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Formulation And Characterization Of A Novel Quinoa Seed Oil-Based Oleogel As A Nutrient-Rich Replacer Of Margarine In Cookies

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ABSTRACT

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Quinoa seeds were processed to produce oil via solvent extraction, followed by oleogel preparation by mixing QSO with beeswax. This study aimed to prepare oleogel-based cookies using QSO, replacing margarine with beeswax as a natural structuring agent. The quality of QSO was determined through physicochemical analysis, revealing a saponification value of 192.78 ± 3.09 mgKOH/g, free fatty acid content of $0.683 \pm 0.035\%$, iodine value of 126.53 ± 0.90 g I₂/100g, peroxide value of 2.50 ± 0.35 meq O₂/kg, specific gravity of 0.91 ± 0.011 , refractive index of 1.45 ± 0.0725 , and p-anisidine value of 1.34 ± 0.11 mg/kg. Antioxidant activity measured as DPPH radical scavenging was $62.8 \pm 3.14\%$, with a total phenolic content (TPC) of 58.30 ± 2.92 mg GAE/g. The fatty acid profile showed 60-80% unsaturated fatty acids, primarily linoleic acid (61%). Oleogels were assessed for rheological parameters, color, firmness, and physicochemical tests, showing improved antioxidant properties with a DPPH activity of 82.80%, TPC of 51.80 ± 2.59 mg GAE/g, iodine value of 129.04, and peroxide value of 0.28 meq O₂/kg at 20°C. Margarine had higher viscosity and firmness, with values of 4.57 ± 0.23 Pa.s at 50°C and 8.46 ± 0.57 N, respectively. Cookies were prepared by substituting margarine with oleogel at varying percentages (0% to 100%). Quality was evaluated through proximate analysis, color analysis, textural analysis, and sensory analysis using a 9-point hedonic scale. QSO-based oleogels could successfully replace traditional fats in cookies, offering comparable or superior qualities, especially in antioxidant potential and sensory attributes.

INTRODUCTION:

Quinoa, technically identified as *Chenopodium quinoa* Willd, is a yearly plant that belongs to the Chenopodiaceae family. It originates from the Andean area in South America (Canaviri Paz *et al.*, 2020). The leaves of this plant exhibit polymorphism, starting out green and transitioning to yellow, red, or purple as they develop (Hussain *et al.*, 2021). The desiccated seed is ingested like a cereal grain and is notable for its exceptional nutritional content (Afzal *et al.*, 2022). The composition of necessary amino acids, fatty acids, minerals, vitamins, and antioxidants in this food is believed to be superior to that of the primary cereals. This pseudocereal is a gluten-free food grain with a low glycemic index, making it a suitable option for particular diets and sectors (Nhamo and Talabi, 2024). It is used in baking to increase the pliability of dough and give baked items a crumbly texture (Ghosh *et al.*, 2023). Butter, lard, vegetable oils, margarine, and processed shortenings are examples of foods that contain animal or vegetable fats (Li *et al.*, 2022). Oleogels are considered a healthy alternative to shortenings because they contain low levels of saturated fatty acids and eliminate the possibility of trans fatty acids (TFAs) while improving the level of unsaturated fatty acids in the diet. Oleogels are semi-solid substances that contain liquid oils that are confined within a thermally reversible lattice structure. This structure is formed using gelators, such as animal waxes, vegetable oils, monoglycerides, and phospholipids (Muhammad Nabeel Sharif *et al.*, 2025). Oleogels preserve the nutritional properties and benefits of oils while exhibiting the attributes of solid fats (Gao *et al.*, 2023). The utilization of oleogels as a substitute for conventional shortening, such as margarine, in food, presents itself as a potentially healthier and more advantageous option (Temkov and Mureşan, 2021). Additionally, they can produce gels at very low concentrations (Pang *et al.*, 2023). More investigation is required to refine processing and formulations so that every variety of cookie has the ideal textural fit (Sarkisyan *et al.*, 2023; Pradhan *et al.*, 2023).

Beeswax is a frequently employed gelling agent for structuring vegetable oil due to its high efficiency in gelling and its relatively low crystal mass fraction. The fundamental arrangement of BW crystals, which are composed of molecular units, facilitates the emulation of the interconnected structure of fat crystals. Hence, the structural characteristics of wax crystals exert a significant influence on the final configuration of oleogels (Sarkisyan *et al.*, 2021). The implementation of oleogels as a replacement for shortening and spread in the manufacturing of trans-free and low-saturated fat products holds significant potential for the baking industry (Rabiya Javaid *et al.*, 2025). Cookies are a widely consumed food product under the bakery category, often consisting of a mixture of flour, sugar, and oil (Fariha *et al.*, 2025). Dietary fat contributes to the desired sensory attributes of mouthfeel and texture, enhances taste perception, imparts stability and aeration, and enhances the structural integrity and shape of cookies. Consequently, the incorporation of fat substitution in cookies may potentially help to the development of a more health-conscious product that exhibits an extended shelf life.

To meet their nutritional demands, bakery items have become a common diet in many nations. Cookies are baked, little, flat dessert snacks that are typically shaped into circles and made from flour. Different techniques are being used by the modern baking business to extend shelf life, improve nutrient content and improve flavor of baked goods (Khan *et al.*, 2024).

Replacing traditional shortening with quinoa seed oil oleogel can provide a healthier option while reducing the quantity of saturated fat in cookie manufacturing. Quinoa seeds are an excellent source of beneficial chemicals and oleogels, which, due to their semi-solid state, enhance the quality of cookies while also providing health benefits such as the elimination of trans fatty acids and improved heart health. This study aims to increase the nutritional value of cookies by using oleogel generated from quinoa seed oil, known for its health advantages (Guine, 2022).

METHODOLOGY

Extraction of quinoa seed oil

Constructed thimble with 15g sample, then put them in the round bottom flask of the Soxhlet apparatus, add

250-300ml of n- hexane. When the heat source was turned on, the solvent began to boil or vaporize. These drops landed on the sample, dissolving the oil. The cycle was repeated until all of the oil from the sample was removed. The solvent with oil was collected after 7-8 washings. The solvent was evaporated using rotatory evaporator to obtain oil. It was then filtered through filter paper to remove coarse particles.

Physicochemical analysis of quinoa seed oil

Free fatty acid

An approximate 10 mL of QSO was placed in a conical flask, followed by 25 mL of 95% ethanol, and mixed thoroughly until the oil sample was fully miscible with the ethanol. Next, 2-3 drops of phenolphthalein indicator were added and the mixture was vigorously shaken. 0.1N NaOH was used to titrate the mixture while stirring continuously until the pink color appeared.

