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Development and Characterization of Flaxseed and Lemon Peel Crackers against Non- Alcoholic Fatty Liver Disease

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ABSTRACT

In the modern era, the rise of chronic diseases including Non-Alcoholic Fatty Liver Disease (NAFLD), heart disease and diabetes are primarily linked to unhealthy dietary habits, lack of physical activity and environmental factors. To tackle this growing health problem, it's important to focus on eating better and making healthier lifestyle choices. In the current study, crackers were developed using different combinations of flaxseed powder and lemon peel powder to evaluate their nutritional, functional and sensory qualities. Crackers were prepared using different combinations of the ingredients and were assessed for their proximate composition, antioxidant activity and sensory evaluations over a 30-day storage period. Proximate analysis shows a significant difference among the treatments. Proximate analysis shows protein content $8.15 \pm 0.03\%$, fiber $4.22 \pm 0.01\%$, ash $2.24 \pm 0.02\%$ content, fat content ($6.79 \pm 0.04\%$). the Antioxidant activity was measured by using DPPH method recorded values were 30.65 ± 0.08 mg to 30.18 ± 0.10 and total phenolic content 41.3 ± 0.04 to 39.57 ± 0.06 with significant level of $p < 0.05$. The total phenolic content (TPC) followed a similar trend. Overall, the results indicate that the addition of flaxseed powder and lemon peel powder to crackers improved their nutritional and functional properties with T3 showing the best results in terms of antioxidant activity and color maintaining a good balance in moisture protein and fiber content throughout the 30-day storage period. All the data was subjected to appropriate statistical analysis.

Keywords: Crackers, Flaxseed powder, Lemon peel powder, Non-Alcoholic Fatty Liver Disease.**Corresponding Author:** Nida IqbalEmail: dr.nida@riphahfsd.edu.pk

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INTRODUCTION

Metabolic syndrome, is a condition characterized by obesity, insulin resistance and dyslipidemia. Growth in the impact of metabolic syndrome and being obese observed in the fatty liver diseases are rapidly increasing the population that leads to the chronic liver diseases (Qidwa, 2020). Worldwide prevalence of (NAFLD) is around 35%. It is estimated that around 47% of population in Pakistan is suffering from non-fatty liver disease (Shah et al., 2018). The prevalence of this syndrome is closely linked to modern lifestyle choices, particularly the consumption of high-fat

diets and reduced physical activity. Syndrome Metabolic has to do with an oxidative stress condition happening when there is an imbalance between the formation and destruction of ROS, which are reactive oxygen species. ROS is one of the risk components of non- alcoholic fatty liver disease (NAFLD). Non-alcoholic fatty liver is a variety of diseases that result from an accumulation of fat in the liver (Man et al., 2022). NAFLD develops as a key aspect of metabolic syndrome related to resistance to insulin, as well as the pathophysiological closure of the liver, pancreas and cardiovascular cell disorder conditions. In NAFLD, death is

most prevalent because of cardiovascular disease and commonly related to non-hepatic malignancies apart from liver disease (parra et al., 2020). NAFLD is considered the liver symptom of the metabolic disorder. A lack of exercise and eating Western foods have led to severe problems for managing patients with NAFLD. Beneficial drug treatments, along with lifestyle changes such as nutritional modifications, exercise and healthy eating habits, can be very effective. Nutrients included in meals strongly modify conditions and consequences throughout various severe health issues. Polyphenols, carotenoids, flavonoids and terpenoids are known to effect inflammation, proliferation, apoptosis and angiogenesis. Dietary and lifestyle adjustments have been demonstrated to prevent 30% to 40% of all malignancies (Perdomo et al., 2019).

Flaxseed (*Linum usitatissimum* L.), which comes from the group Lineaceae, is a blue-blooming herb that develops little flat seeds ranging from golden yellow to a rosy brown colour. Flaxseed features a crunchy texture and a nut-like flavor. Flaxseed has a germ sheath or actual shell (sometimes termed the testa), a thin endosperm, two embryos and an embryo axis. Flaxseed continues to push further in its recognition as a nutritious meal, getting high in the essential omega-3 fatty acids, alpha-linolenic acid and numerous phytochemicals. Flaxseed also supplies dietary fibre and protein. Almost all components of the flaxseed plant are utilised for several functions. Seed contains oil which after refining is used for edible purpose. Seed oil can be processed and used for food purposes. The stem creates high-quality fibre that is extremely strong and durable. Human beings have been eating flaxseed since prehistoric times. It has been farmed for fibre, health benefits and nutritional products. Flaxseed serves as a functional food since it contains the primary bioactive elements of alpha-linolenic acid, lignans, nutritional fibre and protein. (Yang et al., 2021). Flaxseed has antioxidant properties that can help lower total cholesterol and reduce platelet clumping. Dietary management is an essential strategy for enhancing the wellness of the liver. Flaxseed (*Linum usitatissimum* L.) has beneficial properties that make it an excellent adjunctive therapy for decreasing irritation and liver steatosis in NAFLD patients.

