**Article**

# CHARACTERIZATION OF BEESWAX-BASED OLEOGELS WITH PUMPKIN SEED OIL AND RAPESEED OIL

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***Abstract:*** *The present research studied some oleogels based on cold-pressed pumpkin seed oil (PO), refined rapeseed oil (RO), and their mixtures in different combinations: PO:RO (3:1) and RO:PO (1:1), formulated with 7% and 10% beeswax (BW) as oleogelator. Several physicochemical properties were analysed, such as crystal formation time (CFT) and oil bonding capacity (OBC), along with oxidative stability, texture and sensory properties. The developed oleogels based on PO and PO:RO (3:1) with 10% BW were found to be stable regarding texture and oxidation, exhibiting high OBC and good consumer acceptability.*

**Keywords**: oleogel; oleogelator; beeswax; pumpkin seed oil; rapeseed oil

## INTRODUCTION

Oleogels are an innovative form of structured oils that have recently gained significant attention in food science and nutrition. They are semi-solid systems where an oil phase is immobilized within a three-dimensional network. This network is formed by the molecules of oleogelators, which can trap large amounts of oil and transform the liquid oil into a gel-like structure (Sivakanthan et al, 2023). Oleogelators include waxes, cellulose derivatives, fatty alcohols, and specific proteins or carbohydrates. Oleogels have a wide range of applications, particularly in the food industry as animal fat replacers, but with an obvious extension to pharmaceuticals and cosmetics (Sivakanthan et al., 2022). Recent studies have mentioned the applicability of oleogels as fat replacers in bakery products, spreads, confectionery, meat products, ice cream, sauces, and dressings (Liu et al., 2024). These applications capitalize on oleogels' ability to structure oils, improve stability, control texture, and provide healthier alternatives to traditional solid fats.

A large amount of evidence from the scientific literature shows that the structure and properties of oleogels are strongly affected by their two components, namely oils and oleogelators, as well as the interactions between them (Sivakanthan et al., 2023). Beeswax is one of the most studied oleogelators due to its ability to gel at low concentrations. In addition, beeswax offers a natural solution for structuring oils, aligning with clean-label trends and healthier fat alternatives in food products. Its compatibility, applicability, and high efficacy make it a valuable tool in food science (Gao et al., 2021).

Several types of vegetable oils have been used for the preparation of oleogels. However, to our knowledge, oleogels based on pumpkin seed oil have been little studied, and oleogels based on mixture of pumpkin seed oil with rapeseed oil not at all.

The pumpkin seed oil has gained popularity recently for its unique flavour profile and potential health benefits. This oil, extracted from pumpkin seeds, has various applications in the food industry. Several studies emphasize its beneficial health effects, such as preventing prostate enlargement and cardiovascular disease, reducing inflammation or improving diabetes (Borriello et al., 2021). These positive health effects are attributed to the abundant presence of bioactive compounds such as essential fatty acids, sterols, polyphenolic compounds and vitamin E (Singh et al., 2023).

Rapeseed oil, extracted from the seeds of *Brassica napus* or *Brassica rapa* (Banaś et al., 2023), is known in two varieties: canola oil, with reduced levels of erucic acid, and tradition al

rapeseed oil or "virgin rapeseed oil" (Chew, 2020). By its chemical composition, rapeseed oil is considered an essential source of unsaturated fats, with a balanced ratio of omega-6 and omega-3 fatty acids and vitamins E and K (Amiri et al., 2020).

Due to the high nutritional value and complementary sensory properties of pumpkin seed oil (nutty and woody flavour, deep green colour) and rapeseed oil (light, neutral flavour and colour), we have considered these oils optimal for obtaining oleogels with suitable sensory, textural and technological properties. Therefore, this study aimed to formulate and characterize new beeswax-based oleogels prepared with pumpkin seed oil used alone and in different combinations with rapeseed oil, suitable for substituting saturated fats in various food products.

## MATERIALS AND METHODS

### Materials

The lipid sources, including cold-pressed pumpkin seed oil (PO) and refined rapeseed oil (RO), were carefully selected for their unique properties and potential to yield diverse results. These were bought from local markets in Arad, Romania. Food-grade beeswax (BW) was obtained from Sigma-Aldrich (Germany). All chemicals used in this study, including sodium thiosulfate, chloroform, acetic acid, thiobarbituric acid, potassium iodide, trichloroacetic acid, and 1,1,3,3-tetra-methoxypropane were of analytical grade and purchased from Sigma-Aldrich ltd. (Germany), Merck (Germany) or Carl Roth (Germany).