Peroxide value (PV)

5ml of QSO sample was carefully transferred into a 250 mL conical flask. Next, a 30mL solution of acetic acid-chloroform (3:2) was added and gently swirled for about 1 minute until complete dissolution occurred. Next, approximately 0 to 5 mL of standard potassium iodide solution was carefully pipetted using a Mohr pipette. Next, perform a titration of the sample solution using a 0.1N Na₂SO₃ solution, specifically sodium thiosulphate. Stir the solution consistently until the mixture takes on a yellow hue. Afterwards, the starch solution was used as an indicator and it was shaken continuously until the blue color disappeared. The formula used to calculate the PV is as follows:

$$\text{PV (meq/kg)} = \frac{(\text{B-S}) \times \text{N} \times 1000 \times 100}{\text{Weight of oil (g)}}$$

Iodine value

A sample of approximately 5 mL of QSO was placed in an Erlenmeyer flask. Then, a solution of carbon tetrachloride (CCL) of about 25 mL was added, and the sample was continuously stirred. Next, Wj,s solution was added, and the contents were thoroughly mixed. Distillation was carried out using a 10% potassium iodide solution and 2 mL of distilled solution. The flask contents were then titrated against 0.1N sodium thionphate, with starch solution serving as an indicator. A blank reading was also taken, and the provided formula was used to calculate the iodine value of the QSO.

$$\text{Iodine value} = \frac{(\text{B-S}) \times \text{N} \times 12.69}{\text{Weight of the sample}}$$

Specific gravity

First of all, fully dry the pycnometer, then it was fill with the oil sample in such a manner that avert enmeshed to the air bubbles when once removed the cap of the side arm. The stopper was inserted and pycnometer was placed into the water bath for 30 minutes the cautiously, the oil was wiped off that was come out from the opening of capillary. The pycnometer was completely cleaned and dried and cap was removed from the side arm remove it from the water bath. The cap was taken off from the side arm and immediately weigh the sample accurately, it was ensured that the temperature should be 30 °C at the time of Specific gravity of QSO was computed by the following formula:

$$\text{Specific gravity} = \frac{\text{Weight of oil sample (g)}}{\text{Weight of the water (g)}} = \frac{\text{C-A}}{\text{B-A}}$$

Saponification value

A precisely measured 5 g sample of QSO was added to a 300 ml Erlenmeyer flask with the help of a Mojonnier pipette and carriage. 50 mL of alcoholic potassium hydroxide was added into the Erlenmeyer flask with the help of a pipette by allowing the pipette to drain for a certain time. The flask was connected to the air condenser to saponify the sample completely and a solution free of sample globules was obtained. A 0.5 N solution of hydrochloric acid was used to titrate the high concentration of potassium hydroxide in the

flask. As an indicator, phenolphthalein was used and the endpoint of the test disappeared pink color. To calculate the saponification value, the formula was used:

$$\text{Saponification value} = \frac{(B - S) \times N \times 28.05}{\text{Weight of sample (g)}}$$

***p*-Anisidine value**

A 2g sample of the QSO was dissolved in 25 mL of iso-octane. To this solution, 1 mL of 25% *p*-anisidine reagent in acetic acid was added. The reagent reacted with aldehydic oxidation products in the oil, forming a yellowish complex. The absorbance of the reaction mixture was measured at 350 nm using a UV-visible spectrophotometer. This wavelength was optimal for detecting the anisidine-aldehyde complex. A blank solution containing iso-octane and *p*-anisidine without oil is used to set the zero of the spectrophotometers to correct for any background absorption.

Antioxidant activity analysis

DPPH radical scavenging assay

2g QSO sample was immersed in 10 mL n-hexane for 4 hours to ensure that the oils in the samples dissipated. The sample was agitated for 4 hours at varying intervals of 30 seconds. 100 µL of KOH of normality 2N was prepared in methanol and it was introduced into the sample, and again for another 30s the sample was shaken. A solution of 1 mM of DPPH in ethanol was formed, from which 4 mL solution was injected into 4 mL of each sample supernatant. From the interval of 1 to 24 hours, the solutions were vortexed carefully, and then were placed in an incubator in the dark. 100µl of the mixture was added to each well of a 96-well plate after each time point. The absorbance was measured in a 96-well microplate reader at a wavelength of 517 nm against a blank sample without scavenger. In a 96-well microplate reader, the absorbance was measured at 517 nm against a blank sample without a scavenger (Spectra-Max 13-x, Molecular Devices San Jose, CA, USA). The antioxidant potential was measured using the following equation:

$$\text{AA (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Thiobarbituric acid (TBA) assay

10 g of materials were homogenized in 10 ml of a 7.5% TCA 0.1% EDTA solution. After being repeatedly shaken for half an hour using a mechanical shaker, the sample was filtered. In a 25 ml colorimetric tube, exactly 5 ml of filtrate was mixed with 5 ml of TBA (2.88 g/l) solution, and the combination was heated in a water bath (90 °C) for 40 minutes to obtain the pink color. After chilling for one hour, the tube was centrifuged at 3000g for five minutes. In a separate tube, 5 ml of chloroform was added to the supernatant solution, and the tube was stirred. This combination was permitted to stand for a minimum of one hour. A spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) was used to determine the absorbance at 532 nm.

Total phenolic content

20 µL of QSO, 100 µL of the Folin-Ciocalteu reagent, 80 µL of 1 N sodium carbonate, and 10 µL distilled water were sequentially mixed, and the 96-well plate was placed in dark for incubation with constant stirring on microplate titer shaker for 25 minutes before reading on microplate reader. The absorbance was measured at optical density of 765 nm using a UV spectrophotometer (BioTek Synergy H1 Multi-Mode Microplate Reader), and the results were reported as milligram gallic acid equivalent per ml oil (mg GAE/ml QSO).

Fatty acid profile by GC-FID

The 100 µl of QSO was vortex within the 5ml of heptane after that, added sodium methoxide approximately 250 in the current mixture. Then vortex that mixture until three layers were formed. This shows that epical

layer was consist of methyl-esters. The process of transesterification used the boron trifluoride methanol complex (20% BF₃ in methanol). A 30ml screw-cap test tube containing 1g/L of pentadecanoic acid (C_{15:0}) n-methanol was filled with 20mg of an oil sample as an internal reference. The mixture was dried using nitrogen (N₂), and the residue was then dissolved in 6ml of a 0.5M methanolic NaOH solution. The tube was sealed with a cap and warmed to 80 °C while being stirred for about 30 minutes. Then, in aliquots in an auto sampler, the methylation samples were examined using GC-FID. The following temperature programs were used to separate the methyl esters of fatty acids: 5 minutes at 140°C and 30 minutes at 240°C at a 4-minute rate. The concentration of fatty acids was represented as a percentage.

Preparation of Oleogel

A Pyrex glass beaker of capacity 500 ml was taken, weighed on weighing balance, and its weight was tarred to zero. QSO was at a level of 95% on weight basis was taken into the beaker and mixed properly with the help of a stirrer. Beeswax was weighed and added to QSO at level 5% on a weight basis. The hot plate was switched on and maintained at constant temperature. The beaker containing oil and beeswax was put on the hot plate, along with a thermometer and then the heating was begun until the temperature reached 90°C.

Analysis of Oleogel

Solid fat content

The SFC of oleogel was measured by nuclear magnetic resonance (NMR) (Oxford MOC+Oxon, UK). The oleogel sample was placed in an NMR tube with a diameter of 10-millimeters, heated at 90 degrees Celsius for 15 minutes until it melted, then maintained at 60 degrees Celsius for 10 minutes, followed by 10 degrees Celsius and 0 degree Celsius for an hour each. After that the temperature was raised to 20 degrees Celsius, then data was recorded from NMR.

Rheological measurements of Oleogel

The flow behavior of oleogel was examined as a function of temperature. The viscosity was measured by melting oleogel at 50°C and then loading it onto the Rheometers Peltier Plate. The temperature was then raised progressively from 50-90 °C at a rate of 2 °C /min, and the steady share viscosity was determined at a rate of 100/s.

Colour (L*, a*and b*) analysis

After the calibration of the device by using a black and white ceramics plate, the color values L* (positive value demonstrates lightness, while a negative value indicates darkness), a* (specifies red and green color variation) and b* (denotes yellow and blue color) were recorded.

