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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Impact of Carnauba Wax–Structured Olive Oil Oleogels Enriched with Thyme Essential Oil on the Physicochemical, Rheological, and Textural Properties of Biscuits

Karnauba Mumu ile Yapılandırılmış Zeytinyağı Bazlı Oleojellere Kekik Uçucu Yağı İlavesinin Bisküvilerin Fizikokimyasal, Reolojik ve Tekstürel Özellikleri Üzerine Etkisi

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Abstract

This study investigated the effects of using olive oil-based oleogels as an alternative to traditional solid fats in biscuit formulations on their physicochemical, rheological and textural properties. The fat content in the biscuit formulations was standardised to 24 per cent, and all production conditions were kept constant to ensure comparability between samples. The oleogels developed were characterised using oscillatory rheological measurements; the textural properties of the biscuit doughs were determined by Texture Profile Analysis (TPA); and the relationships between the variables were evaluated using Principal Component Analysis (PCA). Furthermore, the baked biscuits were evaluated using Principal Component Analysis (PCA) in terms of physicochemical and textural change parameters, such as water activity (aw) and moisture content, over a 14-day storage period. In the rheological analyses, it was determined that the storage modulus (G′) was higher than the loss modulus (G″) across the entire frequency range under investigation, thereby demonstrating that solid-like viscoelastic behaviour predominated in all structured systems. An increase in CW concentration systematically raised the values of G′, G″ and complex viscosity, leading to the formation of denser, mechanically stronger and more deformation-resistant lipid networks. In all samples, the decrease in complex viscosity with increasing angular frequency demonstrated the shear-thinning behaviour characteristic of structured lipid systems. TPA results revealed that all textural parameters examined were significantly influenced by the formulation. The CW10K05 sample stood out with the highest values for hardness, gumminess and chewability, whilst VOO-based samples exhibited the lowest structural strength. PCA results, the first two principal components explained 87.8 per cent of the total variance. PC1 explained 70.1 per cent of the total variance and was found to be primarily related to hardness, gumminess, chewability, cohesion and resilience, whilst PC2 explained 17.7 per cent of the variance and was mainly related to the flexibility parameter. During storage, water activity increased from a range of approximately 0.12–0.159 on day 1 to a range of 0.411–0.438 on day 14, indicating a significant gain in moisture. Changes in moisture content were also observed, depending on the formulation. Although the hardness of the biscuits generally decreased with storage, CW10K05 retained the highest hardness value on day 14. The brittleness values, which initially differed between formulations, became statistically similar by the end of storage. Whilst oleogels formulated with CW improve dough strength and product texture, the control of moisture transfer during storage is of critical importance for maintaining product quality.

Keywords: Oleogel, Olive oil, Trans fat, Carnauba wax, Biscuit

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Öz

Bu çalışma, geleneksel katı yağlara alternatif olarak zeytinyağı bazlı oleojellerin bisküvi formülasyonlarında kullanımının fizikokimyasal, reolojik ve tekstürel özellikler üzerindeki etkilerini incelemiştir. Bisküvi formülasyonlarında yağ oranı %24 olacak şekilde standardizasyon sağlanmış ve örnekler arasındaki karşılaştırılabilirliği korumak amacıyla tüm üretim koşulları sabit tutulmuştur. Geliştirilen oleojeller osilasyonlu reolojik ölçümlerle karakterize edilmiş, bisküvi hamurlarının tekstürel özellikleri Tekstür Profil Analizi (TPA) ile belirlenmiş ve değişkenler arasındaki ilişkiler Temel Bileşenler Analizi (PCA) kullanılarak değerlendirilmiştir. Ayrıca pişirilmiş bisküvilerin temel bileşenler analizi (PCA) ile 14 günlük depolama süresindeki su aktivitesi (aw) ve nem içeriği gibi fizikokimyasal ve tekstürel değişim parametreleri açısından değerlendirilmiştir. Reolojik analizlerde, incelenen frekans aralığının tamamında depolama modülünün (G′) kayıp modülünden (G″) yüksek olduğu belirlenmiş ve böylece tüm yapılandırılmış sistemlerde katı benzeri viskoelastik davranışın baskın olduğu ortaya konulmuştur. CW konsantrasyonundaki artış G′, G″ ve kompleks viskozite değerlerini sistematik olarak yükselterek daha yoğun, mekanik olarak daha güçlü ve deformasyona daha dirençli lipid ağlarının oluşmasını sağlamıştır. Tüm örneklerde açısal frekans arttıkça kompleks viskozitenin azalması, yapılandırılmış lipid sistemlerine özgü kayma incelmesi davranışını göstermiştir. TPA sonuçları, incelenen tüm tekstürel parametrelerin formülasyondan anlamlı düzeyde etkilendiğini ortaya koymuştur. CW10K05 örneği en yüksek sertlik, sakızımsılık ve çiğnenebilirlik değerleriyle öne çıkarken, VOO bazlı örnekler en düşük yapısal dayanımı göstermiştir. PCA sonuçlarında ilk iki temel bileşen toplam varyansın %87,8'ini açıklamıştır. PC1 toplam varyansın %70,1'ini açıklayarak başlıca sertlik, sakızımsılık, çiğnenebilirlik, kohezyon ve rezilyans ile ilişkili bulunurken, PC2 %17,7'lik varyansı açıklamış ve esas olarak esneklik parametresiyle ilişkili olmuştur. Depolama süresince su aktivitesi 1. günde yaklaşık 0,12–0,159 aralığından 14. günde 0,411–0,438 aralığına yükselerek belirgin nem kazanımını göstermiştir. Nem içeriğinde de formülasyona bağlı değişimler belirlenmiştir. Bisküvilerin sertliği depolama ile genel olarak azalmasına rağmen CW10K05, 14. günde en yüksek sertlik değerini korumuştur. Başlangıçta formülasyonlar arasında farklılık gösteren kırılganlık değerleri depolama sonunda istatistiksel olarak benzer hale gelmiştir. CW ile yapılandırılmış oleojeller hamur dayanımı ve ürün dokusunu iyileştirmekle birlikte, depolama sırasında nem transferinin kontrolü ürün kalitesinin korunması açısından kritik öneme sahiptir.