### Preparation of the oleogels

Oleogel samples were obtained using two different BW concentrations, as follows: 7% (OG7) and 10% (OG10). For oleogelation, PO and RO were used alone and in varied combinations, as indicated in Table 1.

**Table 1.** Oleogels formulation

| Sample code | BW [%] | PO [%] | RO [%] |
|---|---|---|---|
| P-OG7 | 7 | 100 | 0 |
| R-OG7 | 7 | 0 | 100 |
| RP-OG7 | 7 | 50 | 50 |
| PR-OG7 | 7 | 72 | 25 |
| P-OG10 | 10 | 100 | 0 |
| R-OG10 | 10 | 0 | 100 |
| RP-OG10 | 10 | 50 | 50 |
| PR-OG10 | 10 | 72 | 25 |

To prepare the oleogels it was applied the method described in the scientific literature (Gao et al., 2021; Han et al., 2022), schematically represented in Figure 1.

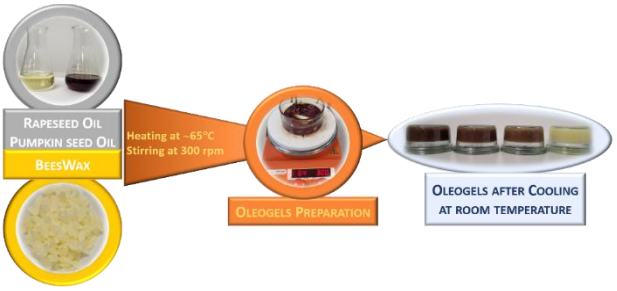



**Figure 1.** Oleogels preparation

Thus, BW was directly dispersed into preheated oils to ~65°C, under constant stirring at 300 rpm, until a clear solution was obtained. The samples were then cooled at room temperature for 24 h to form the gels (Figure 2) and stored at 4°C until specific physicochemical, textural, and sensory analyses were performed. All determinations were performed in triplicate.

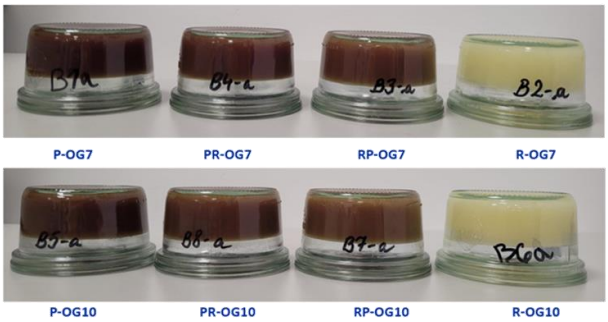



**Figure 2.** Oleogels formulated with BW

### Crystal formation time

The crystal formation time (CFT) method, a crucial aspect of our research, was applied under controlled conditions to identify the time required for the oils to solidify due to the formation of the beeswax crystalline network. CFT was determined by completely pre-melting

the oleogels placed in glass tubes in a water bath at 70°C for 1 hour. The time to complete gelation at room temperature (21±3°C) was then measured in minutes, while the tubes were rotated 180° to observe the flow (Keskin et al, 2021).

**Oil binding capacity**

Oil binding capacity (OBC) is a quality parameter of oleogels that indicates the ability of the gel structure to retain the liquid phase. The OBC was determined for the formulated samples by subjecting them to a centrifugal force under controlled speed, time and temperature conditions. The method described by Giacomozzi et al. (Giacomozzi et al., 2021) was adopted with some modifications and applied.

Approximately 15 g of freshly prepared oleogel samples were accurately weighed into previously tared Falcon tubes. After 24 hours, the oleogels stored at room temperature were centrifuged at 10000 rpm for 15 min at 20°C. After centrifugation, the tubes were inverted for 30 min, the separated oil was decanted, and the samples were weighed again.

Calculation of OBC was performed using the equation (1) (Borriello et al., 2021):

$$\text{OBC}\ (\%) = \left[1 - \frac{(m_1 - m_2)}{m_1}\right] \cdot 100 \quad (1)$$

where $m_1$ and $m_2$ represented the mass of the samples before and after centrifugation, respectively.

