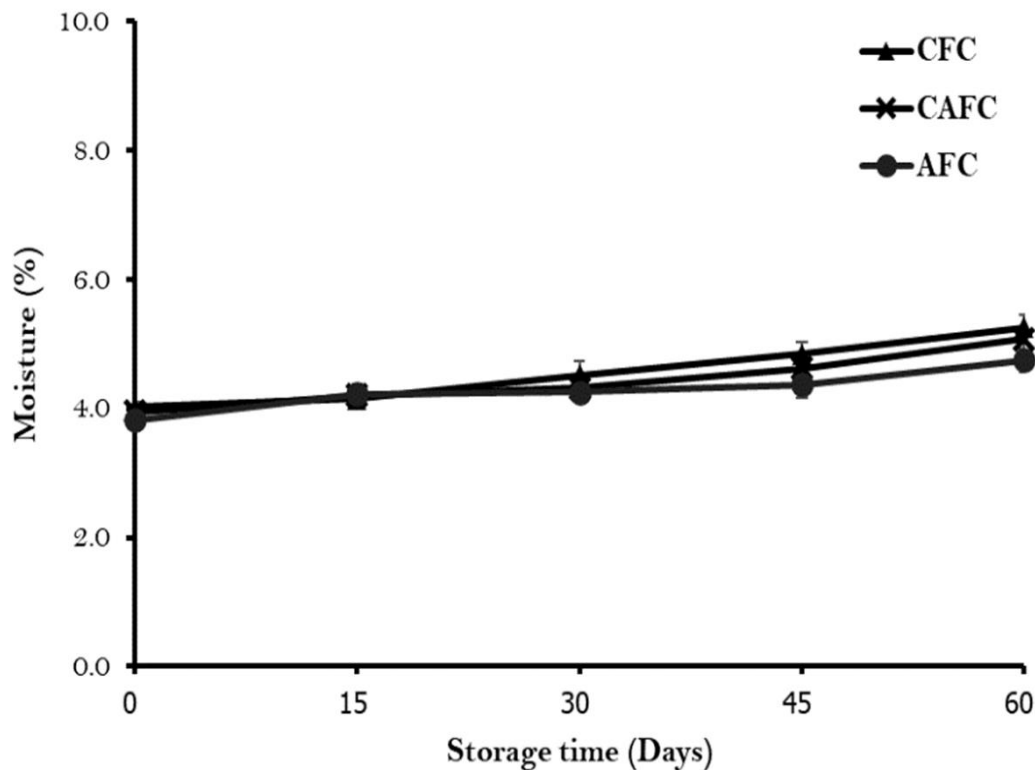


Figure 5. Changes in moisture during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies.

3.4.4. Microorganisms

3.4.4.1. Total Viable Count (TVC)

Figure 6 presents the changes in TVC of the gluten-free cookie formulations over the storage period. The results showed that at the beginning of storage, the TVC of all three cookie formulations was at similar levels, approximately 2.2-2.4 log CFU/g. Among the formulations, the CAFC had the lowest initial microbial count, followed by the AFC and CFC, respectively. As the storage period increased to 45 days, the microbial counts of all three cookie formulations continued to increase. The CAFC had a slightly higher TVC than the other formulations, while the CFC and AFC were similar. At the end of storage (Day 60), the TVC of all three cookie formulations increased further, reaching approximately 2.6-2.7 log CFU/g, which was the highest value observed during the experiment. The AFC had the highest microbial count, followed by the CAFC and CFC, respectively.