Anahtar Kelimeler: Oleojel, Zeytinyağı, Trans yağ, Karnauba mumu, Bisküvi

1. Introduction

In biscuit production, fat is one of the key ingredients that determines the product's textural properties, flavor, shelf life, and overall consumer acceptance. Traditional biscuit formulations typically use margarine or partially hydrogenated oils, which often contain considerable amounts of saturated and/or trans fatty acids depending on the processing method employed (Erenler et al., 2024). However, the association of excessive intake of trans and saturated fatty acids with cardiovascular disease (CVD), obesity, type II diabetes, and other metabolic disorders has raised significant concerns among both health authorities and consumers (Żarnowski et al., 2022; Roche, 2005). The World Health Organization (WHO) has established and continues to promote global strategies aimed at completely eliminating industrial trans fats from the food supply chain, and legal regulations are being implemented accordingly (WHO, 2018).

Fats and oil systems found in foods are largely composed of triglycerides, which contain monounsaturated, polyunsaturated, and saturated fatty acids, along with trace amounts of other components. In recent years, in response to increasing evidence on the health risks associated with trans fatty acids and excessive saturated fat consumption, various technologies and product reformulation methods have begun to be developed, aiming for extremely low trans and saturated fat content in foods (Berry et al., 2019). Particularly, the industrial use of partially hydrogenated fats has been restricted because of their contribution to trans fatty acid intake and related cardiometabolic risks (Brykczynski et al., 2022). Furthermore, excessive consumption of saturated fats has been shown to negatively impact cardiovascular health, leading food manufacturers to seek alternatives that limit the consumption of these types of fats (Piekarska et al., 2023).

While current practices generally favor natural saturated fats (e.g., PO) to reduce trans fats, this approach has raised both environmental (e.g., deforestation) and health concerns (Mattice and Marangoni, 2017; Holey et al., 2021). Moreover, excessive intake of saturated fats is associated with elevated plasma cholesterol levels and an increased risk of metabolic and cardiovascular disorders (Alizadeh et al., 2019; Pinto et al., 2021). Therefore, alternative structuring strategies are needed to minimize trans fatty acids and reduce reliance on highly saturated solid fats while maintaining the desirable technological properties of bakery products. In this context, oleogelation is presented as a current and developing method for converting liquid vegetable oils into a solid or semi-solid-like structure (Mattice and Marangoni, 2017; Alizadeh et al., 2019; Holey et al., 2021; Brykczynski et al., 2022; Noonim et al., 2022).

Through the formation of crystalline, fibrillar, or polymeric networks, oleogelators immobilize the liquid oil phase and provide solid-fat-like functionality, including consistency, spreadability, shape retention, and structural stability (Zhang et al., 2021). Oleogels have the potential to preserve the essential functions of solid oils, such as flavor, mouthfeel, and shelf life, while reducing traditional saturated fat or trans fat content (Patel et al., 2014; Piekarska et al., 2023). A wide range of oleogelators, including waxes (e.g., beeswax and carnauba wax), fatty acids, fatty alcohols, monoglycerides, and phytosterols, have been employed depending on the desired structural and functional properties of the system. It has been reported that the microstructure, rheological properties, and texture of the product are significantly altered as a result of the interaction of builders with each other or with other food ingredients (Brykczynski et al., 2022; Chen et al., 2022).

Among these structuring agents, carnauba wax (CW) is particularly attractive due to its high melting point, strong oil-binding capacity, and ability to form stable crystalline networks at relatively low concentrations. Similarly, virgin olive oil (VOO) is a nutritionally valuable lipid source rich in monounsaturated fatty acids and bioactive minor compounds; however, its liquid nature limits its direct application in solid-fat-based bakery systems. Therefore, structuring VOO using CW represents a promising technological strategy for incorporating healthier lipids into biscuit formulations without compromising processing performance or final product quality.

Furthermore, it has recently been determined that oleogels can be used not only as solid fat substitutes but also as functional food ingredients. It has been stated that they may have benefits such as transporting and increasing the stability of potentially beneficial ingredients (e.g., bioactive substances) that may be beneficial for health (Wang et al., 2021; Contreras-Ramirez et al., 2023). However, for large-scale industrial application, several factors (including cost, sensory attributes (flavor and texture), potential synergistic or antagonistic interactions, shelf life, and storage stability) must be thoroughly evaluated (Du et al., 2021; Contreras-Ramirez et al., 2022). In addition,

the incorporation of functional components such as thyme essential oil into oleogel systems may provide additional bioactive benefits; however, their effects on rheological behavior, dough structure, and storage stability remain insufficiently investigated. Although previous studies have explored olive oil-based oleogels and their applications in bakery products, limited information is available regarding the combined use of VOO, carnauba wax, and thyme essential oil in biscuit formulations, particularly in relation to their effects on rheological properties, dough texture, and storage-dependent physicochemical changes. This gap highlights the need for further systematic investigation.

