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# Evaluation of Physicochemical Properties and Oxidative Stability of Oleogel Based on Rice Bran Oil and Beeswax

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ABSTRACT: Due to the health risks associated with saturated and Trans fats, the food industry is seeking healthier fat alternatives. This study developed oleogels using rice bran oil (RBO) and beeswax (BW) at concentrations of 4%, 7.5%, and 10% (w/w), evaluating their physicochemical and functional properties. Key tests included slip melting point, colorimetry, peroxide value, thiobarbituric acid value, oil binding capacity, and oxidative stability via Rancimat assay. The results showed that higher BW concentrations significantly improved oleogel performance. The 10% BW sample had the highest slip melting point (54.7°C), lowest peroxide value (2.1 meq/kg), longest induction time (6.9 hours), and highest oil binding capacity (92%). Color analysis indicated increased lightness (L*) with more BW. These enhancements are attributed to the formation of a dense crystalline network and antioxidant compounds in beeswax. Overall, the 10% BW oleogel exhibited superior oxidative stability and physicochemical characteristics, making it a promising substitute for solid fats in low-fat food formulations.

**Keywords**: Beeswax, Oleogel, Physicochemical Properties, Rice Bran Oil (RBO).

## Introduction

Animal fats and hydrogenated vegetable oils (HVOs) are widely used in the food industry; however, epidemiological studies have shown a strong correlation between the consumption of these fats and increased risks of cardiovascular diseases, elevated levels of low-density lipoprotein (LDL), and obesity (Cabrera *et al*., 2020). Consequently, global health organizations such as the WHO and FDA have imposed strict regulations on the use of Trans and saturated fats, emphasizing the urgent need for healthier alternatives. In the past, liquid vegetable oils such as olive, canola, and sunflower oils were recommended as substitutes for solid fats due to their high

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content of unsaturated fatty acids and positive health effects (Luukkonen *et al*., 2018). However, their liquid nature limits their functionality in many food applications such as baked goods, chocolates, and meat products, where they fail to replicate the performance of confectionery fats, hydrogenated oils, or shortenings. This leads to issues such as oil migration, reduced oxidative stability, and diminished sensory quality (Puscas *et al*., 2020).These limitations have directed researchers toward the development of structured systems like oleogels. Oleogels are semi-solid systems formed by stabilizing liquid oils within a three-dimensional network created by gelators (Zhao *et al*., 2022). These structures retain the nutritional benefits of unsaturated oils while mimicking the physical properties of semi-solid fats, such as plasticity and stability, thereby fulfilling their functional roles in food products. In oleogels, a small amount of one or more gelator molecules transforms the liquid oil into a structured three-dimensional network. Unlike the polymers used in hydrogels, oleogels require small amphiphilic molecules that self-assemble through specific non-covalent interactions and trap the oil within their structure via capillary forces (Singh *et al*., 2019). The mechanism of oleogel formation is based on the self-assembly of gelator molecules in the oil phase, forming a stable network through non-covalent interactions such as hydrogen bonding and van der Waals forces (Davidovich-Pinhas *et al*., 2015). In addition to reducing the intake of saturated and Trans fats, oleogels can carry lipophilic bioactive compounds (e.g., vitamins E and D) and enhance the oxidative stability of oils, especially at high temperatures (Mert & Demirkesen, 2016). Today, oleogels are widely used as healthy alternatives to saturated and Trans fats in the food industry. Studies have shown that in meat products such as sausages and salami (Da Silva *et al*., 2019), oleogels based on vegetable oils can reduce animal fat content by up to 50% while improving oxidative stability and maintaining desirable sensory attributes like juiciness and flavor (Martins *et al*., 2016). In low-fat ice creams, oleogels made with rice bran wax have been shown to create a creamy and stable texture with rheological properties similar to milk, resulting in higher sensory acceptance (Ning *et al*., 2024). This study investigates the quality of oleogels containing rice bran oil (RBO) and beeswax. The use of RBO in oleogel formulation is beneficial due to its bioactive compounds such as gamma-oryzanol, tocopherols, and tocotrienols, which enhance the nutritional and health-promoting properties of the oleogel. RBO also exhibits high oxidative stability and, when combined with suitable structuring agents, forms a stable gel with desirable rheological characteristics. Moreover, utilizing RBO a byproduct of rice processing, especially in Gilan Province—promotes environmental and economic sustainability and has broad applications in food production. Beeswax, on the other hand, offers strong structuring capabilities, resulting in stable gels with favorable rheological behavior. The synergy between these two components not only improves oxidative stability and nutritional quality of the final product but also adds value to agricultural waste processing. Therefore, the combined use of RBO and beeswax in oleogel preparation represents a strategic and essential approach for developing functional and eco-friendly formulations in the food industry. This study explores the feasibility of producing oleogels from RBO using beeswax as a gelator, aiming to reduce unhealthy fat consumption and promote the development of healthier food products.

