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Exploring the impact of storage and packaging materials on gluten-free corn cookies supplemented with *Chenopodium quinoa*

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Abstract
This research investigated how the storage period and packaging materials affect the quality attributes of cookies made from a blend of corn and quinoa flour. Various quality parameters, including moisture content, water activity (a_w), free fatty acid levels, total plate count, texture profile (specifically crispness), and overall acceptability of gluten-free cookies, were examined during ambient storage at 25±2 °C and 50% RH. Throughout the storage period, the moisture content and a_w of the cookies increased, while their hardness decreased. This trend was observed higher in cookies packed in both Polyethylene Terephthalate boxes (PET) than Aluminium laminated packaging (ALP), wherein moisture content raised from 3.70 to 4.56% in PET and 3.70 to 3.95% in ALP. However, hardness decreased from 4207.46gm to 4006.32 gm in PET and 4207.76 to 4186.32 gm in ALP during 90 days of storage. Furthermore, the levels of free fatty acids and total plate count in the cookies increased during storage regardless of the packaging material used. However, the increase was more significant in cookies stored in PET boxes compared to those in ALP. In terms of overall acceptability, cookies packed in ALP received the highest average scores. Thus, ALP was identified as a superior packaging material compared to PET boxes in terms of sensory attributes, texture, and shelf stability of the cookies.

Keywords: ALP, free fatty acid, PET, quinoa, total plate count, storage

1. Introduction

Patients with celiac disease must follow a gluten-free diet due to their lifelong allergy to ingested gluten. Refined flour, hydrogenated fats, sugar, emulsifiers, and other additives are utilised in commercial bakery products including biscuits, crackers, and cookies; these ingredients are deficient in essential nutrients. Dietary fibre and micronutrients, which are crucial components in supporting health, are absent from the refined wheat used. Additionally, in order to achieve nutritional and therapeutic food security, it is imperative that all sectors of society take advantage of pseudocereals beneficial nutritional qualities and spread awareness of them (Saleh *et al*., 2012) [37].

Low moisture content ensures baked good's resistance to microbial deterioration and extends their shelf life for items like bread, cookies, cakes, and biscuits. These product attributes took advantage for potential mass manufacture and distribution (Lean & Mohamed, 1999) [31]. They are also desirable for fortification and other nutritional improvements due to their good eating quality. Due to their excellent shelf life at ambient circumstances, cookies are favoured even in Indian food traditions. For the wide range of consumers, simplicity, convenience of use, transportation, and affordability are important. If appropriately adapted, cookies are probably the greatest means of delivering nutrients to satisfy the dietary needs of typical customers (Noorfarahzilah *et al.*, 2014) [36].

According to (Baixauli *et al*. 2008) [4] it is possible to define storage stability or the shelf life of baked goods as the preservation of the physical and sensory qualities linked to freshness. In contrast to biscuits, which tend to break after baking, cookies generally have the property of bending.

Due to their increased water activity and moisture content, items like cookies are a major contributor to this fact reliance (Dhankhar, 2013) [16]. The standard of hygiene and nutritional quality; contact with air (Oxygen) which can speed up lipid oxidation; enzymatic activity which contributes to the acceleration of shelf-life; and, c) the contaminant bacteria, moulds, and yeasts which represent a true microbiological risk are the three main obstacles that cookies must overcome in order to maintain their fragrance during storage. The shelf life of other cereal-based items (Toast, frozen goods, biscuits, cakes, pastas) can be extended by using packaging materials that limit or postpone texture changes and flavour loss that occur over the course of the product's shelf life. The physical, sensory, and nutritional properties of the cookies are impacted by the multigrain powder and additions used in the cookies. In the current study, using an alternative material to wheat flour, such as a composite flour blend of corn and quinoa flour in different proportions based on our previous studies, cookies were prepared and storage stability based on physicochemical, textural, and sensory criteria was evaluated.