One of its key components, a lignan called Secoisolariciresinol Diglucoside (SDG), along with the mammalian lignans enterolactone (EL) and enterodiol (ED) lignans, has been demonstrated to protect against destruction of DNA and prevent fat oxidation. These lignans from flaxseed are efficient in delaying or slowing the development of type-2 and type-1 diabetes. SDG's ability to

reduce blood sugar in those with type 2 diabetes is thought to be related to its antioxidant qualities (Soni et al., 2016). Lemon (Rutaceae), it is a fruit that can be eaten. Lemons are the third most important citrus fruit species, after oranges, and are grown in excess of 4.4 million metric tonnes annually. The lemon plant was brought to southern Italy about 200 A.D., and by 700 A.D., it had also reached Iraq and Egypt. With 1.2 million metric tonnes produced, Argentina is currently the world's largest lemon producer. Ascorbic acid, minerals, citric acid, essential oils, and flavonoids are among the many vital natural substances found in large quantities in lemons. Lemon peels are also rich in a variety of phytochemicals, including volatile oils, glycosides, and α and ϵ -sitosterol. Lemons have a number of important properties that are unique to other plants, such as polyethylene, ascorbic acid, flavones, and phenolic compounds (Rafique et al., 2020).

The liver is a big organ that carries out many different functions, primarily metabolic ones, but also biliary secretion, detoxifying from xenobiotic and elimination. However, a number of toxic external agents, such as industrial products, drugs, and heavy metals, can compromise the liver's integrity and cause conditions like cirrhosis, steatosis, and nonalcoholic fatty liver disease (NAFLD), all of which are collectively referred to as hepatic damage. Because of the excessive formation of free radicals caused by chronic exposure to hepatotoxic substances, the hepatic pro/antioxidant balance becomes unstable. These free radicals have the power to change any kind of molecule or cell, resulting in extremely harmful effects that could eventually be irreversible at both the structural and functional levels. As a result, maintaining the liver's health is critical for preserving the body's homeostasis because it is an important metabolic junction in the structural or functional level. The liver is a critical metabolic junction in the body's functioning, it must be in good condition to maintain equilibrium. Lemon is known to have several favorable physiological impacts, such as an anti-inflammatory effect, lipid-lowering activity, detoxifying activity, anticancer, and antimicrobial tasks, which aid in alleviating metabolic diseases. (Bekkouch et al., 2022). The baked snack item known as crackers is typically thin, crunchy, and crispy.

They consist of flour, water, and additional ingredients like lemon peel, salt, olive oil, and ground flaxseed. Crackers can be simple or flavored with herbs, spices, or garlic powder. They are available in a range of sizes and shapes. Variables that affect cracker consumption are availability, price, taste preferences, and cultural norms. Crackers

continue to be a well-liked snack meal all around the world because of its accessibility, durability, and adaptability. Crackers' main component, flour, usually makes up more than 80% of the finished product. Cracker flour types might differ according on the desired texture and flavor profile.