## Materials and Methods

- ***Raw Material Preparation***

Rice bran oil was purchased in 1-liter bottles with the latest expiration date from Vinek Adbil Company, India. Beeswax was obtained from Kahlwax Company, Germany.

- ***Oleogel Preparation***

Oleogels were prepared using a modified version of the method described by Zetzl *et al*. (2012), by heating rice bran oil (RBO) and beeswax (BW), which has a melting point of 62–65°C, at 80°C. After initial stirring for 2 minutes on a cold magnetic stirrer, the mixture was transferred to a hot magnetic stirrer at 155°C and stirred at 300 rpm until complete transparency was achieved. Three concentrations of 4%, 7.5%, and 10% (w/w) were prepared and stored in sealed brown glass containers until testing.

- ***Evaluated Tests***
- ***Slip Melting Point (SMP)***

SMP was determined using the AOCS Cc 3-25 method with open capillary tubes. Samples were heated to 10°C above their melting point, placed in capillary tubes, and rapidly cooled in an ice-water bath. After 16 hours at 6°C, the tubes were attached to a thermometer and placed in 4°C water. The temperature at which the first upward movement of the sample was observed was recorded as the SMP.

- ***Colorimetry***

Color was measured using a HunterLab ColorFlex VA colorimeter (Model 45, USA). The color indices L*, a*, b*, and yellowness index were evaluated. L* indicates brightness (0–100), a* ranges from green to red, and b* from blue to yellow (−120 to +120) (Lothrop, 2012).

- ***Oxidative Satability Test***

Oxidative stability (induction period) was measured using a Metrohm Rancimat device (Herisau, Switzerland) according to AOCS Cd 12b-92 at 110°C.

- ***Oil Binding Capacity***

In order to determine the percentage of migrated oil, an Eppendorf tube was first weighed. Then, 1 g of melted oleogel was added and kept at room temperature for 24 hours. The tube was reweighed, centrifuged at 9000 rpm for 15 minutes, and placed on filter paper for 3 minutes to remove excess oil. Finally, the tube was weighed again, and the percentage of migrated oil was calculated using the following formula (Yılmaz & Öğütcü, 2014):

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

W1: Weight of added oleogel (1 g)

W2: Weight of Eppendorf + oleogel after 24 hours (before centrifugation)

W3: Weight of Eppendorf + oleogel after centrifugation and oil removal

- ***Peroxide Value***

Peroxide value was measured using the Iodometric method according to Iranian National Standard No. 4179.

- ***Thiobarbituric Acid Value***

Thiobarbituric acid value was determined according to Iranian National Standard No. 10494 using a UV-visible spectrophotometer (Varian, Cary 100, Netherlands).

- ***Statistical Analysis***

Data were analyzed using a completely randomized design with three replications. Duncan's multiple range test was used for mean comparison at a significance level of 0.05. Graphs were generated using SPSS software version 19.