2. Materials and Methods

2.1 Packaging and storage studies of cookies

2.1.1 Procedure for the formulation of gluten-free cookies

For the development of cookies, part of the corn flour was replaced by quinoa flour and carboxy methyl cellulose according to the levels as mentioned in Table 1. With the exception of treatments A1,A2,A3,A4, Twelve treatment combinations were created to replace corn flour with quinoa flour in the range of 20-40%. Carboxy methyl cellulose in the ratio of 0.25%, 0.50%, 0.75% and 1.0% was also added in all these treatments including A1, A2, A3 and A4. Based on the above mentioned treatment combinations, twelve different types of cookies were prepared. The desirability function approach was used to optimise the process. To acquire optimal values for the answers, desired goals were assigned to all of the parameters. C3 (60% corn: 40% quinoa:0.75% Carboxy methyl cellulose) cookies is the best treatment combination with highest spread ratio, moderate crispness and highest overall acceptability (Anam *et al.*, 2024) [3].

Table 1: Details of treatment combination for development of Carboxy methyl cellulose incorporated gluten free corn quinoa cookies

S. No.	Treatment code	Corn flour (%)	Quinoa flour (%)	CMC (%)
1.	A1	100	0	0.25
2.	A2	100	0	0.50
3.	A3	100	0	0.75
4.	A4	100	0	1.00
5.	B1	80	20	0.25
6.	B2	80	20	0.50
7.	B3	80	20	0.75
8.	B4	80	20	1.00
9.	C1	60	40	0.25
10.	C2	60	40	0.50
11.	C3	60	40	0.75
12.	C4	60	40	1.00

2.1.2 Packaging materials

Two packaging materials had been used for the storage of optimized cookies such as PET boxes (Polyethylene terephthalate) and ALP (Aluminium laminated packaging) and stored for 90 days at room temperature (25±2 °C and 50% RH). Cookies were withdrawn periodically after every 15 days for analysis as per the work plan elaborated in subsequent sections.

2.1.3 Moisture content of cookies

Moisture content of sample was measured in accordance with AOAC (2012) [1]. Approximately 5g sample was taken in previously dried and weighed petridish. The sample was then dried in a hot air oven at a temperature of 70 °C until a constant weight was obtained. The sample was analysed in triplicates and the mean was recorded. The percent moisture content was calculated as:

$$\text{Moisture (\%)} = (W2 - W3)/(W2 - W1) \times 100$$

W1 = weight of container with lid; W2 = weight of container with lid and sample before drying; and W3 = weight of container with lid and sample after drying.

2.1.4 Water activity of cookies.

Water activity (a_w) of the samples was measured using aw measurement device (Hygrolab, Cole Parmer) with an accuracy of ± 0.001 at 25 °C. Prior to each test, the water activity meter was turned on and allowed to warm up for 30 min. Each sample was measured by covering the bottom of a plastic disposable cup, placing the cup into the sample holder, and taking the reading as per instrument protocol (Dawit *et al.*, 2019) [14].

2.1.5 Free fatty acids contents

Standard method of AOAC (2001) was used for determination of free fatty acids in cookies. 10 gm ground sample of stored cookies was taken in flask. 50 ml benzene was added and kept for 30 min for extraction of free fatty acids. 5 ml extract was taken in flask to which 5 ml benzene, 10 ml alcohol and phenolphthalein as indicator was added and titrated against 0.02 N KOH till light pink colour appeared and persisted for 15 sec. Percentage free fatty acid was expressed as oleic acid. The FFA analyses were performed in triplicates

$$\text{FFA (\%)} = \frac{282 \text{ x (ml of alkali used)x (0.02 N KOH)x 100}}{\text{sample taken x 1000}}$$

2.1.6 Total plate count (cfu/ml)

TPC was determined using Potato Dextrose Agar media and a serial dilution process. 1g of a seemingly healthy piece of the material was homogenised and dossolved in 9ml sterlized distilled water. A three-fold serial dilution was obtained in this manner. In three petriplates containing PDA media, 1ml of aliquot of three fold serial dilution was inserted and incubated for 48 hours at 25±2 degrees Celsius. The colonies that resulted were measured in log cfu/ml.

$$\text{TPC (cfu/ml)} = \frac{\text{No.of colonies formedx 1}}{\text{Dilution factor x Standard volume added}}$$

2.1.7 Hardness

The hardness of cookies was measured using a texture analyzer (TA-XT2, StableMicro Systems, UK) and a 3-point bending rig (HDP/M3PB). The pre-test, test and post-test speeds were set to 1.5, 2 and 10 mm/s, respectively, during the analysis. The probe was positioned 5mm away from the cookies. Crispness was determined as the number of positive peaks during the penetration test, which is a textural feature for baked goods (Hamdani *et al.*, 2020) [29].