This study aims to systematically evaluate the physicochemical and textural properties of oleogels produced from VOO (Virgin olive Oil) and carnauba wax (CW) derivatives used as solid fat substitutes in biscuit production. Specifically, the study investigates the rheological properties of the developed oleogels, the effects of formulation on dough textural characteristics, and the changes in water activity, moisture content, and texture of biscuits during storage. The results are expected to contribute to the existing body of knowledge by providing insights into the application of structured lipid systems in bakery products and supporting the development of healthier formulations for industrial use.

2. Materials and Methods

2.1. Materials

Carnauba wax (CW) was used as the oleogel agent, Virgin Olive Oil (VOO) was used as the liquid oil phase. All raw materials were food-grade, and supplier/brand information should be specified to ensure reproducibility. Due to its high melting point and oxidative stability, CW is widely used in oleogelation systems (Hwang et al., 2016; Doan et al., 2017). In biscuit production, wheat flour, granulated sugar, eggs, baking powder, salt, vanillin, and lecithin were used. All ingredients were used in standardized formulations and expressed on a weight basis (% w/w) for consistency across samples.

2.2. Oleogel preparation

Oleogels were prepared by adding CW to VOO base at 5%, and 10% (w/w). ratios. Additionally, thyme essential oil was incorporated at 0.5%, and 1.5% (w/w) in selected formulations to evaluate its functional contribution. The mixtures were heated to 90 °C with continuous stirring until complete melting of CW was achieved. The molten mixtures were cooled under controlled conditions (20 ± 3 °C) to initiate gel formation. Subsequently, samples were stored at 4 °C for 24 h to complete crystallization, and then equilibrated to room temperature prior to analysis (Jang et al., 2015; Patel et al., 2014). The addition of thyme essential oil was performed after complete melting of the wax phase to prevent volatilization losses and ensure homogeneous dispersion.

2.3. Biscuit production

The biscuit dough was prepared using a standardized formulation expressed on a weight basis (% w/w) to ensure reproducibility. Formulations were not reported based on batch size but were normalized as percentage composition; the formulation consisted of fat (24%), lecithin (1%), water (9%), powdered sugar (17%), flour (48%), and baking powder (1%).

During dough preparation, the fat phase and lecithin were mixed at high speed for 2 min, followed by the addition of powdered sugar and water, and further mixing for 5 min to achieve homogenization. Finally, flour and baking powder were incorporated, and the mixture was kneaded at medium speed for 1 min to obtain a uniform dough. All processing steps and mixing conditions were kept constant across all samples to minimize variability and ensure comparability.

The dough was sheeted to a uniform thickness (4 mm), cut into standardized shapes, and baked in a preheated oven under controlled conditions. Biscuit formulations were prepared as a control group using VOO) and an experimental group using oleogels. The fat phase was maintained at 24% (w/w) of the total formulation for all samples to ensure consistency. Dough kneading, shaping, and baking were performed according to a standardized biscuit production protocol at 180 °C for 12 min (Mert and Demirkesen, 2016; Pang et al., 2023). After baking, samples were cooled at room temperature and stored under controlled conditions prior to analysis.

2.4. Rheological analyses

The rheological properties of the oleogels were determined using an Anton Paar MCR302 (Graz, Austria) rheometer in a 50 mm parallel-plate geometry. Samples were conditioned at 25 °C for 30 minutes before analysis. Prior to frequency sweep measurements, amplitude sweep tests (0.01–100% strain) were conducted to determine the linear viscoelastic region (LVR) of the samples. Frequency sweep tests were then performed within the LVR at a constant strain of 0.5%. Frequency sweep testing was carried out over an angular frequency range of 0.1–100 rad/s. The storage modulus (G′) and loss modulus (G″) were used to characterize the viscoelastic behavior of the oleogels, in accordance with standard rheological terminology rather than using general "elastic" and "viscous" descriptors (Brykczynski et al., 2022; Patel and Dewettinck, 2015). All measurements were performed in triplicate to ensure reproducibility.

2.5. Physicochemical tests and texture profile analyses

Physicochemical analyses included moisture determination, water activity (aw), color measurement, and texture analysis. These parameters were selected as key indicators of quality and stability in bakery products containing oleogel-based fat systems (Pang et al., 2023; Yılmaz et al., 2021). Moisture content was determined according to the AOAC 925.10 method, using a 105°C oven drying method until constant weight was achieved. Water activity (aw) was determined using a HygroLab 3 (Rotronic, Switzerland) device. Texture Profile Analysis (TPA) was conducted using a TA.XT2i Texture Analyzer under defined test conditions (probe type, compression ratio, and test speed should be specified). Parameters including hardness, cohesiveness, springiness, gumminess, and chewiness were calculated from the force–time curves. Breaking strength was not determined using TPA; instead, fracture-related properties (e.g., fracturability) were evaluated based on appropriate compression or bending parameters derived from the force–deformation curves. All measurements were performed in triplicate (n=3), and results were expressed as mean ± standard deviation.