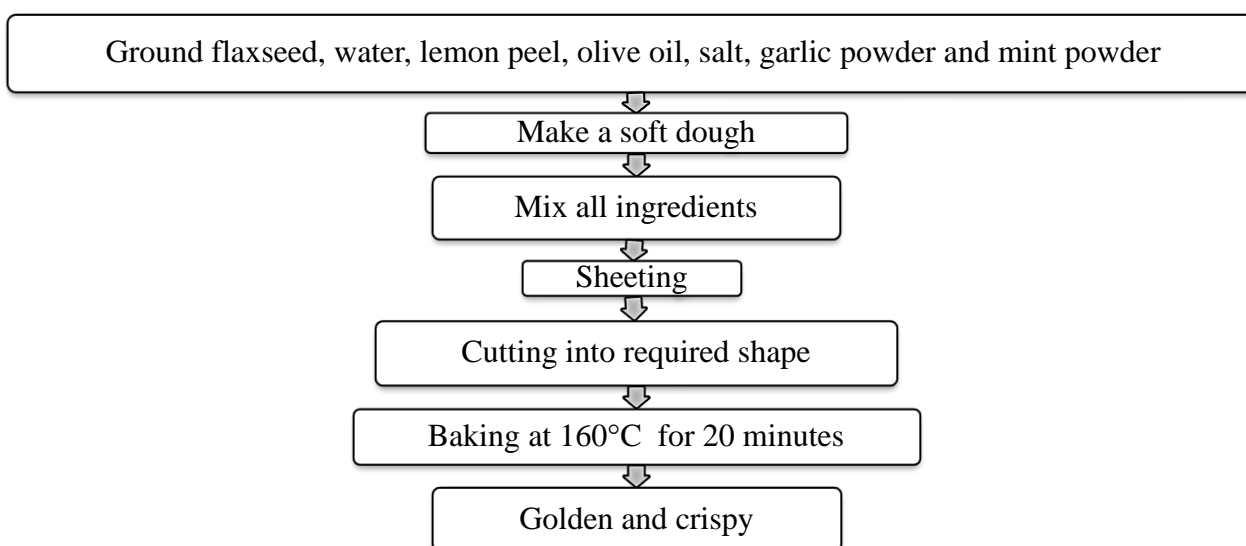
Crackers are a very adaptable snack that can be eaten by themselves or as a foundation for toppings, dips and spreads. Because of this, crackers have become a popular option for customers looking for healthier snack options or quick and simple meal solutions (Ujong et al., 2023).

Treatments	Flaxseed powder%	Lemon peel powder%
T0	100	0
T1	90	10
T2	80	20
T3	70	30

MATERIALS AND METHODS

The current research was conducted at Riphah International University Faisalabad and analysis was carried out using the Product Development and Nutritional Assessment laboratory at Riphah International University. The research

was designed to explore the health benefits of the flaxseed and lemon peel by using it in the form of a crackers to facilitate and enhance the consumption of useful nutrients. Crackers was selected to incorporate with flaxseed and lemon peel powered.



The crackers were made by mixing flaxseed and lemon peel powder in varying ratios using different treatments. For Treatment T0, 100% flaxseed powder and 0% lemon peel powder were used. In Treatment T1, 90% flaxseed powder and 10% lemon peel powder were combined. For Treatment T2, 80% flaxseed powder and 20% lemon peel powder were used while in Treatment T3, 70% flaxseed powder and 30% lemon peel powder were used. The dry ingredients for each treatment were stirred together and olive oil was added to create a smooth dough texture. Water was gradually added to the mixture until a firm dough was formed. The dough was then cut into desired shapes. The shaped dough was placed on the prepared baking sheet and the crackers were baked for 12-15 minutes at a temperature of 160°C until

golden brown and crispy (Zoair et al., 2019). According to the respective techniques the moisture, crude fiber, crude protein, crude fat, ash and NFE (nitrogen free extract) of the cracker samples were measured according to their standard method as described in AOAC, (2016).

The crackers samples were analysed for DPPH method described by (Qiu et al., 2020). The sample extracts (0.0256 mL) were mixed with 10mL of the ethanol after that the 3ml of freshly dissolved 1mg DPPH mixed in 100mL of methanol was added into each test tube. The prepared samples were placed in the dark for 15 mints. The absorbance was estimated at 517nm by using the UV-visible spectrophotometer. During the whole process the highly stable and oxidizing radical, DPPH produced a yellow-

coloured hydrazine which was associated with the removal of free radicals with the hydrogen atom of phenolic compound.

Total polyphenols of flaxseed and lemon peel crackers were estimated method followed by (Deng et al., 2017) using the Folin Ciocalteu. For this purpose, 50 μ L of extract was added in the test tubes and 250 μ L Folin-Ciocalteu reagent along with 750 μ L of sodium bicarbonate were added and a final volume was made using 5mL of distilled water and aluminum foil was used to cover the test tubes in a dark place for about 2 hours. After the mentioned time the absorbance was calculated by using spectrophotometer at a wavelength of 765nm against the control having all reaction reagent except the sample aqueous extract.