2.1.8 Sensory evaluation of cookies

The sensory evaluation of cookies was done on a 5-point scale by a panel of 20 semi-trained assessors. Before the test began, the judges were familiar with the rating criteria and language. The judges were given coded samples at random to evaluate for several sensory aspects such as appearance, mouthfeel, colour and crispness. After analysing each sample, panellists rinsed their tastebuds with potable water. The samples were evaluated under laboratory circumstances according to ISO (1994) criteria by the panellists. The average of sensory scores collected for appearance, flavour, mouthfeel, colour and crispness was used to assess overall acceptability (Mudgil *et al*., 2017 and Naseer *et al*., 2021) [33, 35].

2.1.9 Statistical design and Analysis

The statistical analysis was carried out using standard statistical procedures as per. The SAS software was used for analysing the data.

3. Results and Discussion

The optimized product packed in Aluminium laminated packaging and PET boxes was found shelf stable for a period of 3 months under ambient conditions.

3.1 Moisture content (%)

Over the course of three months of storage, a gradual increase in moisture content was seen. The hygroscopic nature of cookies may be to blame for the increase in moisture content during storage. During a 90 day storage, due to the better barrier protection provided by PET boxes compared to ALP, there was a relatively lower moisture gain from 3.70-3.95% stored in ALP as compared to 3.70-4.56% in cookies stored in PET boxes. According to Butt *et al*. (2009) [6], the relative humidity and hygroscopic characteristics of flours were to blame for the increase in moisture content over the three-month storage period.

Table 2: Effect of storage days and packaging material on moisture (%) of gluten-free corn quinoa cookies

Packaging Mat Storage days	Moisture content (%) of cookies		Mean
	PET boxes	ALP	
0	3.70	3.70	3.70
15	3.79	3.75	3.77
30	3.87	3.79	3.83
45	3.94	3.83	3.88
60	4.06	3.86	3.96
75	4.12	3.90	4.01
90	4.56	3.95	4.25
Mean	4.01	3.82	

ALP = Aluminium Laminated packaging, PET Boxes = Polyethylene Terephthalate
C.D ($p \leq .05$), Storage days= 0.033, Packaging material= 0.014, Storage days x packaging material= 0.047

3.2 Water activity (a_w)

Over the course of three months of storage, a gradual increase in water activity was observed (Table 3). Increased water activity (a_w) value eases diverse microbial growth. The produced cookies were stable from microbial deterioration, according to water activity levels taken near the end of the storage period. There was a relatively lower water activity gain in cookies. Due to the better barrier protection provided by ALP the water activity ranged from 0.296 to 0.328 in 90 days storage as compared to cookies stored in PET boxes that varied from 0.296 to 0.388.

Table 3: Effect of storage days and packaging material on water activity (a_w) of gluten-free corn quinoa cookies

Packaging Mat Storage days	Water activity of cookies		
	PET boxes	ALP	Mean
0	0.296	0.296	0.296
15	0.318	0.305	0.311
30	0.337	0.310	0.323
45	0.349	0.316	0.332
60	0.361	0.320	0.340
75	0.378	0.324	0.351
90	0.388	0.328	0.358
Mean	0.346	0.313	

ALP = Aluminium Laminated packaging, PET Boxes = Polyethylene Terephthalate
C.D ($p \leq .05$), Storage days= 0.002, Packaging material= 0.001, Storage days x packaging material= 0.003

3.3 Free fatty acids (%)

During storage, free fatty acids (FFA) were determined to assess the shelf -life stability of the cookies. FFA were significantly ($p \leq 0.05$) affected by storage time. FFA increased during the storage time of 90 days. The mean FFA during a storage period of 90 days increased from 0.31 to 0.93 irrespective of the packaging material. The similar increase in FFA were also observed by Uma *et al*. 2011 [39]. Production of FFA is due to hydrolysis of triglycerides by lipase or due to non-enzymatic reaction at high temperature (Camire *et al*. 2007) [7]. According to Indian standards, FFA concentration should remain under 10% as this limit is acceptable for human consumption (Amin *et al*. 2016) [2]. Cookies showed a better shelf-life stability during 90 days when packed in ALP (0.31-0.46%) than in PET boxes (0.31-1.28%) and stored at ambient conditions.