2.6. Statistical and chemometric analysis

All biscuit sample data were analyzed using one-way ANOVA using SPSS 25.0 (IBM Corp., USA). Post hoc comparisons were performed using Tukey's multiple comparison test ($p < 0.05$), which provides better control of Type I error compared to the LSD test. All measurements were conducted in triplicate (n = 3), and results were expressed as mean ± standard deviation. Principal Component Analysis (PCA) was applied to the complete dataset rather than individually to each oleogel sample in order to evaluate the overall relationships among samples and variables. The analysis was based on the correlation matrix of the selected variables, and the first two principal components were retained for interpretation. Biplots (PC1 vs. PC2) were constructed to visualize the relationships between samples and their associated variables and to summarize the combined effects of oleogelator composition on TPA parameters. Multivariate chemometric analyses (PCA and biplot) were performed using JMP 11 (SAS Institute Inc., USA). Ward's hierarchical clustering was optionally applied to support pattern recognition among samples. Given the limited number of samples, PCA was used as an exploratory tool rather than for definitive classification, and results were interpreted cautiously. Pearson's correlation coefficients (r) were calculated to evaluate the relationships between major variables. Statistical significance was considered at $p < 0.05$.

3. Results and Discussion

The effects of using oleogels containing VOO and varying amounts of CW in biscuit production on rheological and physicochemical properties were evaluated, with emphasis on structural behavior and its potential implications for dough and product performance.

3.1. Rheological properties

The viscoelastic behavior of the oleogels was characterized using the storage modulus (G′) and loss modulus (G″), obtained from frequency sweep measurements (*Figure 1*).

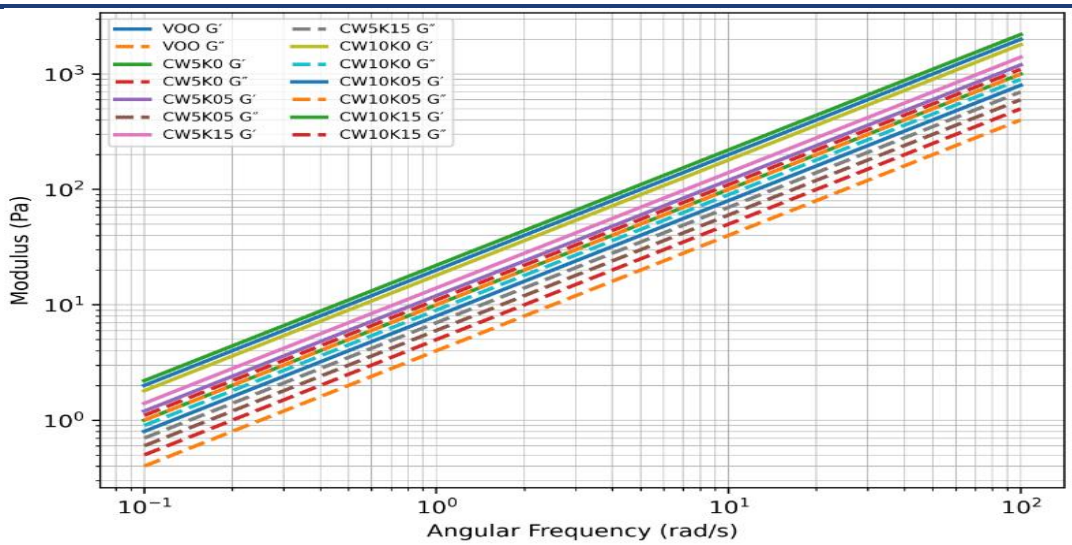


Figure 1. Frequency sweep of oleogel samples

According to *Figure 1*, the storage modulus (G′) values of all samples were consistently higher than the loss modulus (G″) values across the entire frequency range, indicating that all oleogel systems exhibited a dominant elastic (solid-like) behavior. The VOO-based oleogel exhibited the lowest G′ values among all samples, reflecting its comparatively weaker network structure due to the absence of a crystalline structuring agent. With increasing CW concentration, both G′ and G″ values increased in a systematic manner, indicating the progressive development of a stronger and more interconnected three-dimensional network structure. The relatively parallel trends observed among the samples suggest that CW concentration primarily affects the magnitude of the viscoelastic response rather than altering the fundamental rheological behavior of the system. Both G′ and G″ increased with increasing angular frequency, which is characteristic of structured lipid systems and reflects enhanced resistance to deformation under dynamic conditions rather than a direct increase in "hardness." Although these rheological results demonstrate improved structural integrity with increasing CW content, direct implications for biscuit processing performance (e.g., dough stability or final product texture) should be interpreted cautiously and supported by complementary physicochemical and textural analyses.

The complex viscosity (η*) of oleogels containing VOO and varying concentrations of CW was evaluated as a function of angular frequency using oscillatory rheological measurements and is presented in *Figure 2*.