## Results and Discussion

Analysis of variance revealed that increasing the concentration of beeswax had a significant effect on lightness (L**), the b** index (yellow–blue), and yellowness index (YL), while changes in the a* index (green–red) were not statistically significant. Mean comparison showed that among the treatments, the oleogel containing 10% beeswax exhibited the highest values for b* (40.2) and YL (0.57), while its lightness (L*) was the lowest (43.20) compared to other treatments (Tab 1). Significant differences in the color parameters of the oleogels suggest that factors such as environmental pH, presence of esters, hydrocarbons, reducing sugars, amino acid compounds, dry matter content, temperature, and natural pigments in the gelators can influence the product's color characteristics (Buitimea-Cantúa *et al.*, 2021). The yellowness observed in beeswax-containing treatments is likely due to the natural yellow to golden color of beeswax, which contains pigments such as carotenoids and flavonoids. At higher concentrations, these pigments are more incorporated into the gel matrix, increasing the product's yellowness (Moghtadaei *et al.*, 2018). A study by Abdolmaleki *et al.* (2021) also showed that increasing beeswax substitution led to higher yellowness index values, with the control sample showing the lowest yellowness—consistent with the findings of the present study. Regarding the differences in lightness among treatments, Baratian *et al.* (2022) attributed the reduction in lightness in oleogels with increasing beeswax concentration (1–10%) to the formation of denser, larger, and more stable crystalline networks, which enhance light reflection and reduce transparency (L*). This sudden decrease in light transmission and increase in light absorption leads to higher shear viscosity and greater resistance to flow. This phenomenon is likely due to the presence of ester compounds, saturated fatty acids, and long-chain hydrocarbons in beeswax, which form large and orderly crystals, increasing turbidity. Furthermore, XRD patterns showed that in beeswax-containing oleogels, most crystals formed predominantly in the β′ polymorphic form with orthorhombic arrangement, and a very small amount in the β polymorph with triclinic structure. Crystallization in the β′ form creates a dense network of crystals. As beeswax concentration increases, the intensity and number of crystals also increase, resulting in greater light scattering. Therefore, the increased formation of β′ polymorphic crystals in oleogels directly contributes to reduced transparency and lightness, producing a more opaque appearance (Zhang *et al.*, 2026).

Analysis of variance showed a significant difference ($P < 0.05$) in slip melting point (SMP) among the treatments. The control sample exhibited the lowest melting point (30°C), while increasing the beeswax concentration from 4% to 10% raised the melting point from 33°C to 56°C a substantial increase (Tab 1).This rise in melting point in samples with higher beeswax content can be attributed to factors such as the increased proportion of saturated fatty acids, the formation of stronger crystalline networks by the wax, and the influence of the chemical structure of mono acyl glycerols (Saberi *et al.*, 2012). These findings are consistent with the results reported by Qu *et al.* (2024) and Naderi *et al.* (2022), who also observed higher melting points at increased wax concentrations. The chemical composition of beeswax containing approximately 70% wax esters, hydrocarbons, free fatty acids, and small amounts of long-chain saturated alcohols along with their interactions with the oil phase, significantly affects melting behavior, crystallization development, and ultimately the melting point of the oleogel (Martins *et al*., 2016). Similarly, Hwang *et al*. (2024) demonstrated that increasing beeswax concentration up to 12% led to the formation of denser crystals and a significant rise in melting point. Beeswax, due to its high content of saturated fatty acids and esters, exhibits greater thermal stability compared to other plant-based waxes. Studies have shown that the type of crystal formed has a meaningful relationship with the thermal stability of oleogels. Comparative analysis of crystals revealed that those formed in the β′ polymorphic form with orthorhombic structure exhibit high stability even at temperatures above 60°C. Most crystals in beeswax appear in this polymorphic form, while the β polymorph with triclinic structure—which melts at lower temperatures (50–55°C) is present only in trace amounts. Therefore, the increase in beeswax concentration in oleogels and the formation of a denser β′ polymorphic crystalline network are key contributors to the elevated melting point (Zhang *et al*., 2026).