The texture of the crackers was determined by using a texture analyser (Mod.TA-XT2 Stable Micro System, Surrey, UK) with a 5 Kg load cell. The data analysis was done by using the Texture Expert program. The texture was determined by using a 3-bend ridge for a bend test. The crackers were bent to determine whether the structural change happened as a result of the force exerted on the crackers according to the procedure described by (Lekjing & Venkatachalam, 2019).

The colour of the crackers was determined by calorimeter by following the protocol of AOAC, (2016). The colour of the crackers was measured by using a lab colorimeter measuring system (model) and recorded as L α b. The L α b colour system consists of luminance or lightness component 'L' for lightness or darkness the component ' α ' for green (- α) to red (+ α) and 'b' for blue (-b) to yellow (+b) colour. The colorimeter was calibrated using a standard white plate. Values of the white standard α and b Colour were measured in a quartz cuvette of 50 ml capacity. Each measurement was replicated. Crackers samples were evaluated for general appearance color, flavor, taste, texture, softness and overall acceptability by (Najjar et al., 2022) trained taste panel using a 9 point Hedonic Score System following the procedure described with the following individual scores liked extremely-9, liked very much-8, liked moderately-7, liked slightly-6 neither liked nor disliked- 5, disliked slightly-4 disliked moderately-3, disliked very much 2 and disliked extremely-1 to find out the most suitable composition of crackers for commercialization. Crackers samples underwent evaluation for various attributes including general appearance, color, flavor, taste, quality, softness and overall acceptability by a trained taste panel. The product showed different alterations in sensory attributes such as flavor degradation, texture changes or aroma loss which were noted and analyzed to understand the

product's stability over the storage period time. Consumer dissection analysis reveals varying preferences among different demographic groups shows the importance of understanding the overall acceptability of developed crackers.

The data collected from all treatments were analyzed using statistical tools. The results from each treatment were subjected to analysis of variance (ANOVA) to assess the level of significance.

RESULTS AND DISCUSSION

Food is a fundamental part of human health and plays a crucial role in preventing disease and supporting bodily functions. Chronic diseases are long term health conditions that progress slowly over time and can significantly impact a person quality of life. NAFLD is one of them that is leading causes of death and disability worldwide. Maintaining a healthy and balanced diet is essential for supporting overall wellbeing and reducing the risk of disease. These nutrients play a key role in enhancing immune function reducing inflammation and ensuring proper bodily functions. Current research is exploring the potential health benefits of flaxseed seed powder and lemon peel powder which are rich sources of antioxidants. These powerful compounds may play a crucial role in preventing or reducing the impact of diseases such as heart disease, cancer and other lifestyle related conditions. In a current study, crackers were developed using these ingredients to evaluate their nutritional value and health promoting effects. The moisture content of crackers for four different treatments T0, T1, T2 and T3. The moisture content values are reported as means \pm standard deviations. The minimum variation in moisture content across the treatments were indicated. The moisture content of the crackers ranged from 1.85 \pm 0.06% in T3 to 1.91 \pm 0.03% in T0 with T3 showing the lowest moisture content. T1 and T2 moisture values were 1.90 \pm 0.08% and 1.89 \pm 0.04% respectively. The small variation in moisture content suggests that the different treatments did not significantly change the moisture levels of the crackers. In the study conducted by Ujong et al. (2023) a low moisture content of 2.1 \pm 0.02% was reported that supports the current research and shows the general trend that lower moisture content contributes to better product quality and longer shelf life.

Ash content representing the total mineral content showed minor variations across treatments. The values ranged from 2.21% to 2.22%, 2.23, 2.24% suggesting an increase in mineral content in the samples. Hussain et al. (2021) conducted a study in which the ash content ranged from 3.1

$\pm 0.03\%$ which shows the current findings by indicating that comparable samples can also retain a relatively high level of minerals after treatment. According to research trends

results suggest that processing methods help preserve mineral content contributing to the nutritional value of the final product.