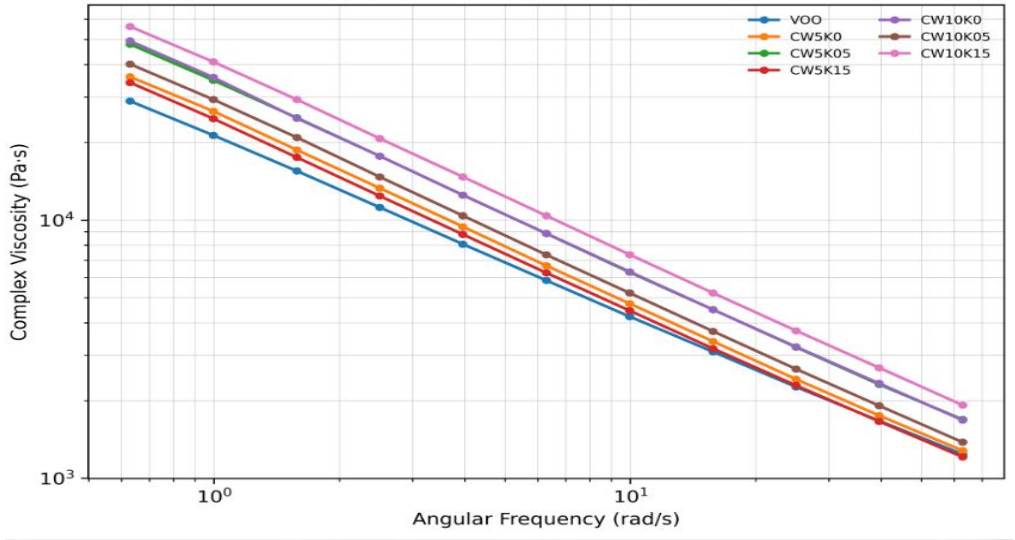


Figure 2. Complex viscosity (η*) of oleogel samples

Figure 2 shows the variation of complex viscosity (η*) as a function of angular frequency for all oleogel samples. All samples exhibited a clear decrease in complex viscosity with increasing angular frequency, indicating typical shear-thinning behavior of structured lipid systems. This behavior reflects the progressive alignment and partial disruption of the internal network structure under increasing deformation conditions. The reduction in viscosity at higher frequencies suggests a decrease in resistance to flow as the applied deformation increases. The VOO-based oleogel showed lower values, indicating a comparatively weaker network structure due to the absence of a crystalline structuring agent. Increasing the CW content (CW5K0, CW5K05, CW5K15, CW10K0, CW10K05, CW10K15) resulted in higher complex viscosity values across the entire frequency range, indicating enhanced network density and improved resistance to flow. Furthermore, the nearly parallel trends observed among the samples suggest that CW concentration primarily influences the magnitude of viscosity without significantly altering the fundamental shear-thinning behavior of the system.

3.2. Textural properties

The TPA results of the dough produced using VOO and carnauba wax (CW) derivatives at different ratios are presented in *Table 1*.

Table 1. TPA properties of of biscuit dough samples

Category	VOO	CW5K0	CW5K05	CW5K15	CW10K0	CW10K05	CW10K15	p
Hardness (g)	10.74^{g}	15.49^{f}	16.78^{e}	14.14^{f}	17.65^{d}	22.47^{a}	18.73^{c}	**0.000**
Adhesiveness (g/s)	-14.99^{g}	-29.44^{c}	-27.26^{d}	-28.94^{c}	-29.45^{c}	-37.70^{a}	-31.58^{b}	**0.000**
Springiness*	0.998^{b}	0.998^{b}	0.998^{b}	0.997^{b}	1.117^{a}	1.013^{b}	0.998^{b}	**0.000**
Cohesiveness*	0.414^{f}	0.480^{b}	0.474^{c}	0.457^{d}	0.471^{c}	0.493^{a}	0.456^{d}	**0.000**
Gumminess (g)	3.60^{e}	2.95^{e}	7.94^{c}	6.47^{d}	8.32^{b}	11.08^{a}	8.54^{b}	**0.000**
Chewiness (g)	3.60^{e}	2.95^{e}	7.94^{c}	6.45^{d}	9.29^{b}	11.22^{a}	8.52^{b}	**0.000**
Resilience*	0.033^{f}	0.037^{e}	0.042^{d}	0.033^{f}	0.054^{c}	0.061^{a}	0.049^{b}	**0.000**

*Springiness, cohesiveness, and resilience are dimensionless parameters expressed as ratios; therefore, they were evaluated within the 0–1 range.
** Formulations: VOO: Virgin olive oil sample; CW5K0: oleogel containing 5% carnauba wax; CW5K05: oleogel containing 5% carnauba wax and 0.5% thyme essential oil; CW5K15: oleogel containing 5% carnauba wax and 1.5% thyme essential oil; CW10K0: oleogel containing 10% carnauba wax; CW10K05: oleogel containing 10% carnauba wax and 0.5% thyme essential oil; CW10K15: oleogel containing 10% carnauba wax and 1.5% thyme essential oil.

Table 1 indicates that all TPA parameters were significantly influenced by formulation ($p < 0.001$). Hardness increased notably with the incorporation of carnauba wax, reaching its highest value in CW10K05 (22.47 g) and lowest in the VOO sample (10.74 g), demonstrating that higher wax concentrations enhance the structural rigidity of the dough matrix. A similar trend was observed for adhesiveness, where CW10K05 exhibited the most negative value (-37.70 g·s), indicating increased stickiness, while VOO showed the lowest adhesiveness. In contrast, springiness remained relatively constant across most formulations (~0.998), with only CW10K0 showing a significant increase (1.117), suggesting that this parameter is less sensitive to compositional changes. Cohesiveness increased with wax addition, with the highest value again observed in CW10K05 (0.493), reflecting improved internal bonding within the dough structure. Likewise, gumminess and chewiness values followed parallel trends, reaching maximum levels in CW10K05 (11.08 g and 11.22 g, respectively), indicating increased resistance to deformation and higher energy requirements during mastication. Resilience values ranged from 0.033 to 0.061, with higher values observed in samples containing greater amounts of carnauba wax, suggesting improved elastic recovery behavior. Overall, these findings demonstrate that both the presence and concentration of carnauba wax play a critical role in enhancing the mechanical and structural properties of biscuit dough, with the CW10K05 formulation consistently exhibiting the most pronounced effects.