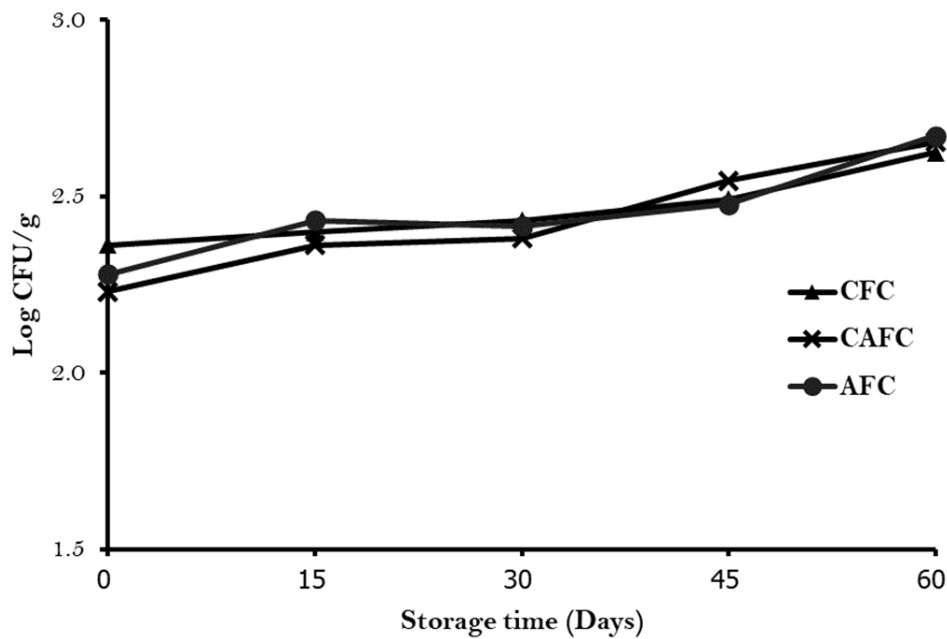


Figure 6. Changes in total viable count (TVC) during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies.

3.4.4.2. Yeast and Mold Counts

The yeast and mold count in gluten-free cookies kept at room temperature for 60 days were analyzed (Figure 7). On Day 0, all three cookie formulations had low levels of yeast and mold, approximately 1.2-1.3 log CFU/g. The CAFC had the lowest yeast and mold levels, while the AFC and CFC had similar levels. The yeast and mold count of all three gluten-free cookie formulations tended to gradually increase throughout the 60-day storage period. On Day 60, the CFC had the highest yeast and mold levels, approximately 1.6 log CFU/g, followed by the CAFC and AFC, respectively. The AFC maintained the lowest yeast and mold levels. However, the yeast and mold count in all formulations remained low, below 2.0 log CFU/g throughout the storage period.

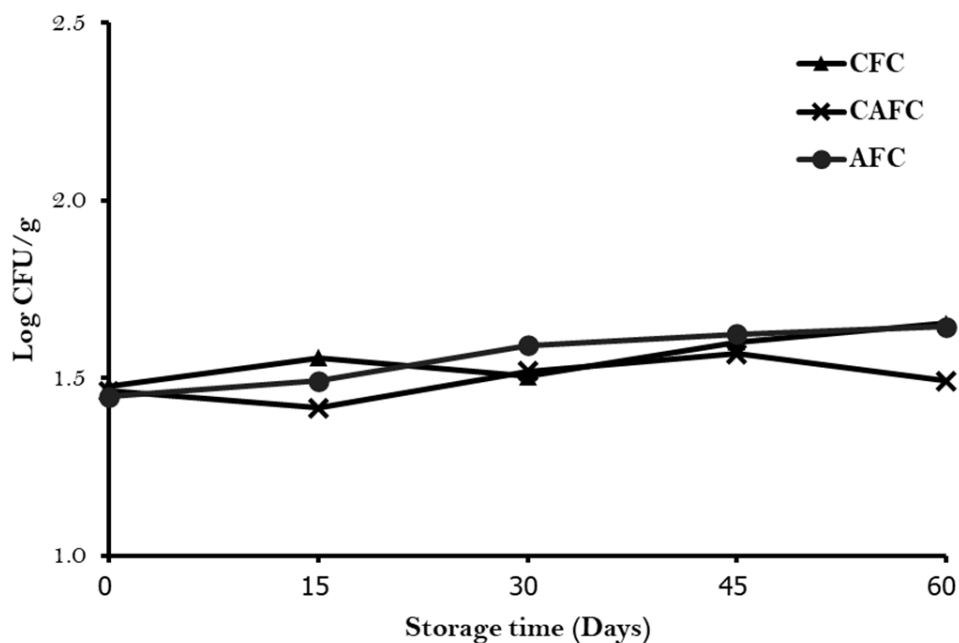


Figure 7. Changes in yeast and mold counts during storage of gluten-free cookies.