**Oxidative stability**

The lipid oxidation of oleogels was evaluated at 24 h after obtaining (T0) and periodically after 7 (T1), 14 (T2) and 21 (T3) days during storage under accelerated oxidation conditions (50°C). The peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were determined to evaluate the degree of primary and secondary oxidation products. As part of a comprehensive assessment, the oxidative stability was also compared for fresh oil samples: pumpkin seed oil (PO), rapeseed oil (RO), PO:RO 3:1 mixture (PRO), and PO:RO 1:1 mixture (RPO).

The peroxide value (PV) was determined to quantify the amount of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. The method is based on the oxidation of potassium iodide by the peroxides present in the oil sample, releasing iodine, which is then titrated with sodium thiosulfate (Jadhav et al., 2022). This was carried out according to the AOCS Official Methods (Giacomozzi et al., 2021). The process involved dissolving oil or oleogel samples in an acetic acid/chloroform mixture (3:1), adding saturated potassium iodide, homogenizing, and keeping it for 15 minutes in the dark. Titration was then carried out with sodium thiosulfate (0.01 N) using 1% starch as an indicator. The PV was calculated by equation (2) (Millao et al., 2023):

$$\text{PV} = \frac{\text{V} \cdot \text{N} \cdot 1000}{\text{w}} \quad (2)$$

where V represented the volume of sodium thiosulfate (mL), N the normality of sodium thiosulfate, and w the sample weight (g).

TBARS (mg malondialdehyde (MDA)/Kg oil) is another important technique to assess lipid oxidation. It measures secondary oxidation products, mainly MDA, formed during the oxidation of polyunsaturated fatty acids. MDA reacts with thiobarbituric acid under acidic conditions and high temperatures to form a pink-colored complex that can be measured spectrophotometrically (Pan et al., 2021). BARS were determined according to the method described by Pan et al. (Pan et al., 2021).). Briefly, 0.5 g of the sample was mixed with 8 mL of TBARS reagent until it was dissolved. The mixture was heated at 100°C for 15 min. After cooling at room temperature, it was centrifuged at 8000 rpm/20 min (Rotina 380R, Hettich, Germany). The lower phase was separated, and the absorbance was measured at 532 nm (Shimadzu UV-2250, Tokyo, Japan). The results were expressed as mg MDA equivalents per kg sample, using a standard curve prepared with 1,1,3,3-tetraethoxypropane.

**Texture**

The textural properties of the oleogels were measured by applying a Direct Compression – Relaxation – Traction test using the TX-700 Texture Analyzer (Lamy Rheology Instruments, France), equipped with a 50 N load cell and a 12.7 mm diameter stainless plunger. After preparation, the oleogel samples were placed in

polypropylene containers (63 mm height, 40 mm inner diameter), and after gelation at room temperature, they were stored at 4°C. The texture evaluation was performed with the following working parameters: compression speed 1 mm/s; relaxation time 10 s; penetration depth 6 mm; lifting speed 1 mm/s (Pang et al., 2020; Sarkisyan et al., 2023). The maximum force after compression, the equilibrium force after relaxation and the retention force of oleogels when lifting the plunger were measured and recorded. Textural parameters were evaluated by using the associated Rheo Tex software.

### Sensory analysis

The sensory analysis was performed only on the oleogels with 10% BW, considering their more stable structural characteristics. Quantitative descriptive analysis (QDA) methodology was applied to ten trained panelists by quantifying some specific descriptive sensory attributes on a 5-point intensity scale (0 - minimum intensity → 5 – maximum intensity). A 9-point hedonic scale (1 – dislike extremely → 9 - like extremely) was used to identify 21 naive consumers' perceptions of the formulated oleogels. A control was also subjected to sensory analysis, meaning pork lard (L-C). XLSTAT Sensory software was used to interpret the statistical data.

## RESULTS AND DISCUSSION

### Crystal formation time

The gelation behavior of the oleogels was consistent in all samples. Crystal formation time ranged from 7.00 to 8.30 (±0.50) minutes. The values fluctuated between samples due to the different compositions of oleogels in the oils and BW concentrations, which affected crystal formation times and, thus, the gelation process. As shown in Figure 3, increasing the BW concentration from 7 to 10% reduced CFT from 8.15 min to 7.00 min for P-OG and from 8.30 min to 7.15 min for PR-OG. Also, CFT was influenced by the type of oils and their mixing in different proportions, with a lower value observed in the gelation time of pure oils compared to their mixtures.