3.3. Chemometric analysis on textural properties of dough samples

In this study, biscuit dough samples obtained from oleogels prepared with different formulations were classified and characterized using a chemometric approach, namely Principal Component Analysis (PCA), based on their oleogel composition and thyme essential oil levels. Selected Texture Profile Analysis (TPA) parameters were used as input variables for multivariate analysis. The resulting data matrix, consisting of dough samples (n = 8) and their corresponding textural attributes, was subjected to PCA to identify the main sources of variation. The

first two principal components (PCs) explained 70.1% (PC1) and 17.7% (PC2) of the total variance, respectively, accounting for 87.8% of the overall variability. This indicates that the majority of the variation in the textural properties of biscuit dough samples can be effectively represented in a two-dimensional PCA space (*Figure 3*). The PCA score and loading plots revealed a clear separation of samples according to formulation, demonstrating the discriminative power of TPA parameters.

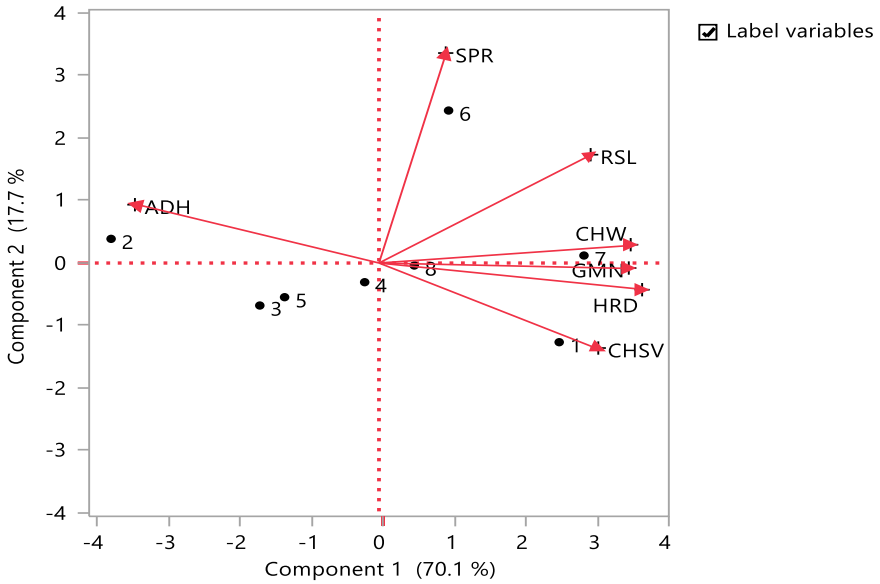


Figure 3. Principal Component Analysis (PCA) biplot scores

The PCA biplot (*Figure 3*) illustrates the relationships between samples and textural variables. Cohesiveness (CHSV) showed strong positive loadings along the lower right quadrant, contributing to the characterization of samples located in this region, whereas adhesiveness (ADV) was associated with the opposite direction, indicating its role in distinguishing softer and more adhesive samples such as VOO. The CW10K0 sample was mainly associated with resilience (RSL), suggesting improved elastic recovery, while CW10K05 was strongly correlated with gumminess (GMN) and chewiness (CHW), indicating a firmer and more resistant structure.

PC1 was primarily associated with hardness (HRD), gumminess (GMN), chewiness (CHW), cohesiveness (CHSV), and resilience (RSL), all of which exhibited high positive loadings, reflecting their contribution to structural strength and mechanical resistance. Samples positioned on the positive side of PC1 were characterized by higher hardness, gumminess, and chewiness values, indicating a more compact and mechanically stable structure. PC2, which explained 17.7% of the variance, was mainly influenced by springiness (SPR), representing elastic behavior. Samples located on the positive side of PC2 exhibited higher springiness values, whereas those on the negative side of PC1 were associated with adhesiveness, reflecting a softer and more deformable texture. The PCA results confirm that formulation variables, particularly carnauba wax concentration and thyme essential oil addition, play a key role in determining the textural differentiation of biscuit dough samples.

3.4. Water activity, moisture, hardness and fracturability analysis of biscuit samples

Water activity (aw) values of biscuit samples are presented in *Table 2*. A consistent increase in aw was observed in all formulations during storage. Initial values ranged between 0.12 and 0.159 on Day 1 and increased to 0.411–0.438 by Day 14. This increase indicates moisture uptake during storage, which may influence both microbial stability and physicochemical changes in the product. Notably, formulations such as CW10K0, CW10K05, and CW10K15 exhibited aw values above 0.42 at Day 14, suggesting a higher susceptibility to quality deterioration during prolonged storage.

Moisture content results (*Table 3*) showed that formulation significantly affected moisture levels on Day 1 ($p < 0.001$). Samples containing 5% carnauba wax (CW5K0, CW5K15) and VOO exhibited higher moisture values, whereas CW10-based formulations (CW10K0 and CW10K05) showed lower initial moisture content. After 14 days of storage, moisture content increased in all samples; however, the extent of this increase varied depending

on formulation ($p < 0.05$). The CW10K0 formulation exhibited the highest moisture content at Day 14, while VOO and CW5K15 samples remained in the lowest statistical group. These results indicate that both formulation and storage duration significantly influence moisture dynamics in biscuit systems.