Note: CFC = Coconut flour cookies; CAFC = Coconut flour-almond flour cookies; AFC = Almond flour cookies.

3.4.4.3. Coliforms and *E. Coli*

Analysis of coliforms and *E. coli* in the cookie samples over a 60-day storage period at room temperature indicated that none of the formulations exceeded microbial safety limits. The levels of coliforms and *E. coli* remained below 3 MPN/g throughout the storage period (data not shown).

3.4.4.4. Pathogenic Bacteria

An analysis of pathogenic microorganisms, including *S. aureus* and *Salmonella* spp., in the CFC, CAFC, and AFC samples over 60 days of storage at room temperature showed that all samples remained within acceptable microbial safety limits. *S. aureus* remained at levels below 3 MPN/g for the duration of the storage period. Furthermore, *Salmonella* spp. was not detected in any of the samples analyzed (data not shown).

4. DISCUSSION

4.1. Effect of Substitution of All-Purpose Flour with Almond Flour on Cookie Qualities

The substitution of all-purpose flour with almond flour affected the physicochemical and microbiological properties of the cookies. Lightness (L) decreased with higher proportions of almond flour, while redness (a^*) and yellowness (b^*) increased. These color changes were caused by the Maillard reaction, due to the presence of reducing sugar in the formulation and the high protein content of almond flour compared to all-purpose flour. The color changes observed in cookies may not only be attributed to the Maillard reaction but also to other non-enzymatic browning mechanisms such as sugar caramelization, lipid oxidation, and variations in formulation and baking conditions, including temperature, baking time, and moisture loss during baking. At baking temperatures, sucrose in cookie formulations remained relatively stable. In contrast, fructose produced higher amounts of 5-hydroxymethylfurfural (HMF), a key intermediate in browning, compared with glucose (Ait Ameer, Mathieu, Lalanne, Trystram, & Birlouez-Aragon, 2007; Qi, Wang, Yang, Ding, & Xu, 2025). However, increasing the amount of protein or amino acids in the formulation by incorporating almond flour promoted the Maillard reaction and led to a darker product color. The dietary fiber content in almond flour also influenced the color parameters. This observation is consistent with the findings of Martínez-Cervera, Salvador, Muguerza, Moulay, and Fiszman (2011), who reported that the addition of soluble cocoa fiber increased the brown color of both the crust and crumb of muffins. They also noted that lightness values significantly decreased as the concentration of soluble cocoa fiber increased. The variation in crumb color was similar to the differences observed in crust color, with the lowest cocoa fiber addition producing the lightest crumb color. Chen, Espinal-Ruiz, Francavilla, Joye, and Corradini (2024) reported that the color indexes (lightness, redness, blueness, and browning index) of cookies (AACC-I standard protocol 10-53.01) baked at 185, 205, and 225°C were strongly correlated with changes in moisture content over time.

The practical significance of a_w in food matrices is due to its role as a primary determinant of microbial growth and spoilage. Consequently, products with higher moisture content and a_w are inherently more susceptible to rapid deterioration through biological and chemical pathways. In our work, a slight decrease in moisture content and a_w was observed when replacing wheat flour with almond flour. The reduction in moisture content and water activity might be due to the lower starch content, absence of gluten, and higher lipid fraction of almond flour compared to wheat flour. This compositional difference affected water retention and water-binding capacity within the food matrix. Furthermore, the water absorption capacity of alternative flours appears to be influenced by their protein and dietary fiber content. These factors may lead to enhanced water-binding properties and contribute to improved dough hydration (Pejcz, Agnieszka, & Zygmunt, 2015; Rao, Ashwath Kumar, & Indrani, 2022). The water absorption capacity of low-carbohydrate, high-fat flours, such as almond flour, is related to their higher protein and fiber content. This characteristic resulted in shelf-life extension, as lower moisture content and a_w values inhibit microbial growth.