Development of Cookies

First, a mixture of sugar, milk powder, and baking powder was prepared using a kitchen-aid blender at a speed of 1 for 2 minutes, and then cream was prepared by blending this mixture with margarine and oleogels for 2 min at a speed of 2. After wiping down, the mixing was resumed for 1 min at a speed of 4. Then the water was added to it and mixed for 3 minutes at a speed of 2. White wheat flour was added to the cream mixture and mixed on speed 2 for 2 minutes after it had been fully incorporated. The dough was formed into a sheet with a 5-6 cm diameter and 10-12 mm thickness.

Product analysis

Cookies was prepared using oleogel made of quinoa seed oil and the following cookies analysis of proximate, textural, color, and sensory were performed.

Proximate analysis

The oleogel cookies were subjected to moisture analysis (AACC Method No. 44-15A), crude fat (AACC

Method No. 30-25), crude protein (AACC Method No. 46-10), total ash (AACC Method No. 08-01), crude fiber (AACC Method No. 32-10), and NFE according to their respective procedures (AACC, 2010).

Moisture content

10.0g of the cookies sample was weighed by using the weighing balance. A pre-weighed China dish containing the sample was used to dry it in the oven for 24 hours at 105°C. After the process of oven drying, the sample was removed and dried for 5-10 minutes in a desiccator, the moisture percentage was determined using the formula:

$$\text{Moisture (\%)} = \frac{\text{Original sample Wt. (g)} - \text{Dried sample Wt. (g)}}{\text{Original sample Wt. (g)}} \times 100$$

Crude fat

The 8-10g of moisture-free sample in finely grounded form was enveloped in a thimble and placed in the Soxhlet tube. The heater was adjusted to a temperature range of 65 to 70 °C, so that the solvent n-hexane falls drop by drop on the sample after condensation through condenser. This process was continued for 4-5 hours until 5-6 washings. The sample was then transferred to a porcelain plate or china dish that had already been weighed and put in the oven to dry. After drying, the sample was moved to desiccator and weighed. Then this sample was oven dried until a uniform weight was attained.

$$\text{Crude fat (\%)} = \frac{\text{Wt. of sample before extraction (g)} - \text{Wt. of sample after extraction (g)}}{\text{Wt. of original sample}} \times 100$$

Crude protein

For digestion, 2.0 g of fine powdered sample was put in digestion tube. 5.0 g of digestion mixture and 25-30ml of 98% conc. H₂SO₄ was added in tube. The mixture was given 3-4 hours of stay time with mild heating until the sample turned clear to light green in color. Remove and cool it, but do not let it crystallize. After being further distilled with 5ml of a 40 percent NaOH solution, 10ml of this diluted sample was placed to a conical flask. 10g of 4% boric acid along with methyl red indicator was added in this flask. After steam distillation in distillation assembly, the ammonia, liberated from distilled sample was collected. The solution was thereafter subjected to titration using a 0.1N H₂SO₄ solution until the endpoint was reached.

Nitrogen free extract

The determination of the nitrogen free extract (NFE) content in cookies was conducted by using the equation:

$$\text{NFE} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fiber})$$

Mineral content

A digestive tube was filled with a 5-g flour sample and 15 mL of a concentrated 3:2:1 mixture. The tube was filled with sulfuric, perchloric, and nitric acids. The tubes were heated to 90 degrees Celsius for 45 minutes and then to 170 degrees Celsius for 40 minutes. Two to three milliliters of the material remained in the tube after 40 minutes at 300C. The digested samples were cooled, and then they were diluted with 100 mL of deionized water in a volumetric flask. An atomic absorption spectrophotometer (Perkin-Elmer Analyst 100, Waltham, MA) was used to evaluate the samples for iron, zinc, copper, and manganese.

Peroxide value

5 grams of the material were placed into a volumetric flask, followed by the addition of 50 milliliters of chloroform. The volumetric flask was put on a shaker for a period of 2 to 3 hours in order to remove fat. Next, the extract was strained using Whatman no. 1 filter paper. 20 ml of the filtered extract was transferred into a flask. Then, 30 ml of glacial acetic acid and 1-2 ml of saturated potassium iodide solution were added to the flask. Subsequently, the flask was allowed to sit undisturbed for duration of 30 minutes. After

duration of 30 minutes, a volume of 50 millilitres of distilled water and 2 millilitres of a 1 percent starch solution were introduced into the flask. The solution underwent a colour change, becoming blue/black. The solution was titrated with 0.01 N sodium thiosulfate until it became colorless. The calculation of PV was performed using the following formula:

$$PV = \frac{(B - S) \times N \times 1000}{\text{Weight of sample (g)}}$$

Phytochemical analysis

Total phenolic compounds (TPC)

0.1 mL of Folin Ciocalteu reagent was obtained and 0.1 mL of aliquot of the extract was added. This mixture was given a stay time of 6 minutes. After 6 minutes 0.3 mL of 2 % Na₃CO₃ solution was added to the mixture. Spectrophotometer was used to measure the absorbance of the sample at 760 nm. Gallic acid was used as a standard for the calibration curve.

Physical analysis

A digital Vernier calliper was used to determine the mean thickness (in millimetres) of six cookies. Similarly, for the diameter, six cookies were arranged in a straight line, touching each other, and the average width (measured in millimetres) was determined by rotating the cookies at a 90-degree angle.

Texture analysis

A texture analyser (T.A.X.T Plus, Stable-Micro-Systems, UK) was employed to examine textural profile of the cookies sample. The breaking force of cookies was tested employing the three-point bending attachment (Stable-Micro-Systems, UK) to determine their hardness. Texture analyzer was used in conjunction with a computer to record the data. Hardness, springiness, and gumminess were determined using the curves.

Color (L*, a* and b*) analysis

With minor adjustments, the technique outlined by Li *et al.* (2021) was applied to an Ultra Scan Pro colorimeter (Hunter Lab, Reston, VA, USA) in order to estimate the colour values L*, a*, and b*.

Energy value

Cookies were tested for energy value by subjecting the samples to a bomb calorimeter as employed by Pasha *et al.* (2002).

Statistical analysis

Data was assessed statistically by using Minitab 18.1 software. Experiments were repeated thrice. Analysis of variance was determined by using two-way factorial design. Mean comparisons were determined by applying Tuckey's test (Montgomery, 2017).

RESULTS AND DISCUSSIONS

Physicochemical analysis of QSO

Free fatty acid value

The findings of this study align with research on the physicochemical and antioxidant characteristics of seed oils which showed a value of FFA of 0.65% expressed in oleic acid being within the maximum limit of 1% for natural crude oils and was found to be rich in polyunsaturated acids to be about above 60% showing it a healthy source for consumption (Castano-Angel *et al.*, 2023). Similarly, the predominance of PUFAs, particularly linoleic acid (C18:2), oleic acid (C18:1), and α -linolenic acid (C18:3), in QSO contributes to its nutritional value and health benefits (Shen *et al.*, 2022).

Peroxide value

The PV is defined as the content of peroxide oxygen that is present in one liter of oil and fat. From the quality assurance perspective, the PV is noticeable among the main quality control parameters for the QSO. The estimated PV (meq/kg oil) of QSO was 2.50 ± 0.35 , represents the degree of oxidative degradation in the oil, with higher values indicating elevated oxidation and likely spoilage. Results of da Silva Lira *et al.* (2020) showed 2.75 (0.39) meq/Kg PV which also indicating high oxidation degradation.