Oil Binding Capacity (OBC) reflects the gel structure's ability to retain and stabilize oil. The analysis of variance showed significant differences among treatments ($P < 0.05$), with OBC increasing notably as beeswax concentration increased. In the 10% beeswax treatment, oil retention reached 99%, close to the control sample, while in the 4% treatment, 24% of the oil was released from the oleogel ( Fig 1). OBC is based on two primary mechanisms: oil entrapment within the crystalline network pores and surface adsorption onto triacylglycerol nanoplates (Flöter *et al*., 2021). These results align with findings by Gao *et al*. (2021), who reported nearly 100% oil retention at higher beeswax concentrations. It appears that dissolving an optimal amount of beeswax in oil followed by cooling leads to uniform distribution and stabilization of microcrystals in the continuous phase. This is due to beeswax's inherent ability to trap oil within a three-dimensional network. Additionally, beeswax's emulsifying and stabilizing properties increase viscosity, reduce oil droplet mobility, accelerate crystallization, and form a crystalline matrix that enhances oleogel viscosity and oil retention (Sintang *et al*., 2017). Patel *et al*. (2015) showed that beeswax oleogels outperform other waxes (e.g., sunflower, carnauba, candelilla) in structural recovery and oil retention due to their spherical building units. These spherical structures maintain integrity even under pressure. Although the gel network may exhibit brittleness, its bonds reform once the force is removed, allowing oil to remain trapped within the gel matrix.

Higher OBC appears directly related to more uniform crystal networks and finer pores, which increase surface contact. Oil bound to the surface of strong wax crystals is better retained. Thus, networks with smaller, evenly distributed crystals offer higher oil retention than those with larger crystals (Wang *et al*., 2023). Han *et al*. (2022) similarly found that increasing beeswax concentration (1–10%) led to more uniform and porous 3D crystal networks that physically trap oil more effectively. The rise in storage modulus (G') in beeswax-rich oleogels also indicates improved oil retention. This may be due to long-chain esters like myricyl palmitate in beeswax, which form needlelike crystals upon cooling and trap oil. Non-covalent bonding and physical entanglement further stabilize this structure (Pange *et al*., 2020).

Analysis of variance revealed significant differences in peroxide values among treatments ($P<0.05$). Mean comparisons showed that peroxide levels increased over the storage period. Under identical time conditions, the lowest peroxide value was observed in the oleogel containing 10% beeswax. The highest peroxide value was recorded on day 60 in the control sample (3.02 meq $O_2$/kg), followed by the 4% beeswax treatment (2.48 meq $O_2$/kg). The lowest value was observed on day 1 in the 10% treatment (0.044 meq $O_2$/kg) (Fig 2). Differences in peroxide levels may be attributed to the presence of phenolic compounds and esters such as apigenin, quercetin, and kaempferol in beeswax, which possess antioxidant properties that neutralize peroxide radicals and protect the gel structure from oxidation (dos Santos Alves *et al*., 2022). Despite the significant differences, all peroxide values remained within acceptable limits according to the Iranian National Standard (4179). Findings by Najibi *et al*. (2025) also support a direct relationship between gel firmness and oxidative resistance in oleogel formulations. Firmer gels, due to denser and more orderly crystal structures, have reduced interparticle spaces, limiting oxygen penetration into the oil phase and slowing oxidation reactions. At higher beeswax concentrations, smaller water droplets form within the oleogel system, enhancing stability. These droplets act as active fillers, reinforcing the gel network and reducing oil–oxygen contact, ultimately improving the oxidative stability of the final product