Table 4: Effect of storage days and packaging material on free fatty acids (%) of gluten-free corn quinoa cookies

Packaging Mat Storage days	Free fatty acids of cookies		
	PET boxes	ALP	Mean
0	0.31	0.31	0.31
15	0.44	0.33	0.38
30	0.58	0.37	0.47
45	0.89	0.38	0.63
60	1.02	0.46	0.74
75	1.13	0.46	0.79
90	1.28	0.59	0.93
Mean	0.80	0.41	

ALP = Aluminium Laminated packaging, PET Boxes = Polyethylene Terephthalate
C.D ($p \leq .05$), Storage days= 0.05, Packaging material= 0.08, Storage days x packaging material= 0.13

3.4 Total plate count (TPC) (CFU/ml)

The total plate count (TPC) is a microbiological test used to estimate the total number of viable aerobic bacteria present in a sample. The cookies packed in aluminium laminate have a lower TPC compared to cookies packed in PET (Polyethylene Terephthalate) boxes, because aluminium laminate typically provides better barrier properties compared to PET. It offers excellent protection against moisture, oxygen, light, and other environmental factors that can promote microbial growth. As a result, cookies packed in aluminium laminate may experience less exposure to external contaminants, leading to lower microbial counts.

TPC was too few to count during 45 days of storage. However, TPC showed significant increase from 60 days of storage. The increase in TPC may be due to increase in moisture content (Frazier *et al*., 1988) [27].

Microbial studies showed that the cookies packed in PET boxes under room temperature over 3 months had better shelf life as the microbial load remained under limit. According to Indian Standards, total bacterial count should not be more than 50,000 in high protein cookies (Nagi *et al.*, 2012) [34].

Table 5: Effect of storage days and packaging material on Total plate count (CFU/ml) x 10^3 of gluten-free corn quinoa cookies

Packaging Mat Storage days	TPC of cookies		
	PET boxes	**ALP**	Mean
0	Nil	Nil	-
15	TFTC	TFTC	-
30	TFTC	TFTC	-
45	TFTC	TFTC	-
60	2.48	2.26	2.37
75	4.32	4.24	4.28
90	5.52	5.10	5.31
Mean	4.10	3.86	

ALP = Aluminium Laminated packaging, PET Boxes = Polyethylene Terephthalate C.D ($p \leq .05$), Storage days = 0.295, Packaging material = 0.027, Storage days x packaging material= 0.322

3.5 Crispness (Hardness)

The changes in the hardness of cookies over time can be influenced by various factors, including moisture content, storage conditions, and packaging material. The cookies packed in aluminium laminate exhibited better crispness compared to those packed in PET boxes over time, it suggests that aluminium laminate may offer superior moisture barrier properties, thereby preserving the texture of the cookies more effectively. Aluminium laminate offers excellent oxygen barrier properties, which can help prevent oxidative reactions that may lead to changes in texture and flavour. PET, although a relatively good barrier material, may allow a small amount of oxygen to permeate over time, potentially affecting the crispness of the cookies. The textural property is one of the major factors contributing to the eating quality of cookies. Crispness decreased with increase in the duration of storage as cereals being hygroscopic in nature absorb moisture over a period of time from 4207.46 g- 4186.32g in ALP and from 4207.46 g to 4006.32g in case of PET boxes (Chevallier *et al.*, 2000) [8] (Table 6). The amount of moisture, the activity of the water, and the composition all have an impact on the texture of baked goods. Ingredient interactions during storage, such as those involving fat, sugar, and flour, have an impact on the microstructure and crispness of dry goods. It's possible that moisture redistribution and migration caused the developed cookies' lack of crispness. Additionally, Rajiv *et al.* (2012) [40] noted that after 90 days of storage, the flaxseed cookie's crispness decreased, indicating a loss of crispness.