Microbiological analysis demonstrated that total viable counts and yeast and mold counts decreased with increasing almond flour substitution. This trend is driven by the reduction in moisture content and a_w values, as well

as by the presence of oils and phenolic compounds in almond flour, which could inhibit microbial growth in food products (Bolling, Dolnikowski, Blumberg, & Chen, 2010; Daglia, 2012; Lacivita et al., 2024; Musarra-Pizzo et al., 2019). Moreover, the absence of pathogenic microorganisms in all formulations confirmed the microbiological safety of the cookies.

4.2. Effect of Substitution of Sucrose with Erythritol on the Cookie Qualities

Our findings indicated that sucrose can be substituted with erythritol in cookies without negatively affecting the physicochemical properties and microbiological safety. Color parameters (L , a^* , and b^*) showed slight variations among formulations, with no significant differences ($p > 0.05$). Generally, the use of polyols as sugar substitutes in baking results in lighter product coloration because they lack a reactive aldehyde group and do not participate in Maillard reactions (Róžańska, Rychlewski, Mildner-Szkodlarz, & Muzolf-Panek, 2025). Although sucrose is completely replaced in a cookie formulation, browning can still occur due to other factors within the food system. Other ingredients may contain components capable of undergoing browning reactions, such as reducing sugars released from starch degradation during baking. These sugars can react with amino acids or proteins present in the ingredients and trigger the Maillard reaction. The thermal degradation of certain carbohydrates can generate browning compounds such as furfural and hydroxymethylfurfural. The oxidation of fats from high-fat ingredients may also contribute to product browning.

Water activity and moisture content remained stable across all cookie samples, while TVC and yeast and mold counts exhibited minor differences. No pathogenic bacteria were found, indicating microbiological safety. This was due to the low moisture content and a_w values of all formulations, which limited microbial growth. Our observation was consistent with the findings of Toledo (1991) and Chowdhury, Khan, Karim, Obaid, and Hasan (2012), who concluded that food products with a_w values below 0.7 are microbiologically safer and possess longer shelf-life. Erythritol has also been reported to affect the physical quality of bakery products. Yan et al. (2023) reported that the addition of erythritol to angel cake resulted in partial structural unfolding of the sugar-egg white complex, which may be related to an improvement in foaming capacity. On this basis, the baking performance of angel cakes formulated with erythritol as a partial replacement for sucrose was further examined by Yan et al. (2023). They reported that the specific volume and baking loss of the cakes remained stable, while both textural and sensory attributes of the samples were maintained at acceptable levels throughout the study. Nevertheless, Laguna, Primo-Martín, Salvador, and Sanz (2013) reported that substituting sucrose with erythritol affected both the appearance and texture of cookies. They also stated that cookies formulated with sucrose substitutes were less crispy and obtained lower consumer acceptability scores. According to texture and acoustic measurements, cookies formulated with erythritol demonstrated increased hardness compared to those formulated with sucrose.

4.3. Effect of Substitution of Almond Flour with Coconut Flour on the Gluten-Free Cookie Qualities

The progressive substitution of almond flour with coconut flour slightly influenced the physicochemical and microbiological properties of gluten-free cookies. As the proportion of coconut flour increased, the L value (lightness) remained stable. Conversely, the a^* and b^* values decreased with higher coconut flour proportions. The results showed that, compared to almond flour, coconut flour made cookies appear brighter. This is because almond flour is high in protein and fat, promoting the Maillard reaction during baking, which results in increased melanoidin formation and a darker cookie color. In contrast, coconut flour is high in dietary fiber and contains fewer Maillard reaction precursors, leading to less browning and a lighter cookie color. Bravo-Núñez, Sahagún, Martínez, and Gómez (2018) found that the incorporation of vital wheat gluten decreased the L^* values of cookies with the highest protein content, although the differences were not significant. In contrast, the addition of Nutralys W wheat gluten (HGP) resulted in lower L^* values, producing darker cookies. As the amount of HGP increased, the luminance values

decreased. Some authors have reported that the darkness of cookies increases with higher protein content due to a high degree of nonenzymatic browning.

Our findings were consistent with Raczky, Kruszewski, and Michałowska (2021), who reported that the lightness of bread crumb made with chestnut flour decreased proportionally with increasing levels of chestnut flour addition. They claimed that the bread looked lighter after the coconut flour was added. Their findings also revealed that the flours with high sugar content, such as chestnut flour, exhibited a darker color due to the caramelization and the Maillard reaction.