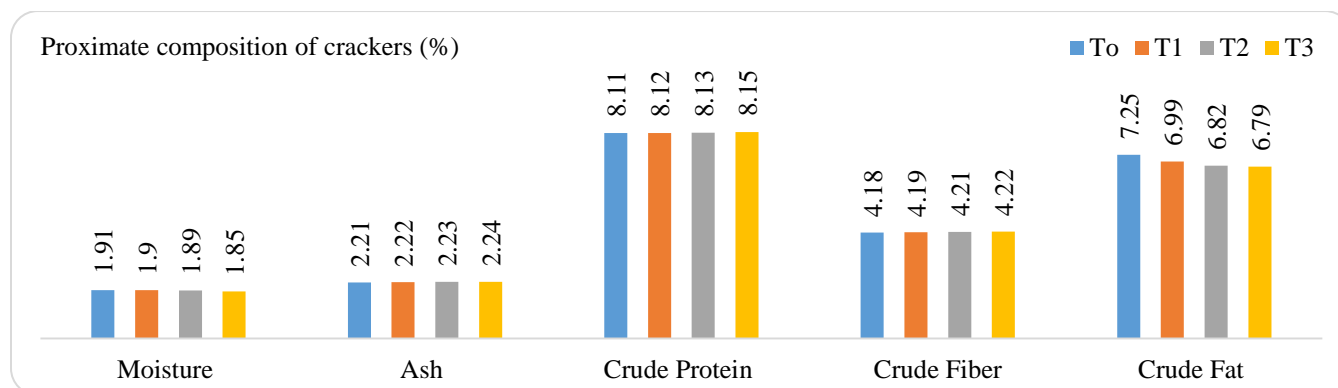


Figure 1: Proximate composition of crackers (%).

The crude protein content (%) of crackers subjected to four different treatments T₀ to T₃. The crude protein values ranging from $8.11 \pm 0.02\%$ in T₀ to $8.15 \pm 0.03\%$ in T₃. Treatment T₁ recorded $8.12 \pm 0.02\%$, T₂ had $8.13 \pm 0.02\%$. The present study findings are following Ujong et al. (2023) who conducted a study on effect of lemon basil powder (LBP) on nutritional and sensory properties of crackers the incorporation of lemon basil powder (LBP) into crackers significantly enhanced their

nutritional profile mainly the protein content. Three levels of LBP (1.0%, 2.5% and 5.0%) were tested in combination with 20% cashew kernel flour (CKF) and the results showed a distinct improvement in crude protein compared to the control sample made with refined wheat flour. The control cracker had a crude protein content of 7.85% while crackers enriched with 1.0%, 2.5% and 5.0% LBP recorded protein values of 9.21%, 9.94% and 10.68% respectively.

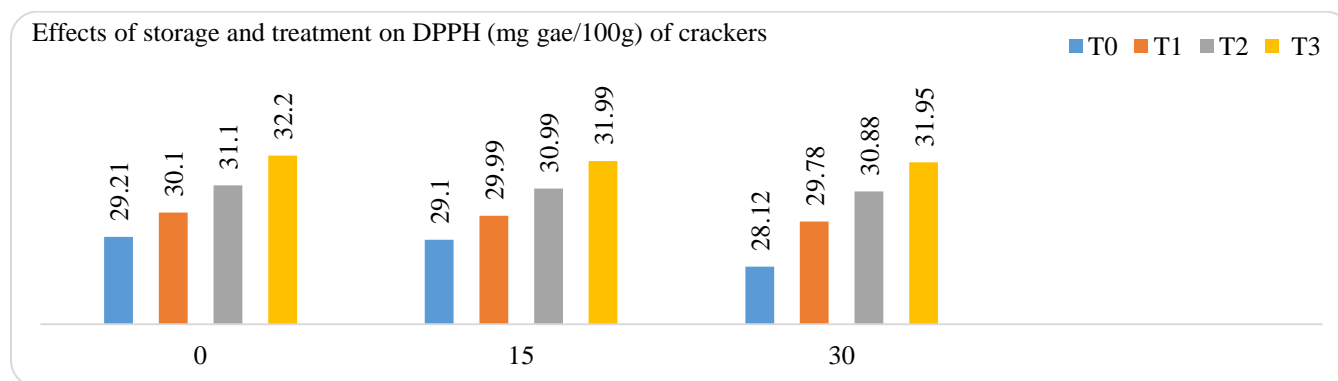


Figure 2: Effects of storage and treatment on DPPH (mg gae/100g) of crackers.

The crude fiber content (%) of crackers using different treatments (T₀ to T₃). The fiber content increased slightly across the treatments from $4.18 \pm 0.02\%$ in T₀ (control) to $4.22 \pm 0.01\%$ in T₃. Treatments T₁ and T₂ had values of $4.19 \pm 0.06\%$ and $4.21 \pm 0.04\%$ respectively. This gradual increase suggests that treatments shows improvement in the fiber content of the crackers. Ujong et al. (2023) reported a crude fiber content of $5.8 \pm 0.09\%$ which supports the findings of the current study and follows the trend.