Iodine value

The high iodine value of QSO indicates a higher degree of unsaturation because a percentage of 72.92% for unsaturated fatty acids was observed, which may impact its oxidative stability and susceptibility to rancidity, emphasizing the importance of monitoring and understanding the iodine value in edible oils. This result is in accordance with De Souza Aquino *et al.* (2012), who found 82.70% of unsaturated fatty acids and Iodine value of 102.87 cg I₂g⁻¹, inferior to that reported by Repo-Carrasco *et al.* (2003) which was 127.81 cg I₂g⁻¹.

Specific gravity

The value of specific gravity of oils always lies below and the normal value lies between 0.85-0.959 g/cm³ for fats and oils. The results aligned with the study of Nadiya Jan *et al.* (2019) that found the specific gravity of 0.905 g/cm³, falling in the normal range of fats and oils. The findings is supported by the principles of specific gravity analysis and its significance in assessing the quality of edible oils.

Fatty acid profile by GC-FID

The results of our finding matched with the Matias *et al.* (2022) research, which showed the same findings for QSO consisting of Lauric Acid 0.2, Acid myristic 0.73, C16:0 palmitic acid 11.7%, palmitoleic 0.11, stearic acid 10.3, C18:1 oleic acid 14.1, linolenic acid 61.4, arachidic acid and Paulinic acid 0.49 and 0.19 prospectively, Other 0.68%, fats that are saturated Unsaturated fatty acids (23.12) 76. QSO has these fatty acids.

Analysis of Oleogel

Solid fat content

The findings indicate that the specific heat capacity (SFC) of margarine is significantly higher than that of the oleogel. The findings indicated that the solid fat content (SFC) of oleogels exhibited a reduction as the temperature increased, measuring 10.2% at 10 °C and 7.9% at 25 °C. The study conducted by Esmailifard *et al.* (2016) demonstrated the solid fat content (SFC) of margarine at various temperatures ranging from 0 to 35 °C. Margarine has the highest Solid Fat Content (SFC) at a low temperature of 0°C, with a value of 94.21%, compared to the SFC of 0.23% observed at a higher temperature of 35°C. The oleogel containing 3% wax achieved a solid fat content (SFC) of 1.96%, whereas the oleogel containing 7% wax exhibited an SFC of 4.88%..

Iodine value

The iodine value of the margarine was assessed and found to have a mean value of 59.68 ± 2.98 g I₂/100g fat. The iodine value of the oleogel was much greater than that of the margarine, indicating a higher concentration of double bonds in the oleogel and thus a higher level of unsaturation compared to margarine. The study conducted by Chandana and Navaratne (2015) further supported these findings, namely in the development of soft dough cookies using highly unsaturated oils. In a further investigation carried out by Demirkesen and Mert (2019), the characteristics of an oleogel formed from a combination of beeswax and sunflower oil were assessed, yielding the same findings.

Table 1- Mean value of Iodine value (g I/100g fat) of Oleogels, QSO and Margarine

Sample	Iodine Value
Oleogel	130.75 ± 1.47
Margarine	59.68 ± 2.98
QSO	126.53 ± 1.90

Saponification value

The saponification number of the oleogel was determined and was compared with the margarine and QSO. These findings were reinforced by the study conducted by Holey *et al.* (2021a) to formulate oleogels with rice bran wax and sunflower wax. Another study was performed by Putri *et al.* (2020) to determine the oil's saponification value.

Table 2- Mean value of Saponification value (mg KOH/g fat) of Oleogels, QSO and Shortening

Sample	Saponification value (mg KOH/g fat)
Oleogel	187.24 ± 2.45
Margarine	205.87 ± 4.29
QSO	192.78 ± 3.09

Peroxide value

The oleogel stored at 20 °C had an average PV of 2.05 ± 0.72 meqO₂/kg, whereas the oleogel held at refrigerated temperature had an average PV of 1.85 ± 0.51 meqO₂/kg. Similarly, the margarine kept at a temperature of 20 °C and a chilled temperature of 4 °C had average PVs of 1.21 ± 0.33 meqO₂/kg and 0.75 ± 0.12 meqO₂/kg, respectively. The data revealed that the PV of both oleogel and margarine was somewhat higher at high temperatures compared to low temperatures. The PV of the monoglyceride oleogel increased from 0.27 meqO₂/kg to 0.29 meqO₂/kg when the storage temperature was raised from 4 °C to 20°C. Yilmaz and Ogutcu (2014) conducted a study to evaluate the oxidative stability of oleogels made with hazelnut oil, beeswax, and monoglyceride oleogelator at temperatures of 4°C and 20°C.

Table 3- Mean values of Peroxide (meq O₂/kg) of Oleogel and Margarine at 20 °C and 4° C

Temperature	Oleogel	Margarine	QSO
At 20° C	2.05 ± 0.72	1.21 ± 0.33	2.50 ± 0.35
At 4° C	1.85 ± 0.51	0.75 ± 0.12	1.94 ± 0.29

Rheological measurements of Oleogel

The margarine showed a 4.57 ± 0.23 Pa.s mean value of viscosity at 50 °C which was decreased to 3.12 ± 0.16 Pa.s at 60 °C and finally reduced to 2.45 ± 0.12 at 70 °C. In comparison to margarine, the oleogel showed lower values of viscosity. Oleogel showed a 0.80 ± 0.04 Pa.s mean value at 50 °C, 0.46 ± 0.02 Pa.s at 60 °C and 0.31 ± 0.02 Pa.s at 70 °C. From 50-70 °C the viscosity of oleogel decreased in a non-linear pattern and reduced quickly while slow reduction. Results were supported by the study performed by Holey *et al.* (2021) using the oleogel of sunflower oil, prepared by using different waxes at different concentrations in cookie formulation. The findings indicate that the decrease in oleogel was influenced by the concentration of wax. Oleogels prepared with 9% rice bran wax showed higher viscosity (0.18 Pa.s) than the oleogels prepared with 3% rice bran wax (0.10 Pa.s).

Table 4- Mean value of Viscosity (Pa.s) of Margarine and Oleogel

Sample	Temperature °C	Viscosity (Pa.s)
Margarine	50	4.57 ± 0.23
Margarine	60	3.12 ± 0.16
Margarine	70	2.45 ± 0.12
Oleogel	50	0.80 ± 0.04

Oleogel	60	0.46 ± 0.02
Oelogel	70	0.31 ± 0.02
QSO	50	0.037 ± 0.04
QSO	60	0.031 ± 0.02
QSO	70	0.027 ± 0.01

Antioxidant activity analysis

DPPH radical scavenging assay

The results indicate that the oleogel exhibited a greater DPPH scavenging activity compared to QSO. The oleogel had a mean value of 66.50 ± 3.33 , whereas the QSO demonstrated mean values of 62.80 ± 3.14 . The findings of Onacik-Gur and Zbikowska (2022) were consistent with the results of this investigation.

Table 5- Mean values of DPPH (% inhibition) of Oleogel and Oils

Sample	DPPH
Oleogel	66.50 ± 3.33
QSO	62.80 ± 3.14
Margarine	40.00 ± 2.05

Total phenolic content

The oleogels sample had a total phenolic content (TPC) mean value of 51.80 ± 2.59 mg GAE/g, which was somewhat lower than the TPC mean value of 58.30 ± 2.92 mg GAE/g displayed by the QSO. The difference in TPC between the oleogels and QSO was not significant. The findings were corroborated by the study conducted by EL-Shafey *et al.* (2018) to assess the resistance to oxidation and ability to remove free radicals of corn oil and mixtures held under oxidizing conditions for 15 days. Combining corn oil with sunflower oil enhanced the antioxidant activity of the oleogel.