In terms of chemical quality, both moisture content and water activity increased with higher levels of coconut flour, which may be attributed to its higher fiber content and water-holding capacity. Similar findings were reported by Dhankhar and Tech (2013), who demonstrated that the moisture content of coconut flour-based cookies was higher than that of soft wheat flour cookies. This difference was attributed to the high water absorption capacity of coconut fiber. Their study further concluded that gluten-free, grain-free cookies formulated with coconut powder as the primary ingredient were standardized and deemed acceptable based on sensory evaluation, nutritional quality, storage stability, microbial safety, and physical and textural characteristics. The different particle sizes of roasted almond flour also affected the physical and sensory properties of reduced-gluten muffins. Finer almond flour enhanced water absorption, gas retention, and overall batter uniformity, resulting in improved baking characteristics and sensory properties (Nasir, Abidin, & Cheng, 2025). Consequently, TVC and yeast and mold counts also increased in formulations containing higher proportions of coconut flour. Although no pathogenic microorganisms (e.g., *Salmonella spp.* and *S. aureus*) were detected in any formulation, the results suggest that a high level of coconut flour may shorten the shelf life of gluten-free cookies.

4.4. Quality Changes in Gluten-Free Cookie Products During Storage

4.4.1. Color

Bakery products, like other processed foods, are susceptible to physical, chemical, and microbiological spoilage. Physical and chemical deterioration primarily limit the shelf life of bakery products with low to intermediate moisture content. In contrast, microbiological spoilage caused by bacteria, yeasts, and molds is the principal concern in high-moisture products, that is, those with an a_w greater than 0.85 (Smith, Daifas, El-Khoury, Koukoutsis, & El-Khoury, 2004). The reduction in the L value during storage indicates the occurrence of non-enzymatic browning reactions, particularly the Maillard reaction, which commonly occurs in baked products containing proteins and reducing sugars. In addition, lipid oxidation may contribute to surface discoloration over time. In our study on the color quality of gluten-free cookies during storage, the L value was observed to decrease consistently throughout the 60-day storage period. Purlis (2010) reported that during baking, color pigment formation in products results from both the Maillard reaction and sugar caramelization. Similarly, Onacik-Gür and Zbikowska (2022) studied the effect of green tea extract and oleogels on the physicochemical properties and oxidative stability of short-dough biscuits during storage. They concluded that the color formation of biscuits results from non-enzymatic browning reactions, particularly the Maillard reaction and lipid oxidation. Small variations in the a^* and b^* values were observed in our work. However, no consistent trend was found across the samples. Onacik-Gür and Zbikowska (2022) reported that biscuits formulated with green tea extract exhibited higher in a^* values, which may be related to the inherent brownish color of the extract. In contrast, the biscuits containing palm fat maintained the lowest a^* values throughout the storage period, suggesting lighter color development. This observation may indicate reduced formation of Maillard reaction products in these biscuits.

4.4.2. a_w and Moisture Content

The moisture content and a_w of all gluten-free cookie formulations tended to increase during storage. This is because cookies are low-moisture products that can easily absorb moisture from the environment through packaging.

Additionally, structural changes in cookies during extended storage may enhance water absorption, leading to increased moisture content and a_w values over time. The CFC formulation exhibited the highest values after 60 days, while the AFC formulation showed the lowest values. This might be due to the higher water-absorption capacity of coconut flour compared to almond flour in cookie formulations. Dhankhar and Tech (2013) similarly reported that the water activity of coconut flour-based cookies was higher than that of cookies made from all-purpose flour. This difference was attributed to coconut flour's higher fiber content and greater water-holding capacity, which resulted in greater moisture retention compared with wheat flour. Nevertheless, the a_w values of both types of gluten-free cookies remained within the safety threshold for microbial stability. In our study, both moisture content and a_w increased across all formulations. However, the values remained below 0.70. This threshold is regarded as a safe limit for the inhibition of microbial growth (Toledo, 1991). It implies that all gluten-free cookie formulations maintained microbiological safety during the storage period. However, the CFC formulation may have a shorter shelf life than the almond flour-based formulation due to its higher moisture absorption. The addition of almond flour alone appeared to improve storage stability. In contrast, coconut flour not only increased dietary fiber content but also raised a_w and moisture levels. The increase in moisture-related parameters is a key factor leading to a shorter shelf life.