Table 6: Effect of storage days and packaging material on crispness of gluten-free corn quinoa cookies

Packaging Mat Storage days	Hardness of cookies			
	PET boxes		**ALP**	
	Hardness (g)	**Crispness (Positive peaks)**	**Hardness (g)**	**Crispness (Positive peaks)**
0	4207.46	6	4207.46	6
15	4191.40	5	4204.40	6
30	4153.37	5	4200.37	6
45	4115.33	4	4195.33	5
60	4095.30	4	4193.30	5
75	4049.29	3	4189.29	4
90	4006.32	3	4186.32	4

ALP=Aluminium Laminated packaging, PET Boxes= Polyethylene Terephthalate

3.6 Sensory evaluation of stored product

Nonsignificant changes were noted for the appearance, colour, crispness and overall acceptability (OA) values after up to 15 days of storage (Table 7). However, the appearance, colour, crispness, mouth feel and OA significantly ($p \leq 0.05$) dropped from 4.3 to 3.5, 4.7 to 3.8, 4.6 to 3.0, 4.8 to 3.3, and 4.6 to 3.4, respectively in case of ALP and from 4.3-3.0, 4.6-3.1, 4.7-2.9, 4. 8-3.0 and 4.6-3.0 in PET boxes with the advancement of storage from 30 to 90 days (Table 7). Color deterioration during storage is to blame for the decline in appearance and colour scores. The crispness of the cookies may have also deteriorated as a result of the moisture accumulation during storage. After 30 days of storage, cookie's flavour and mouthfeel ratings may have decreased as a result of lipid hydrolysis that may have happened as a result of air getting into the packaging. Even 90 days after being stored, the produced cookies were only marginally appreciated. During storage, sensory scores for attractiveness declined. Some panellists claimed that the product began to taste rancid on the 90th day. An rise in FFA during storage may be the cause of a sour flavour (Uma *et al.*, 2011) [39]. Crispness was shown to be greater on the first day of storage, declining as time went on due to moisture gain. The length of storage time had an impact on overall acceptability.

Table 7: Effect of storage days and packaging material on sensory evaluation of developed gluten-free corn quinoa cookies stored in PET boxes and ALP

	ALP							PET						
Storage days	0	15	30	45	60	75	90	0	15	30	45	60	75	90
Appearance	4.3	4.3	4.0	4.0	3.9	3.7	3.5	4.3	4.1	4.0	3.8	3.4	3.3	3.0
colour	4.7	4.7	4.5	4.3	4.3	4.0	3.8	4.6	4.4	4.1	3.9	3.5	3.3	3.1
crispness	4.6	4.1	4.0	3.9	3.6	3.2	3.0	4.7	4.3	4.0	3.7	3.4	3.1	2.9
Mouthfeel	4.8	4.5	4.3	4.0	3.8	3.6	3.3	4.8	4.6	4.3	4.0	3.7	3.3	3.0
OAA	4.6	4.4	4.2	4.0	3.9	3.6	3.4	4.6	4.3	4.1	3.8	3.5	3.2	3.0

4. Conclusion

It can be concluded from this study, that quality attributes of corn and quinoa flour cookies packed in aluminium laminate packaging demonstrates superior quality retention of cookies over a storage period of 90 days compared to PET packaging. This is primarily attributed to the excellent barrier properties of aluminium laminate, including its ability to effectively block moisture and oxygen ingress. By providing a robust barrier against external factors, aluminium laminate packaging helps preserve the texture, flavour, and overall freshness of cookies for an extended duration. The moisture barrier offered by aluminium laminate prevents softening of cookies, maintaining their desired hardness and crispness over time. Additionally, the superior sealing capability of aluminium laminate ensures the integrity of the packaging, minimizing the risk of contamination and oxidation. Overall, the findings suggest that aluminium laminate packaging is a preferable option for extending the shelf life and enhancing the quality of cookies during prolonged storage periods, thus offering consumers a better product experience.

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