The crude fat content (%) of crackers across four treatments

T₀ to T₃. A decreasing trend in fat content was observed. The control sample T₀ recorded the highest fat content $7.25 \pm 0.03\%$ while the lowest value $6.79 \pm 0.04\%$ was observed in T₃. Treatments T₁ and T₂ showed values of $6.99 \pm 0.01\%$ and $6.82 \pm 0.04\%$ respectively. This gradual reduction suggests that the treatment may have shown to a slight decrease in the fat content of the crackers. Hussain et al. (2021) reported a crude fat content of 8.11% which supports the current findings and shows a similar trend of reduced fat in treated samples.

The DPPH (mg GAE/100g) values of crackers over three storage durations 0, 15 and 30 days for four treatments T0, T1, T2 and T3. Show that both storage time and treatment significantly impact the DPPH values. The DPPH values decreased over time across all treatments. At day 0 the mean value was 30.65 ± 1.12 which declined to 30.52 ± 1.08 at day 15 and further to 30.18 ± 1.42 at day 30. This suggests a gradual reduction in the antioxidant activity over the storage period. Barthet et al. (2014) evaluated the antioxidant

potential of flaxseed meal using DPPH and ORAC assays finding that methanolic flaxseed extracts demonstrated strong free radical scavenging activity with recorded antioxidant values of 44 ± 0.08 mg GAE/100g flaxseeds potential as a natural antioxidant source. Ahmed et al. (2023) investigated the strong antioxidant properties of lemon peel with DPPH scavenging values ranging from 40 to 55 mg GAE/100g depending on extraction methods and cultivar supporting its use as a functional ingredient in food products.

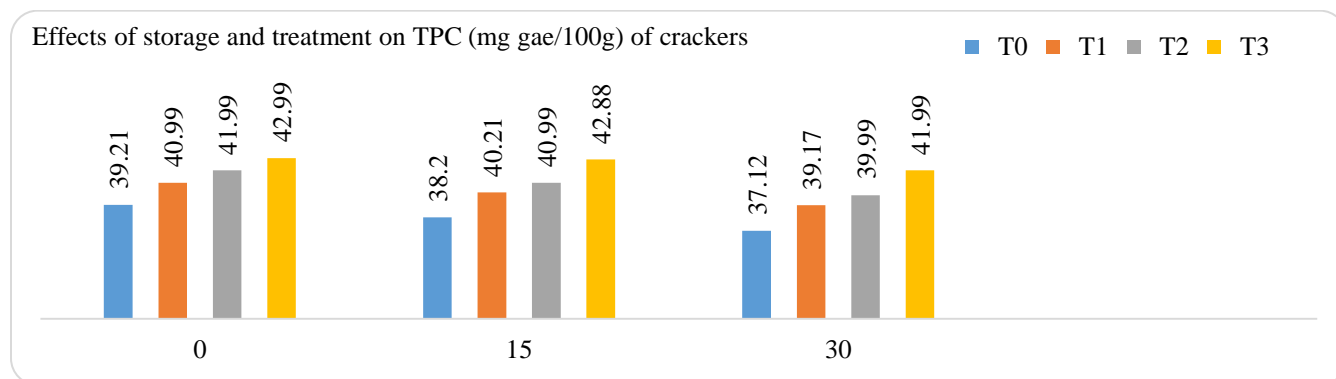


Figure 3: Effects of storage and treatment on TPC (mg gae/100g) of crackers.

The effect of different treatments and storage durations on the measured treatments was evaluated across 0, 15 and 30 days. Results indicated a significant decline in the treatments over time with mean values decreasing from 41.3 ± 0.04 at day 0 to 40.55 ± 0.05 at day 15 and further to 39.57 ± 0.90 at day 30. Ahmed et al. (2023) investigated that lemon peel contains high levels of phenolic

compounds with TPC ranging from 40 to 55 mg GAE/100g depending on the extraction method and lemon variety. Phenolic content during storage showing that adding both flaxseed and lemon peel together gives better antioxidant effects. This combination helps improve the health benefits of the crackers and keeps them fresh for a longer time.