Table 2. Water activity values of biscuits samples during day 1 and day 14 storage

Formulation	Day 1	Day 14
VOO	0.159	0.438
CW5K0	0.138	0.431
CW5K05	0.135	0.411
CW5K15	0.12	0.433
CW10K0	0.14	0.437
CW10K05	0.13	0.422
CW10K15	0.121	0.421

*Formulations: VOO: Virgin olive oil sample; CW5K0: oleogel containing 5% carnauba wax; CW5K05: oleogel containing 5% carnauba wax and 0.5% thyme essential oil; CW5K15: oleogel containing 5% carnauba wax and 1.5% thyme essential oil; CW10K0: oleogel containing 10% carnauba wax; CW10K05: oleogel containing 10% carnauba wax and 0.5% thyme essential oil; CW10K15: oleogel containing 10% carnauba wax and 1.5% thyme essential oil.

Table 3. Moisture levels of the biscuit samples during day 1 and day 14 storage

Formulation	Day 1 (Mean±SD)	Day 14 (Mean±SD)	*p*
VOO	5.44 ± 0.03^{a}	5.02 ± 0.56^{e}	0.5865
CW5K0	5.51 ± 0.16^{a}	5.82 ± 0.58^{bcd}	0.6811
CW5K05	4.94 ± 0.24^{b}	5.97 ± 0.19^{abc}	0.0838
CW5K15	5.46 ± 0.29^{a}	5.01 ± 1.00^{e}	0.7291
CW10K0	4.40 ± 0.34^{cd}	6.50 ± 0.39^{a}	0.0575
CW10K05	4.34 ± 0.17^{d}	6.26 ± 0.41^{ab}	0.0964
CW10K15	4.69 ± 0.30^{bc}	5.48 ± 0.16^{cde}	0.1837

* Group means indicated by different letters in the same column are statistically different from each other ($p < 0.05$).
** Formulations: VOO: Virgin olive oil sample; CW5K0: oleogel containing 5% carnauba wax; CW5K05: oleogel containing 5% carnauba wax and 0.5% thyme essential oil; CW5K15: oleogel containing 5% carnauba wax and 1.5% thyme essential oil; CW10K0: oleogel containing 10% carnauba wax; CW10K05: oleogel containing 10% carnauba wax and 0.5% thyme essential oil; CW10K15: oleogel containing 10% carnauba wax and 1.5% thyme essential oil.

Hardness results (*Table 4*) demonstrated that formulation had a significant effect on texture at both Day 1 and Day 14 ($p < 0.01$). At Day 1, CW10K05 and CW10K0 exhibited the highest hardness values, indicating increased structural rigidity with higher wax content. In contrast, VOO and CW5K15 samples showed lower hardness values. After storage, hardness values decreased in most formulations; however, CW10K05 maintained the highest hardness at Day 14, indicating improved structural stability over time. The observed reduction in hardness during storage may be attributed to moisture migration and softening of the matrix.

Table 4. Hardness levels of the biscuit samples during day 1 and day 14 storage

Formulation	Day 1 (Mean±SD)	Day 14 (Mean±SD)	*p*
VOO	362.97 ± 79.14^{b}	177.74 ± 59.37^{bcd}	0.015
CW5K0	402.10 ± 53.63^{ab}	117.84 ± 48.13^{de}	0.001
CW5K05	390.13 ± 75.69^{ab}	126.16 ± 31.35^{cde}	0.004
CW5K15	364.33 ± 100.02^{b}	229.52 ± 24.05^{ab}	0.020
CW10K0	454.37 ± 90.32^{a}	106.66 ± 50.34^{e}	0.003
CW10K05	463.22±126.08a	288.70 ± 11.93^{a}	0.001
CW10K15	403.72 ± 64.74^{ab}	190.04 ± 49.58^{bc}	0.006

* Group means indicated by different letters in the same column are statistically different from each other ($p < 0.05$).
** Formulations: VOO: Virgin olive oil sample; CW5K0: oleogel containing 5% carnauba wax; CW5K05: oleogel containing 5% carnauba wax and 0.5% thyme essential oil; CW5K15: oleogel containing 5% carnauba wax and 1.5% thyme essential oil; CW10K0: oleogel containing 10% carnauba wax; CW10K05: oleogel containing 10% carnauba wax and 0.5% thyme essential oil; CW10K15: oleogel containing 10% carnauba wax and 1.5% thyme essential oil.

Fracturability results (*Table 5*) showed significant differences among formulations at Day 1 ($p < 0.01$), with CW10K05 exhibiting the highest values, followed by CW10K0 and CW10K15. VOO samples showed the lowest fracturability, reflecting a weaker and less brittle structure. However, after 14 days of storage, no significant differences were observed among formulations ($p > 0.05$), indicating a convergence of fracturability values over time. This suggests that storage time has a dominant effect on fracturability, reducing formulation-based differences.