4.4.3. Microorganism

In this study, the microbiological analysis revealed a progressive increase in TVC across all gluten-free cookie formulations over the 60-day storage period. Yeast and mold counts exhibited a similar increasing trend. Our findings were consistent with the report of Mushtaq, Salim-ur-Rehman, Zahoor, and Jamil (2010). They found that cookies formulated with xylitol as a sugar substitute exhibited lower microbial counts compared to those containing sucrose. Nevertheless, TVC in all cookie formulations increased progressively throughout the 60-day storage period. Similarly, Pangestu, Wulandari, and Soenarno (2025) found that the initially low moisture content and a_w of egg-yolk-powder cookies inhibited microbial growth, although a gradual increase in moisture was observed during storage. They also reported that molds were a predominant microbial group throughout the storage period. Extended storage duration was positively correlated with increased microbial count. Comparable results were reported by Asadi, Khan, and Zaidi (2022), who examined cookies formulated with beetroot leaf powder, sapota fiber powder, and control cookies stored at room temperature for 15 months. They concluded that a prolonged storage period increased total microbial counts, including aerobic bacteria as well as yeasts and mold counts, across all cookie samples.

The findings of our study showed the absence of coliform bacteria, *E. coli*, *S. aureus*, and *Salmonella* spp. in all cookie formulations during the entire storage period. The absence of these pathogenic bacteria revealed the microbiological safety of all gluten-free cookie formulations under the study conditions. The results aligned with the findings of Khalil, Elbeltagy, Aljutaily, Ali, and Gadallah (2023). They reported that the biscuits contained various dietary fibers; neither *E. coli* nor *Salmonella* spp. was detected in any sample, and no growth of these pathogenic bacteria was observed. The findings were also supported by De Oliveira, Costa, and De Sousa (2021), who formulated bread and biscuits partially substituted wheat flour with cassava flour. Their results exhibited that the coliform count was below 3 MPN/g at both 35°C and 45°C. Furthermore, coagulase-positive Staphylococci and *Salmonella* spp., were not detected in any sample. The microbiological quality of buttermilk biscuits produced from orange-flesh sweet potato flour infused with coconut flour was studied by Ramsaran, de Gannes, and Eudoxie (2024). They also demonstrated that the growth of bacteria, yeasts, or molds was not observed in the tested samples. Therefore, our study confirmed that the use of almond flour and coconut flour as primary ingredients contributed positively to the microbiological safety and shelf life of gluten-free cookie formulations.

Although our research provides useful information for developing gluten-free cookies, several limitations should be acknowledged. The formulations were developed using only two types of gluten-free flours, almond flour and coconut flour, and a single sugar substitute, erythritol, which may influence the applicability of the findings.

Additionally, the present study primarily evaluated physicochemical properties and microbiological quality during storage, without incorporating sensory evaluation. Future research should investigate different formulations and include sensory evaluation to better understand consumer acceptance of gluten-free cookie products.

5. CONCLUSION

The application of almond flour and coconut flour as substitutes for wheat flour affected the physicochemical and microbiological safety of gluten-free cookies. The substitution of wheat flour with almond flour and sucrose with erythritol had only minor effects on the quality of the cookies, both in terms of physicochemical and microbiological properties. However, increasing the proportion of coconut flour as a substitute for almond flour led to changes in product characteristics, including an increase in lightness and decreases in redness and yellowness. Additionally, moisture content and water activity tended to increase with higher proportions of coconut flour. Nevertheless, no pathogenic microorganisms were detected in any formulation, indicating that the developed products maintained microbiological safety throughout the storage period. These findings provide practical guidance for the formulation of gluten-free cookies with improved shelf-life stability using almond-based and coconut-based flour. However, because this work used only two types of gluten-free flours and one sugar substitute, the findings may limit the applicability of the results. Future research should study additional gluten-free flours and sweeteners and include sensory evaluation to better understand consumer acceptance and optimize product quality.

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