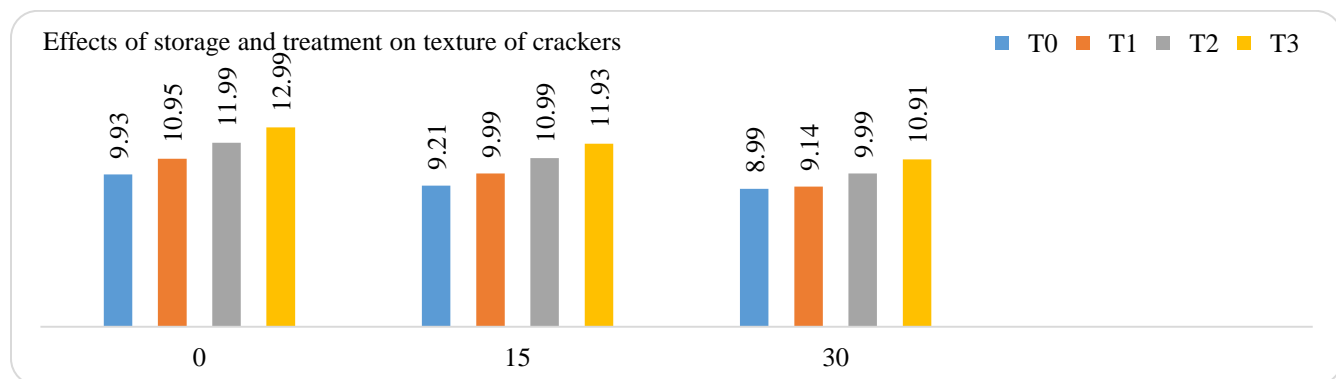


Figure 4: Effects of storage and treatment on texture of crackers.

The effects of storage duration and different treatments on the texture of crackers. Values are presented as mean \pm standard deviation. At day 0, texture values ranged from 9.93 ± 0.05 to 12.99 ± 0.09 . By day 15, texture values decreased slightly ranging from 9.21 ± 0.06 to

11.93 ± 0.07 . By day 30, the texture further declined with values between 8.99 ± 0.08 and 10.99 ± 0.08 over time. Khouryieh and Aramouni (2017) studied the use of flaxseed flour as a partial replacement for wheat flour in making cookies. They found that using up to 12% flaxseed

flour maintained good texture and taste. The recorded value was 13.3 ± 0.02 .

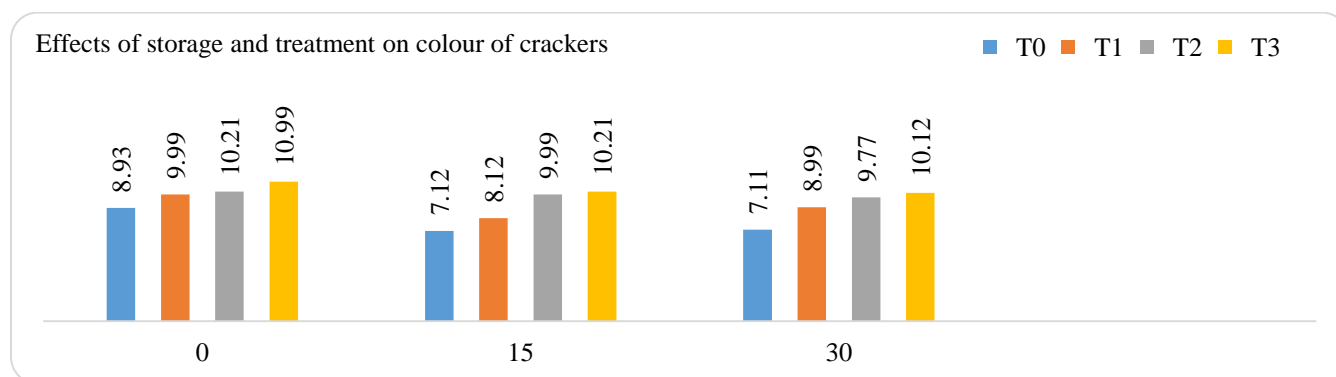


Figure 5: Effects of storage and treatment on colour of crackers.