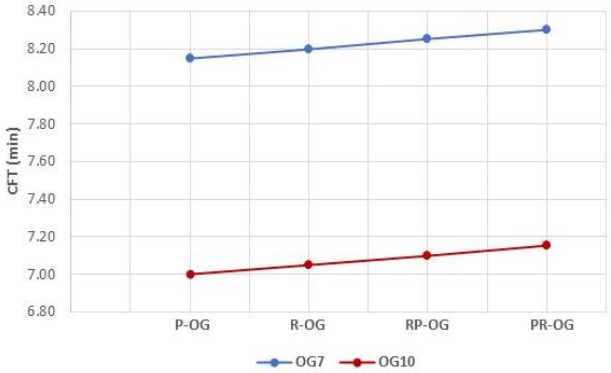



**Figure 3.** CFT of BW formulated oleogels

### Oil binding capacity

The OBC results for the prepared oleogel samples are shown in Figure 4.

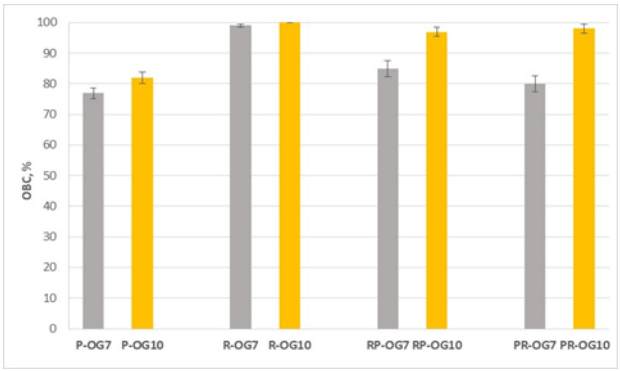



**Figure 4.** OBC of BW formulated oleogels

All formulated oleogels indicated high levels of OBC, which depended on the type of oil and clearly on the concentration of BW. The OBC values of the prepared oleogels were found to be higher with increasing BW concentration. The OBC was also influenced by the type of oil. P-OG7 and P-OG10 oleogels, formulated with cold-pressed and unrefined PO, showed significantly lower OBC (77% and 82%) than R-OG7 and R-OG10 oleogels, formulated with refined RO (99% and 100%). The OBC of oleogels based on the mixture of the two oils was also higher than those containing only PO.

### Oxidative stability

Figure 5 shows the PV evolution of oils and oleogels during storage. The initial PV of the oils and oleogels (T0) was between 1.48 meq $O_2$/kg oil (RO) and 4.45 meq $O_2$/kg oil (RP-OG10), suggesting that the primary oxidation levels were low. It can be noted that all the oleogel samples showed higher PV compared to the oils, probably because the oils were heated at 65°C through the oleogel preparation.

During storage at T1, a linear increase of PV can be observed for all the samples. The results showed that PO (3.15 meq $O_2$/kg oil) was the most stable oil, while P-OG10 (3.15 meq $O_2$/kg oil) was the most stable oleogel. The highest PV was recorded for RP-OG7 (7.53 meq $O_2$/kg oil). Still, this value is lower than the maximum limit for edible oils by international regulations (10 meq$O_2$/kg oil) (Pignitter et al., 2016).