Table 6- Mean values of TPC (mg GAE/100 g oil) of Oleogel and Oils

Sample	TPC
Oleogel	51.80 ± 2.59
QSO	58.30 ± 2.92
Margarine	5.3 ± 0.23

Fatty acid profile by GC-FID

The margarine had the following concentrations of fatty acids: palmitic acid (12.07 ± 0.05), stearic acid (9.67 ± 0.01), oleic acid (20.82 ± 0.08), and linoleic acid (41.19 ± 0.4). The composition of margarine includes (31.34 ± 0.1) percent saturated fatty acids, (21.13 ± 0.06) percent mono-unsaturated fatty acids, and (44.59 ± 0.3) percent PUFA. The mean value of linoleic acid in corn oil was the greatest at 54.05 ± 0.2 , followed by oleic acid at 28.06 ± 0.06 , palmitic acid at 11.66 ± 0.01 , stearic acid at 1.47 ± 0.04 , and a little quantity of other fatty acids. The findings were corroborated by Oh *et al.* (2017) study, which utilized sunflower oil, carnauba wax, and candelilla wax to create oleogels for the production of cookies.

Analysis of Cookies

Proximate analysis

The levels of moisture, fat, protein, fiber, and NFE value varied significantly between cookie compositions with varying oleogel percentages. The cookies were also submitted to a two-month storage study at five different intervals (0, 15, 30, 45, and 60 days).

Ash content

The highest mean ash content was observed in treatment T5 ($1.24 \pm 0.10\%$), with the following order: T4 (1.22%), T3 (1.17%), T2 (1.15%), T1 (1.13%), and the lowest in T0 (1.04%). During storage, cookies were kept in a cool place, and the ash content was measured at intervals of 0, 15, 30, 45, and 60 days. No significant decrease in ash content was noted over time. For instance, the ash content of T₀ decreased from $1.08 \pm 0.052\%$ to $1.01 \pm 0.049\%$, while for T₅, it reduced from $1.28 \pm 0.072\%$ to $1.20 \pm 0.06\%$ after 60 days of storage. These findings align with a study by Ishera *et al.* (2021) which evaluated the storage stability of breadfruit cookies. This study further supports that oleogels can maintain a stable ash content over time.

Table 7- Mean values showing the effect of different treatments on ash content (%) of cookies during 60 days of storage study

Treatment	Storage days					Mean
	0 th	15 th	30 th	45 th	60 th	
T ₀	1.08±0.03	1.07±0.07	1.05 ± 0.05	1.03 ±0.03	1.01 ± 0.09	1.04 ^a
T ₁	1.16 ±0.07	1.14 ±0.09	1.11 ±0.07	1.09 ±0.07	1.11 ±0.02	1.13 ^a
T ₂	1.18 ±0.09	1.15 ±0.05	1.16 ±0.09	1.13 ±0.09	1.14 ±0.04	1.15 ^a
T ₃	1.22 ±0.01	1.21 ± 0.04	1.20 ± 0.06	1.17 ±0.03	1.16 ± 0.06	1.17 ^a
T ₄	1.26 ±0.03	1.25 ± 0.06	1.22 ±0.02	1.19 ±0.07	1.18± 0.08	1.22 ^a
T ₅	1.28 ±0.02	1.26 ± 0.01	1.23 ±0.03	1.21 ±0.09	1.20 ± 0.05	1.24 ^a
Mean	1.19 ^a	1.17 ^a	1.14 ^a	1.13 ^a	1.09 ^a	

Crude fat

The maximum decline in fat content was observed in T0, which dropped from $24.05 \pm 1.20\%$ to $21.15 \pm 1.10\%$, whereas the minimum reduction was seen in T5, which decreased from $31.04 \pm 0.88\%$ to $28.93 \pm 1.44\%$. Results are consistent with the study conducted by Yılmaz and Ogutcu (2015), which formulated cookies using different waxes with hazelnut oil. In their study, the fat content decreased from $22.77 \pm 1.56\%$ to $21.69 \pm 1.11\%$ during storage. Further supporting these findings, Mert and Demirkesen (2016) investigated replacing shortening with oleogel in cookies. They reported that the fat content of cookies prepared with oleogels ranged from $25.14 \pm 0.55\%$ to $28.36 \pm 0.68\%$, depending on the oleogel formulation. They found that the cookies made with oleogels had a stable fat content initially but showed a decline during storage. The initial fat content values ranged from $25.60 \pm 0.32\%$ to $28.15 \pm 0.45\%$, with a notable decrease observed over the storage period.

Table 8- Mean values showing the effect of different treatments on fat content (%) of cookies during 60 days of storage study

Treatment	Storage days					Mean
	0 day	15 days	30 days	45 days	60 days	
T ₀	24.05±1.20	23.09±1.19	22.45±1.15	21.39±1.12	21.15±1.10	23.09 ^e
T ₁	27.76±1.38	27.56±1.36	26.93±1.34	25.65±1.24	24.02±1.21	26.80 ^d
T ₂	27.97±1.39	28.74±1.38	27.83±1.35	26.58±1.28	25.67±1.24	27.00 ^{cd}
T ₃	29.25±1.46	29.40±1.45	28.18±1.41	27.35±1.32	26.56±1.34	28.28 ^{bc}
T ₄	30.50±1.48	30.11±1.50	29.19±1.49	28.98±1.38	27.56±1.38	29.56 ^{ab}
T ₅	31.04±0.88	30.90±1.01	29.23±1.54	29.11±1.46	28.93±1.44	29.90 ^a
Mean	28.34 ^a	28.09 ^{ab}	27.47 ^{abc}	26.91 ^{bc}	26.39 ^c	

Crude protein

The maximum value of crude protein was observed in the cookies of treatment T5 ($8.56 \pm 0.49\%$), while the minimum value was found in T0 ($8.05 \pm 0.40\%$), showing an increasing trend from T0 to T5. The results of the present investigation are consistent with a study carried out by Chandana and Navaratne (2015), in

which biscuits were prepared using sunflower oil and corn oil. Additionally, research by Mert and Demirkesen (2016) examined replacing shortening with oleogel in cookies. They found that the protein content in oleogel-incorporated cookies ranged from $7.85 \pm 0.35\%$ to $8.67 \pm 0.42\%$, depending on the formulation.