The effects of storage time and treatment on the color of crackers were evaluated. Color which is a significant sensory attribute is influenced by the ingredients used and the oxidation process during storage. At day 0, the colour values for T0, T1, T2 and T3 were 8.93 ± 0.05 , 9.99 ± 0.03 , 10.21 ± 0.08 and 10.99 ± 0.09 respectively. By day 15, the values were 7.12 ± 0.06 , 8.12 ± 0.08 , 9.99 ± 0.09 and 10.21 ± 0.07 . On day 30, the values recorded were

7.11 ± 0.08 , 8.99 ± 0.05 , 9.77 ± 0.08 and 10.12 ± 0.08 . Hernández et al. (2019) examined the effect of natural antioxidants in citrus peel on maintaining the color of baked products by reducing oxidative damage. They recorded color values of 13.1 ± 0.04 . The study showed that adding flaxseed and lemon peel not only improved the antioxidant content of the crackers but also helped preserve their color over time.

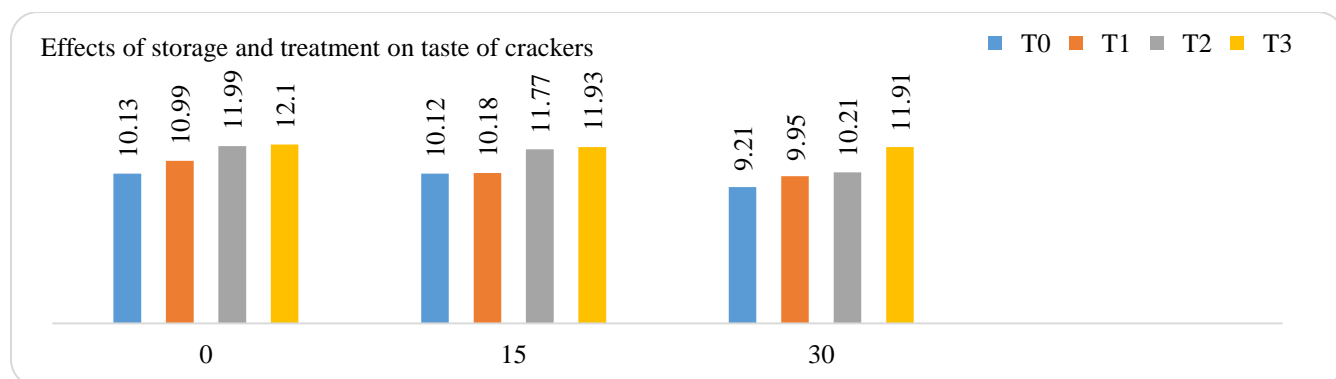


Figure 6: Effects of storage and treatment on taste of crackers.

The sensory evaluation of crackers shows differences in taste scores across treatments and storage durations. At day 0, taste values were 10.13 ± 0.05 (T0), 10.99 ± 0.03 (T1), 11.99 ± 0.08 (T2) and 12.10 ± 0.09 (T3). After 15 days of storage values declined to 10.12 ± 0.06 (T0), 10.18 ± 0.08 (T1), 11.77 ± 0.09 (T2) and 11.93 ± 0.07 (T3). By day 30 reduction was observed with values at 9.21 ± 0.08 (T0), 9.95 ± 0.05 (T1), 10.21 ± 0.08 (T2) and 11.91 ± 0.08 (T3) indicating a gradual decline in taste over time. A study by Hussain et al. (2021) found that lemon peel improves the flavor of baked foods because it contains healthy oils and natural compounds. Their crackers had taste scores as high

as 13.97 ± 0.08 which supports the good taste results seen in the treatments with lemon peel in this study.

CONCLUSION

The findings of this study clearly show the potential of flaxseed and lemon peel powders as valuable functional ingredients in baked products like crackers. Their presence significantly enhanced the nutritional quality by increasing protein and fiber content while reducing fat, aligning with the goals of healthier dietary formulations. These ingredients contributed to improved antioxidant activity and phenolic content which are crucial in preventing oxidative

stress related to Non Alcoholic Fatty Liver Disease (NAFLD). Sensory evaluation shows that the crackers remained acceptable and appealing in terms of taste, texture and color particularly in the T3 treatment which combined both flaxseed and lemon peel at optimal levels. Overall the results indicate that the addition of flaxseed powder and lemon peel powder to crackers improved their nutritional and functional properties with T3 showing the best results in terms of antioxidant activity and color while also maintaining a good balance in moisture protein and fiber content throughout the 30 day storage period. These findings shows the importance of using natural plant based ingredients flaxseed and lemon peel in food products. They not only help improve health by providing important nutrients and antioxidants but also help keep the food fresh and good in quality during storage. Adding these powders to crackers is a simple and effective way to create healthier snacks that may help reduce the risk of chronic diseases and meet the growing demand for nutritious food choices.

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