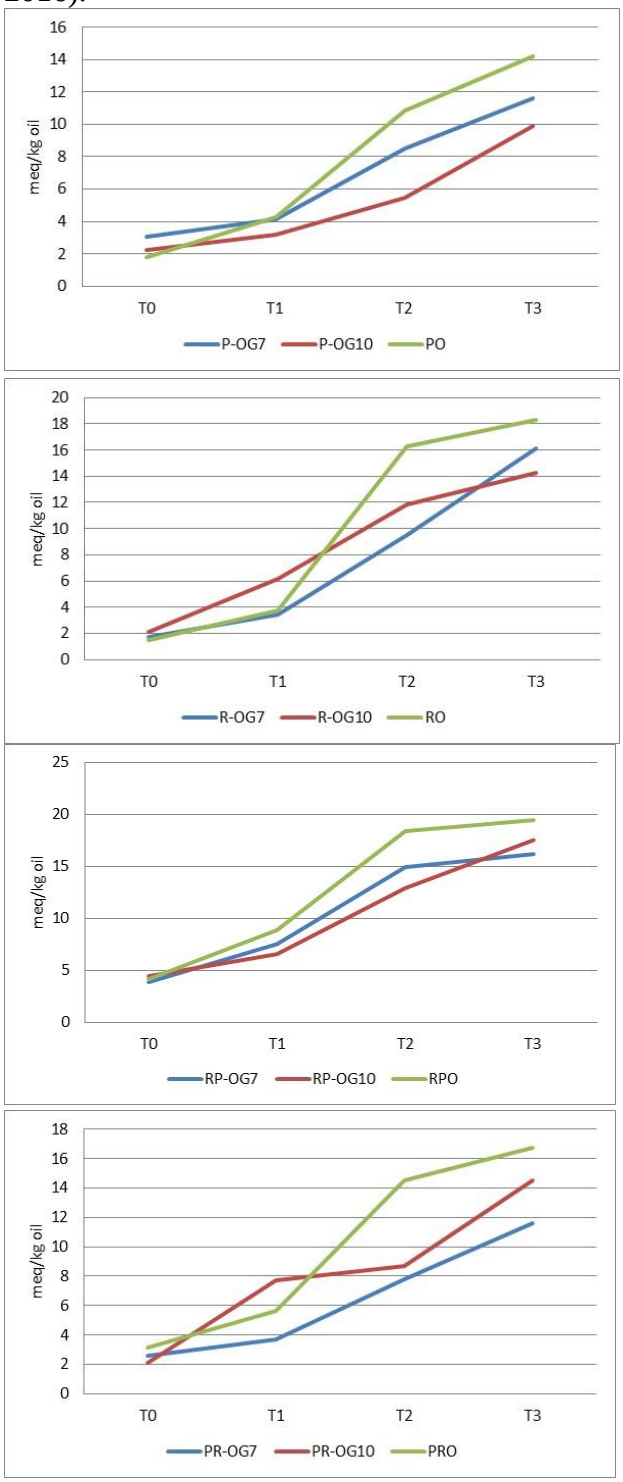
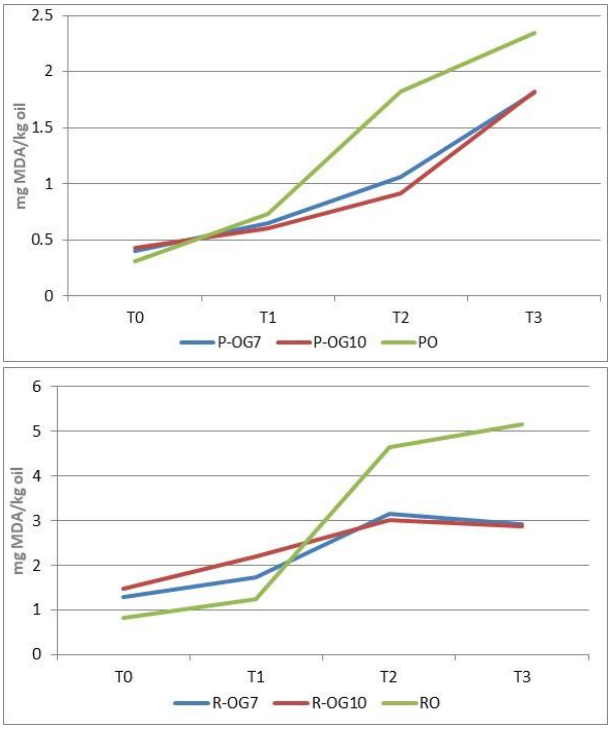



**Figure 5.** Changes in PV of formulated BW oleogels during storage at 50°C

PV significantly increased for all samples at T2 due to accelerated oxidation processes. For all the oil samples and some oleogels, such as RP-OG7 (14.95 meq $O_2$/kg oil), RP-OG10 (12.86 meq $O_2$/kg oil) and R-OG10 (11.81 meq $O_2$/kg oil), PV exceeded the acceptability level. PV continued to increase for all samples during storage and recorded maximum values when T3 was reached, exceeding the acceptability limit in all cases. After storage, PV for all oleogels was lower than that of oils, due to the the compact structure of BW oleogels that reduced the oxygen permeability and diffusion rate within the samples.