Analysis of the mean data revealed a statistically significant difference (at the 5% level) between the control and oleogel samples throughout the storage period. The control sample exhibited the highest TBA value, while the TBA values in oleogels decreased progressively with increasing beeswax concentration from 4% to 10%. However, TBA values increased over time during storage. The minimum and maximum TBA values were observed on day 1 in the 10% beeswax treatment (0.007) and on day 60 in the control sample (0.09), respectively (Fig 3). The TBA test evaluates oil stability based on the production of malondialdehyde (MDA), a final product of unsaturated fatty acid oxidation. The results of this study showed a decrease in TBA values with increasing beeswax concentration in oleogels, likely due to the formation of a dense gel network with hydrogen bonding that limits oxygen access to the oil phase. Higher beeswax concentrations led to denser crystalline networks, which restricted oxygen diffusion and reduced MDA formation by up to 35%, resulting in significantly lower TBA values. This reduction indicates higher oxidative stability in formulations with greater beeswax content (Tanislav *et al*., 2024). According to literature, the acceptable limit for MDA production is 0.5 mg/kg, and values exceeding 1 mg/kg indicate advanced oxidation. None of the treatments in this study exceeded this threshold (Shahidi & Zhong, 2010). Fathi *et al*. (2023) also demonstrated that beeswax effectively reduces TBA values due to its phenolic compounds—mainly flavonoids, phenolic acids, and their esters—which possess antioxidant properties that inhibit the formation of free radicals such as superoxide, hydroxyl, and peroxyl.

The Rancimat test assesses the oxidative stability of oleogels at a standard temperature of 110°C by blowing air and measuring the conductivity of volatile compounds. Analysis of variance revealed significant differences among treatments ($p < 0.05$). Oxidative stability increased with beeswax concentration, reaching 11.9 hours at 10% beeswax treatment, while the control sample showed the lowest stability of 8.3 hours. The results indicated that samples with higher beeswax content had greater thermal stability. As beeswax concentration increased from 4% to 10%, the oxidation induction period significantly extended, delaying the onset of oxidation and slowing the formation of oxidation products (Fig 4). It appears that free hydroxyl groups in the gelator structure play an antioxidant role by donating hydrogen atoms to neutralize peroxides during the early stages of oxidation (Mahjoub & Hakimzadeh, 2021). These findings are consistent with those of Patel *et al*. (2015), who reported that higher beeswax concentrations can extend the induction period and improve oxidative resistance in lipid-based products. Sobolev *et al*. (2022) also found that firmer gels slow the oxidation process, as the dense physical structure formed by beeswax reduces oxygen penetration into the oleogel, thereby increasing oxidative stability. Pang *et al*. (2020) further showed that beeswax contains not only tocopherol antioxidants but also phenolic compounds such as coumaric acid derivatives, which exhibit antioxidant activity up to 30% greater than α-tocopherol. These derivatives, when present at concentrations above 5%, form a surface layer over the oil, reducing oxygen contact and enhancing oil resistance (Naderi *et al*., 2022).

## Conclusion

This study demonstrated that oleogels formulated with beeswax and rice bran oil—particularly at higher beeswax concentrations (10%)—can serve as effective substitutes for solid fats in the production of low-fat food products with desirable functional properties. Increasing the concentration of beeswax significantly improved the physicochemical characteristics of the oleogels, including enhanced lightness (L*), slip melting point, oil binding capacity (OBC), and especially oxidative stability. These improvements are primarily attributed to the formation of a dense crystalline network, the presence of phenolic compounds with antioxidant activity, and the physical barrier effect of beeswax in limiting oxygen penetration. Moreover, the synergistic interaction between beeswax and the natural antioxidants present in rice bran oil enhances the potential of this combination for designing stable and safe lipid systems. Given the availability, affordability, and safety of beeswax, along with the nutritional benefits of rice bran oil, the use of this combination offers a promising strategy for developing healthier and functional food products.