Table 9- Mean values showing the effect of different treatments on protein content (%) of cookies during 60 days of storage study

Treatment	Storage days					Mean
	0 day	15 days	30 days	45 days	60 days	
T ₀	8.05 ± 0.40	8.02 ± 0.40	7.98 ± 0.39	7.93 ± 0.38	7.80 ± 0.39	7.96 ^c
T ₁	8.10 ± 0.42	8.06 ± 0.41	8.03 ± 0.40	7.98 ± 0.39	7.99 ± 0.41	8.06 ^{bc}
T ₂	8.17 ± 0.45	8.12 ± 0.42	8.16 ± 0.41	8.10 ± 0.40	8.16 ± 0.46	8.13 ^{bc}
T ₃	8.35 ± 0.46	8.32 ± 0.43	8.27 ± 0.42	8.21 ± 0.42	8.23 ± 0.48	8.26 ^{ab}
T ₄	8.45 ± 0.48	8.42 ± 0.44	8.33 ± 0.43	8.28 ± 0.43	8.25 ± 0.41	8.36 ^a
T ₅	8.56 ± 0.49	8.48 ± 0.45	8.42 ± 0.45	8.38 ± 0.45	8.34 ± 0.44	8.46 ^a
Mean	8.27 ^a	8.22 ^a	8.15 ^a	8.09 ^a	8.01 ^a	

Nitrogen-free extract

The maximum mean value of NFE was calculated for the treatment T₀ 60.56±1.70, followed by T₁, T₂, T₃, T₄ and T₅ with a minimum value of 54.36±1.35. The data from the mean values illustrated that the NFE decreased from T₁ to T₅ as the percentage of the oleogel increased. The NFE values showed a decreasing trend from 0 to 60 days of storage in all treatments. The maximum decline was found in the treatment T₀ from 60.56±1.70 to 55.99±2.20 while the minimum escalation was observed in T₅ from 54.36±1.35 to 53.24±2.59. These results were reinforced by the study conducted by Brito *et al.* (2022).

Table 10- Mean values showing the effect of different treatments on nitrogen-free extract (NFE (%)) of cookies during 60 days of storage study

Treatment	Storage days					Means
	0 th	15 th	30 th	45 th	60 th	
T ₀	60.56±1.70	59.87±2.01	58.66±2.07	57.50±2.13	55.99±2.20	58.52 ^a
T ₁	57.77±2.16	57.44±2.15	57.24±2.14	56.60±2.21	55.36±2.28	56.49 ^{ab}
T ₂	56.98±2.16	56.88±2.16	56.29±2.20	55.71±2.17	55.01±2.25	56.14 ^{bc}
T ₃	56.14±2.23	55.46±2.23	55.03±2.29	54.96±2.23	54.54±2.27	55.35 ^{bc}
T ₄	55.35±2.04	54.80±2.28	54.30±2.34	54.02±2.28	53.85±2.29	54.30 ^{bc}

T5	54.36±1.35	54.26±1.69	54.06±2.34	53.85±2.19	53.24±2.59	54.07 ^c
Means	56.35 ^a	56.32 ^{ab}	56.28 ^c	55.56 ^{cd}	54.55 ^d	

Mineral Analysis

Potassium

Among the treatments, T₅ exhibited the highest mean potassium content indicating that this formulation maintained superior potassium levels over time with the addition of QSO. These findings are in line with the study conducted by Karyantono *et al.* (2016), which demonstrated that potassium fortification in food products significantly improved their mineral content, leading to enhanced nutritional benefits and showed a decreasing trend in the mineral contents from 0 to 90 days.

Table 11- Potassium content (mg/100g) of biscuits

Treatment	Storage days					Means
	0 days	15 days	30 days	45 days	60 days	
T ₀	221.45±2.33	220.02±3.71	219.10±3.34	213.43±2.86	211.34±3.47	217.06 ^a
T ₁	224.65±4.46	222.14±1.14	220.40±2.36	215.93±1.35	213.99±3.26	219.42 ^a
T ₂	226.54±3.59	223.98±3.41	221.19±3.87	219.09±3.34	215.78±2.12	221.36 ^a
T ₃	229.31±2.14	228.06±3.68	222.83±1.39	221.59±1.41	218.18±2.48	223.17 ^a
T ₄	232.15±3.53	229.96±3.13	226.37±2.26	223.55±3.19	221.68±2.45	226.25 ^a
T ₅	233.77±1.31	232.66±2.46	229.41±2.74	227.02±1.42	226.78±2.50	229.35 ^a
Means	227.43 ^a	226.17 ^a	223.13 ^a	220.41 ^a	217.37 ^a	

Calcium

The interaction between treatment and days was found non-significant (F = 0.95, p > 0.05). Among the treatments, T₅ exhibited the highest mean calcium content (29.88^a), maintaining high levels from Day 0 to Day 60. These results align with the findings of Stellamaris *et al.* (2018), who reported that calcium fortification in food products significantly enhanced their nutritional profile and stability.

Table 12- Calcium content (mg/100g) of biscuits

Treatment	Storage days					Means
	0 days	15 days	30 days	45 days	60 days	
T ₀	25.08±0.38	23.59±0.16	23.95±0.36	21.21±0.85	21.34±0.47	23.33 ^d
T ₁	26.47±0.67	25.56±0.50	24.07±0.69	23.82±0.15	22.89±0.35	24.58 ^{cd}
T ₂	28.30±0.69	27.26±0.42	25.03±0.19	25.43±0.39	23.89±0.60	25.24 ^c
T ₃	29.27±0.02	28.31±0.34	27.92±0.17	26.54±0.67	24.99±0.26	27.97 ^{bc}
T ₄	30.22±0.46	29.30±0.36	28.27±0.43	27.71±0.63	26.38±0.58	28.81 ^{ab}
T ₅	31.08±0.48	30.53±0.72	29.19±0.24	28.13±0.18	27.91±0.31	29.88 ^a
Means	28.31 ^a	27.95 ^{ab}	26.43 ^{bc}	25.07 ^{cd}	24.99 ^d	

Iron

Among the treatments, T₅ exhibited the highest mean iron content (11.69^f) showing that with increased oleogel percentage the mineral content of the product increased to some extent, maintaining high levels particularly on Day 0 and Day 15. These findings align with the research by Robertson *et al.* (2012), who demonstrated that iron fortification in food products significantly improved their nutritional quality and

stability.

Table 13- Iron content (mg/100g) of biscuits

Treatment	Storage days					Means
	0 days	15 days	30 days	45 days	60 days	
T ₀	1.80±0.02	1.71±0.23	1.66±0.03	1.57±0.20	1.48±0.02	1.64 ^a
T ₁	1.86±0.07	1.82±0.01	1.78±0.08	1.65±0.01	1.58±0.04	1.73 ^a
T ₂	1.92±0.26	1.90±0.03	1.82±0.25	1.77±0.03	1.71±0.06	1.82 ^{ab}
T ₃	2.01±0.48	1.96±0.08	1.93±0.06	1.88±0.08	1.78±0.23	1.91 ^b
T ₄	2.10±0.03	2.03±0.29	1.98±0.02	1.93±0.26	1.85±0.09	1.97 ^{bc}
T ₅	2.14±0.12	2.09±0.02	2.01±0.09	1.98±0.03	1.89±0.11	2.02 ^c
Means	1.97 ^a	1.91 ^a	1.86 ^a	1.81 ^a	1.76 ^a	

Phytochemical analysis

Total phenolic compound (TPC)

The maximum mean value of TPC was calculated for the treatment T₅ 110.21±5.51, showing an increasing trend from T₀ (99.54 ± 4.98) and T₅ (110.21±5.51). The TPC values showed a decreasing trend from 0 to 60 days of storage in all treatments. The maximum decline was found in the treatment T₀ (0 % oleogel) while the minimum decline was observed in T₅ showing oleogel had higher antioxidant potential and stability. Bayat *et al.* (2022) worked on the preparation of gluten-free cookies fermented with *Saccharomyces cerevisiae*. It was concluded that TPC content increased from 7.04 to 7.46mg/g by 7.08% of cookies also relatable with the study of Aktaş and Akın (2020).