Table 5. Fracturability levels of the biscuit samples during day 1 and day 14 storage

Formulation	Day 1 (Mean±SD)	Day 14 (Mean±SD)	*p*
VOO	0.35 ± 0.04^{c}	1.52 ± 0.90^{a}	0.1542
CW5K0	0.39 ± 0.07^{bc}	1.30 ± 0.24^{a}	0.0181
CW5K05	0.41 ± 0.06^{abc}	1.03 ± 0.18^{a}	0.0207
CW5K15	0.40 ± 0.09^{abc}	1.09 ± 0.25^{a}	0.0341
CW10K0	0.45 ± 0.03^{ab}	1.23 ± 0.05^{a}	0.0142
CW10K05	0.47 ± 0.08^{a}	1.50 ± 0.87^{a}	0.1754
CW10K15	0.44 ± 0.09^{ab}	1.31 ± 0.56^{a}	0.0514

* Group means indicated by different letters in the same column are statistically different from each other ($p < 0.05$).
**Formulations: VOO: Virgin olive oil sample; CW5K0: oleogel structured with 5% carnauba wax (vontrol); CW5K05: oleogel structured with 5% carnauba wax and containing 0.5% thyme essential oil; CW5K15: oleogel structured with 5% carnauba wax and containing 1.5% thyme essential oil; CW10K0: oleogel structured with 10% carnauba wax (control); CW10K05: oleogel structured with 10% carnauba wax and containing 0.5% thyme essential oil; CW10K15: oleogel structured with 10% carnauba wax and containing 1.5% thyme essential oil.

The results demonstrate that both formulation and storage time significantly influence the physicochemical and textural properties of biscuit samples. While formulation plays a key role in determining initial properties, storage conditions lead to moisture uptake and structural changes that reduce differences between formulations, particularly in parameters such as fracturability.

3.5. Discussion

The study demonstrated that oleogel formulation, particularly carnauba wax (CW) concentration and thyme essential oil incorporation, plays a critical role in determining the rheological, textural, and storage-related properties of biscuit systems. All oleogel samples exhibited dominant elastic behavior ($G' > G''$), confirming the formation of structured lipid networks. Increasing CW concentration resulted in a systematic increase in both storage and loss moduli, indicating the development of a more interconnected and mechanically stable network structure. This finding is consistent with previous studies reporting that wax-based oleogelators enhance network strength through crystalline structuring mechanisms (Doan et al., 2017; Yılmaz et al., 2021).

Textural analysis further supported these rheological observations. Formulations containing higher CW levels, particularly CW10K05, exhibited significantly higher hardness, gumminess, and chewiness values, indicating increased resistance to deformation and improved structural integrity. In contrast, VOO-based systems showed lower hardness values, which can be attributed to the absence of a strong crystalline network. Similar trends have been reported for wax-based oleogels, where increasing structurant concentration enhances mechanical strength even at relatively low levels (Pang et al., 2023; Li et al., 2022).

PCA results provided additional insight into the relationship between formulation and textural behavior. Samples containing higher CW levels were clearly separated along the positive PC1 axis, which was associated with hardness, gumminess, chewiness, and cohesiveness. This confirms that CW concentration is a key determinant of textural differentiation. In contrast, VOO-based samples were associated with higher adhesiveness and lower structural strength, reflecting a softer and more deformable matrix. These findings demonstrate the usefulness of chemometric approaches in capturing complex relationships between formulation variables and mechanical properties.

During storage, significant changes were observed in both physicochemical and textural properties. Water activity increased in all formulations, indicating moisture uptake from the environment. Despite the hydrophobic nature of oleogels, the presence of hydrophilic components such as flour and sugar likely facilitated water absorption. Moisture content also increased over time, with formulation-dependent differences becoming more

pronounced. These results suggest that while oleogel networks may partially limit moisture migration, they do not act as complete barriers. Similar findings have been reported in bakery systems, where moisture redistribution significantly influences product quality and shelf life (Romani et al., 2016; Silva et al., 2023; Demirkesen and Mert, 2020).

Textural changes during storage were characterized by a general decrease in hardness across most formulations, which can be attributed to moisture migration and plasticization effects within the matrix. Although formulation-dependent differences in hardness remained evident at Day 14, fracturability values converged over time, indicating that storage conditions reduce structural differences between samples. This behavior suggests that storage-induced structural relaxation and moisture equilibration play a dominant role in determining long-term texture. Similar softening effects have been observed in oleogel-based bakery products, where interactions between starch, lipids, and water lead to progressive structural modification (Hwang et al., 2016; Wettlaufer and Flöter, 2022).

4. Conclusions

The study demonstrated that carnauba wax-based oleogels are effective structuring systems for improving the mechanical and rheological properties of biscuit dough. Increasing CW concentration enhanced viscoelastic behavior (G′ and G″), resulting in stronger and more stable network structures. Among the tested formulations, CW10K05 consistently exhibited superior performance in terms of hardness, gumminess, chewiness, and resilience, indicating its potential as an optimized formulation for improving dough strength and product quality. However, storage conditions significantly influenced product stability. Water activity and moisture content increased in all samples during storage, leading to a general reduction in hardness and convergence of fracturability values. These findings indicate that although oleogels improve initial structural properties, they do not fully prevent moisture migration, which remains a critical factor affecting shelf life. In conclusion, oleogel systems based on carnauba wax and olive oil offer a promising alternative to conventional solid fats in bakery applications, enabling the development of structurally stable and potentially healthier products. Nevertheless, future studies should focus on improving moisture barrier properties, optimizing formulation–storage interactions, and evaluating long-term stability under industrial conditions.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

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