**Table 1.** Effect of Wax Concentration Physical characteristics of Oleogels

| **Oleogel treatments** | **Physical characteristics** | | | |
|---|---|---|---|---|
| | **Lightness (L)** | **Yellowness Index (YI)** | **b** | **Melting point (C°)** |
| Oil | 56.4 $^{a}$ | 0.4 $^{d}$ | 37 $^{b}$ | 30 $^{d}$ |
| oil + 4% beeswax | 54.6 $^{b}$ | 0.41 $^{c}$ | 37.3 $^{b}$ | 33 $^{c}$ |
| oil+ 7% beeswax | 50.1 $^{c}$ | 0.47 $^{b}$ | 38.9 $^{ab}$ | 48 $^{b}$ |
| oil+ 10% beeswax | 43.2 $^{d}$ | 0.57 $^{a}$ | 40.2 $^{a}$ | 56 $^{a}$ |

Different superscript letters indicate significant difference (P<0.05).

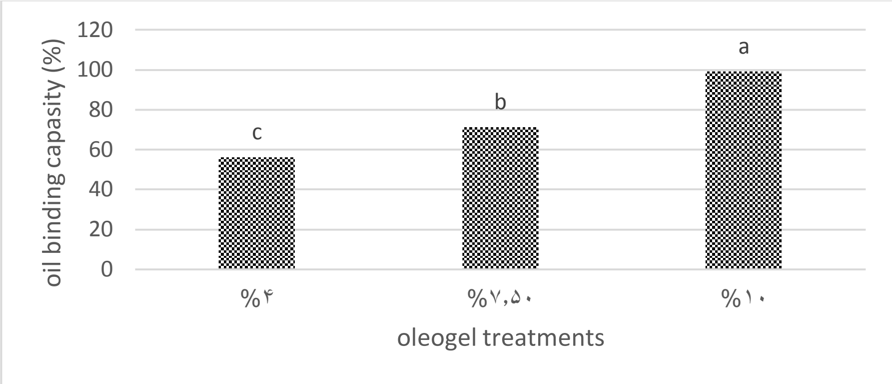
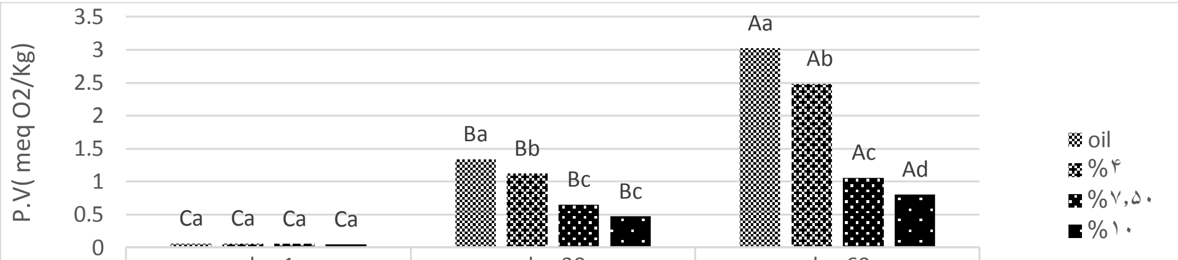
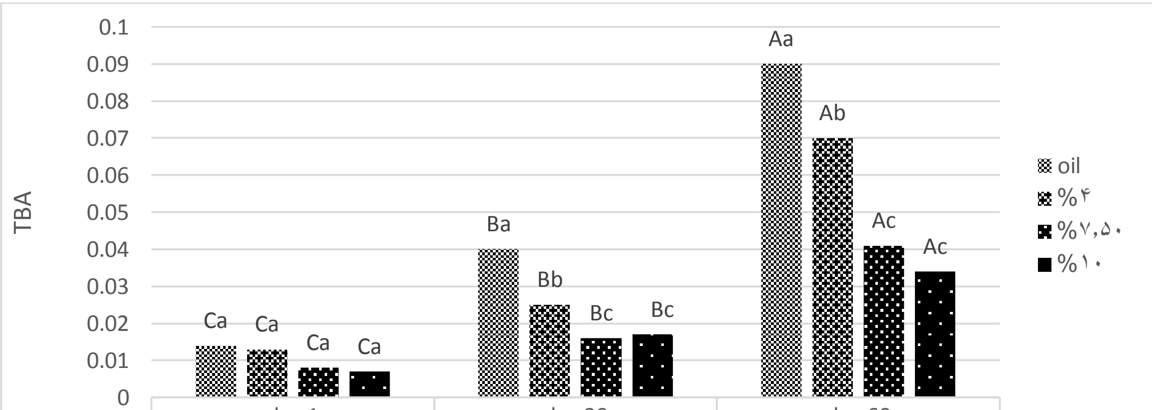