Antioxidant activity analysis

DPPH radical scavenging assay

The maximum mean value of DPPH was calculated for the treatment T₅ 59.41 ±2.97 %, showing an increasing trend from T₀ (40.41 ± 2.02 %) and T₅ (59.41 ±2.97 %). The DPPH values showed a decreasing trend from 0 to 60 days of storage in all treatments. The maximum decline was found in the treatment T₀ (0 % oleogel) while the minimum decline was observed in T₅ showing oleogel higher antioxidant potential and stability. Choudhury *et al.* (2015) illustrated the influence of bamboo shoot powder effect on the properties of biscuits and stated that DPPH content was increased from (3.50 to 17.85%) by adding 15% bamboo shoot powder. Mudau *et al.* (2022) stated that DPPH content dark brown finger millet biscuits increased from 59.66 to 72.99 for 72 hours.

Physical analysis

The effect of storage showed an increasing trend from 0 days to 60 days with maximum change in T₅ showing that with increasing time oleogel becomes less viscous leading to a change in the spread ratio. Study by Singh *et al.* (2017) showed that the spread ratio of cookies was found to increase as the duration of storage progressed for self-rising flour.

Color analysis

The results indicated that the lightness (L*) of cookies decreased with increasing oleogel concentration and storage time, with the highest value observed in the control (T₀) and the lowest in T₅. The yellowness (b*)

values increased with higher oleogel substitution but decreased over the storage period. The greatest reduction in b^* was observed in T0, while T5 showed the least change. These trends are consistent with previous findings reported by Flores-Garcia *et al.* (2023), confirming the impact of oleogel incorporation and storage on cookie color attributes.

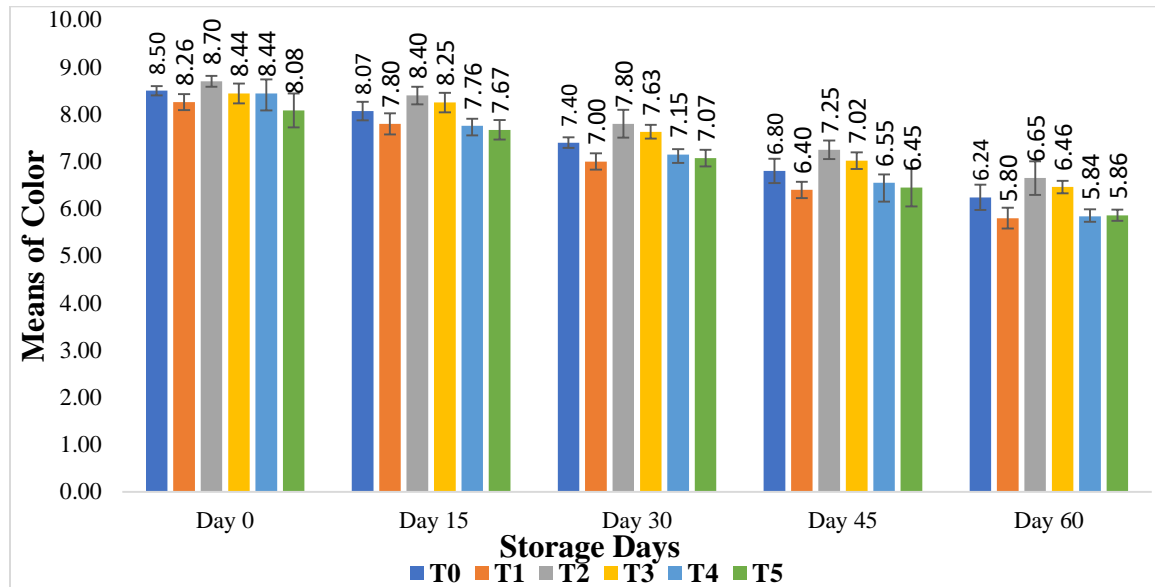


Figure 1- Graphical representation of color parameter of cookies in sensory evaluation during two months of storage

Flavor

The maximum decline was found in the treatment T₅ from 7.95 ± 0.51 to 5.75 ± 0.77 and the minimum was noted in T₂ from 8.65 ± 0.73 to 6.6 ± 0.67 . The values of the flavor showed that there was a very mild decline in the flavor of the T₂ treatment cookies. While the maximum decrease in the flavor score value in the treatment T₅ showed a higher reduction in the flavor of the cookies. These results were supported by the research conducted by Pang *et al.* (2023).

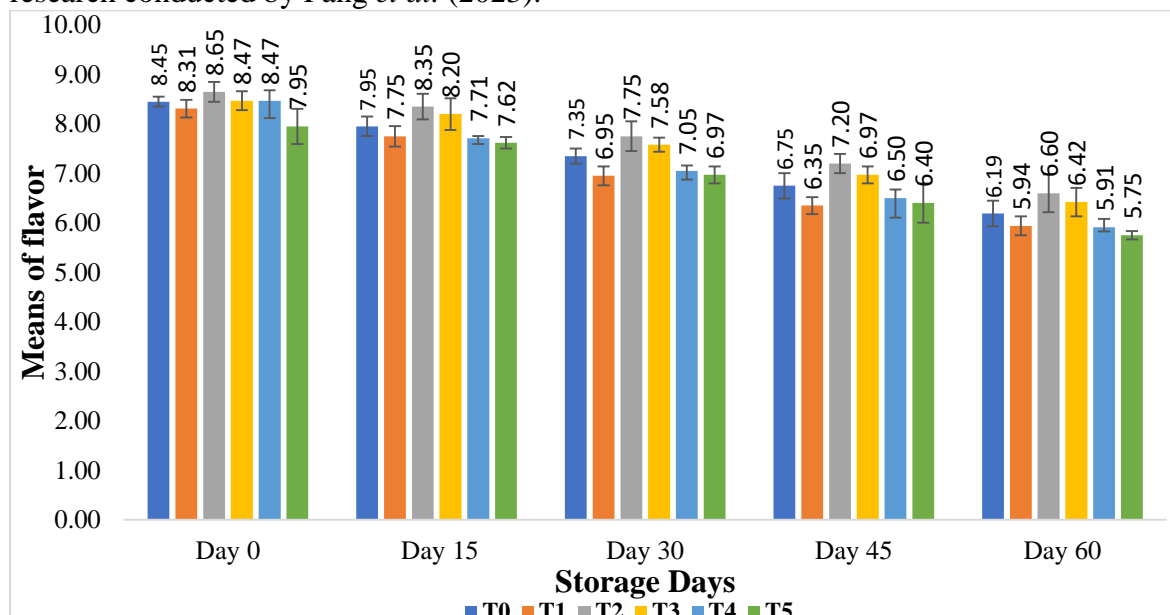


Figure 2- Graphical representation of flavor parameter of cookies in sensory evaluation during two months of storage

Texture

The cookie’s texture also undergoes sensory evaluation during the storage of two months at the 0th, 15th, 30th, 45th, and 60th days. The evaluation of the cookie’s texture resulted in decreasing the assigned score from the panelists from 0th to 60th days. The maximum reduction was noted in the treatment T₅ from 7.64±0.62 to 5.38 ±0.59 while the minimum was found in T₂ from 8.34±0.67 to 6.25±0.85. These findings of the sensory evaluation of cookies texture were reposed by the study organized by Pang *et al.* (2023).

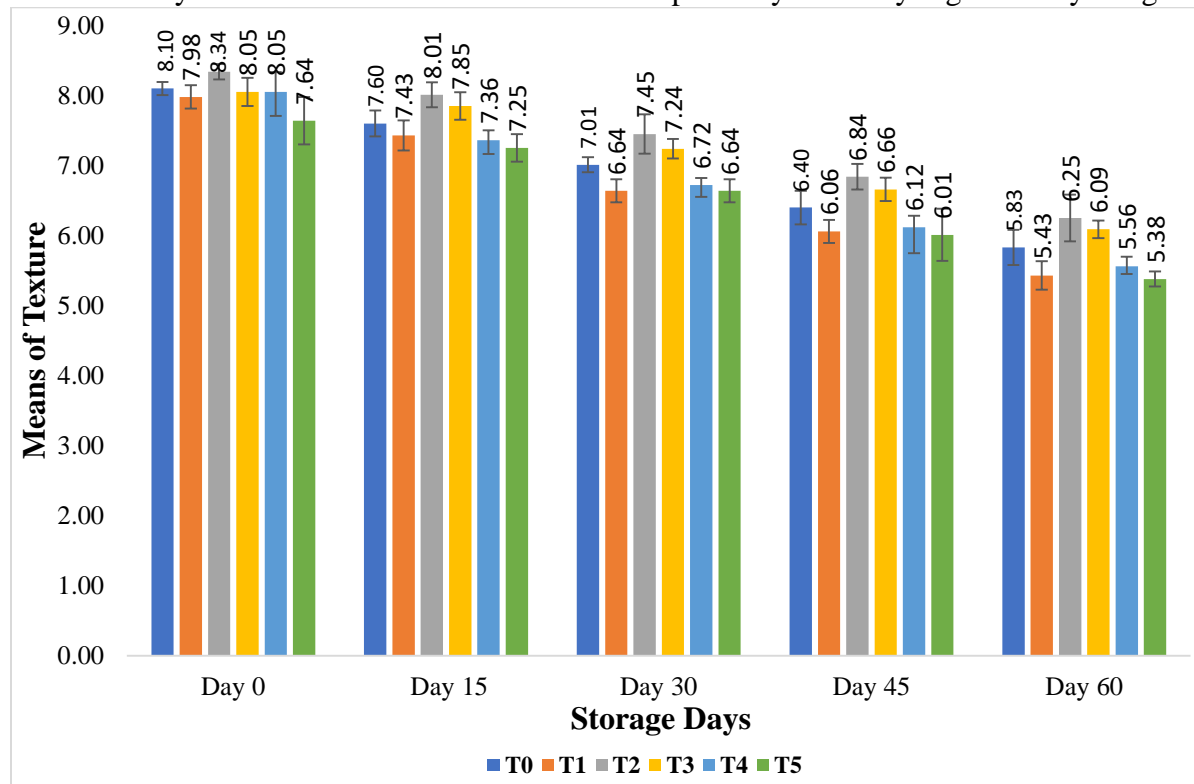


Figure 3- Graphical representation of texture parameter of cookies in sensory evaluation during two months of storage

Overall acceptability

The maximum decline was noted in the treatment T₅ from 7.87 ± 0.63 to 5.65 ±0.72, while the minimum decline was found in T₂ from 8.55 ±0.51 to 6.49±0.55. So, the treatment T₂ with the 40% replacement of margarine with oleogel was selected as the best treatment and the last one was T₅ (with 100% oleogels) due to the score of the parameters about the sensory evaluation of the cookies. The results of the sensory evaluation of the current study were reinforced by the study organized by Pang *et al.* (2023).

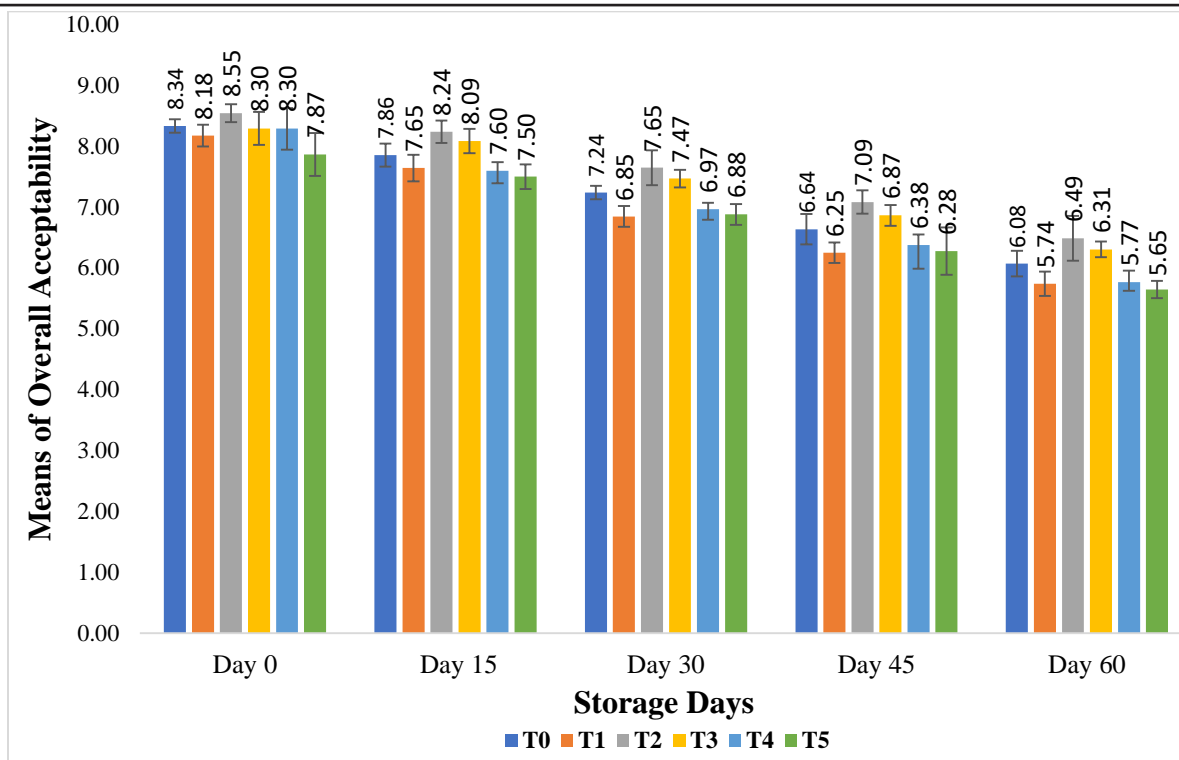


Figure 4- Graphical representation of overall acceptability parameter of cookies in sensory evaluation during two months of storage

Conclusion

Oleogels prepared from quinoa seed oil and blended vegetable oils (corn and sunflower oils) using beeswax as a structuring agent are highly effective substitutes for traditional margarine in cookie formulations. These oleogels successfully replicated the functional, rheological, and textural properties of solid fats while significantly reducing saturated and eliminating trans fatty acids. The oleogel-based cookies exhibited enhanced antioxidant activity, improved oxidative stability, and favorable physicochemical characteristics throughout a two-month storage period. Sensory evaluation revealed that cookies with 40% oleogel substitution achieved the highest overall acceptability, offering an optimal balance between spreadability, firmness, flavor, and texture. Moreover, the high unsaturated fatty acid content, particularly linoleic acid, along with the lower viscosity of oleogels at baking temperatures, contributed to desirable cookie quality and health benefits. Overall, the findings highlight the strong potential of QSO-based oleogels as a sustainable, health-promoting, and technologically viable alternative to conventional shortenings in the bakery industry.

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