**Fig. 1.** Oil binding capacity (OBC) of oleogel produced from beeswax with rice bran oil Different superscript letters indicate significant difference (P<0.05)




| | day 1 | day 30 | day 60 |
|---|---|---|---|
| oil | 0.06 | 1.34 | 3.02 |
| 4% | 0.06 | 1.12 | 2.48 |
| 7.50% | 0.052 | 0.65 | 1.06 |
| 10% | 0.044 | 0.47 | 0.8 |

**Fig. 2.** Peroxide values of the oleogel formulated using beeswax and rice bran oil
The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each oleogel treatment




| | day 1 | day 30 | day 60 |
|---|---|---|---|
| oil | 0.014 | 0.04 | 0.09 |
| 4% | 0.013 | 0.025 | 0.07 |
| 7.50% | 0.008 | 0.016 | 0.041 |
| 10% | 0.007 | 0.017 | 0.034 |

**Fig. 3.** Thiobarbituric acid values (TBA) of the oleogel formulated using beeswax and rice bran oil The difference in uppercase letters in each column represents a significant difference in each storage period, while the difference in lowercase letters indicates a significant difference at each oleogel treatment.

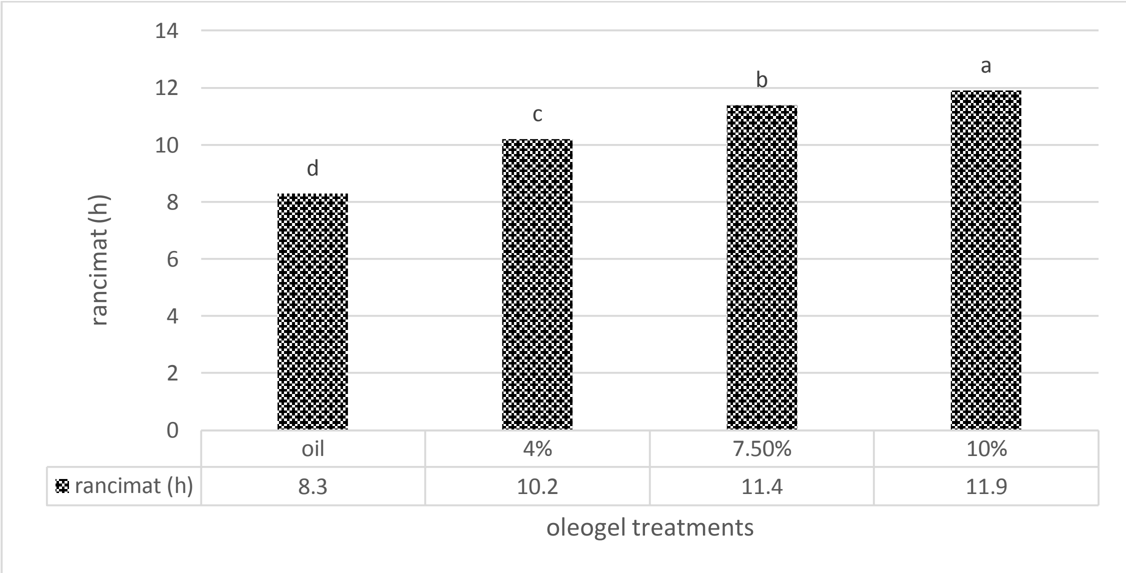



**Fig. 4.** oxidative stability time test (Rancimat) of the oleogel formulated with beeswax and rice bran oil
Different superscript letters indicate significant difference (P<0.05)

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