Figure 6 shows the changes in TBARS values of the samples during storage at 50°C.



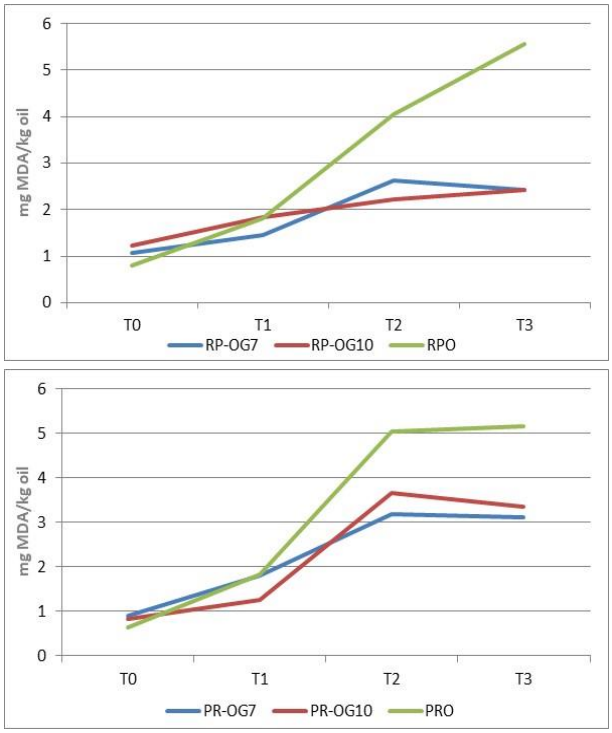



**Figure 6.** Changes in TBARS values of formulated BW oleogels during storage at 50°C

It can be noticed that TBARS values increased for all samples as a result of the secondary oxidation steps of lipids. In addition to PV, the increasing rate of TBARS values of oleogels was slower than that of oils. This demonstrated that the compact and dense structure of the BW network slowed the transfer rate of oxygen, thus preventing the secondary oxidation processes.

## Texture

Texture is an essential property of oleogels, which are used as fat replacers in food products (Tan et al., 2023). The texture of oleogels is influenced by several parameters, including the type of oil due to its fatty acid composition, oleogelator concentration, and working conditions (Frolova et al., 2022; Sarkisyan et al., 2023).

Figure 7 presents the texture parameters obtained for the BW oleogel samples, measured at 5°C. As can be observed, textural properties were significantly influenced by oil type and oleogelator concentration. In line with other authors (Zbikowska et al., 2022), the results showed a high correlation between oleogel firmness and BW concentration. Regardless of oil type, 10% BW oleogels showed significantly higher firmness and better mechanical properties than 7% BW oleogels. Differences were also found in the firmness of BW oleogels depending on the type of oil. R-OG10 was the firmest gel (3.25 N), while P-OG10 was considerably softer (1.05 N). The combination of PO and RO induced firmness values as a function of the mixing ratio. PR-OG10 (2.36 N) showed higher firmness than P-OG10 (1.05 N) and RP-OG10 (1.56 N).

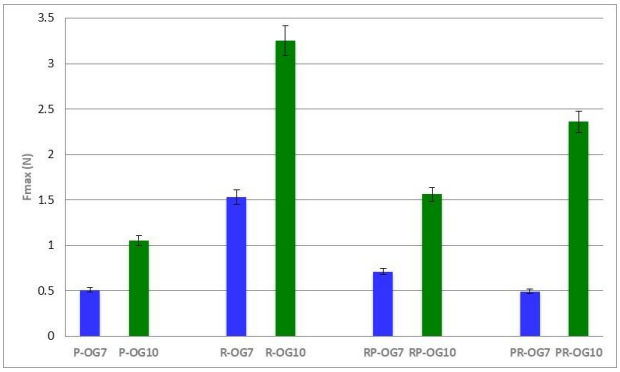



**Figure 7.** Firmness of formulated BW oleogels

## Sensory analysis

The QDA applied on the oleogels with 10% BW led to the quantification of 15 sensory attributes, as shown in Figure 8. Significant differences between the samples were recorded for most descriptors, except those related to mouthfeel, rancid taste and granularity ($\alpha$=0.05).

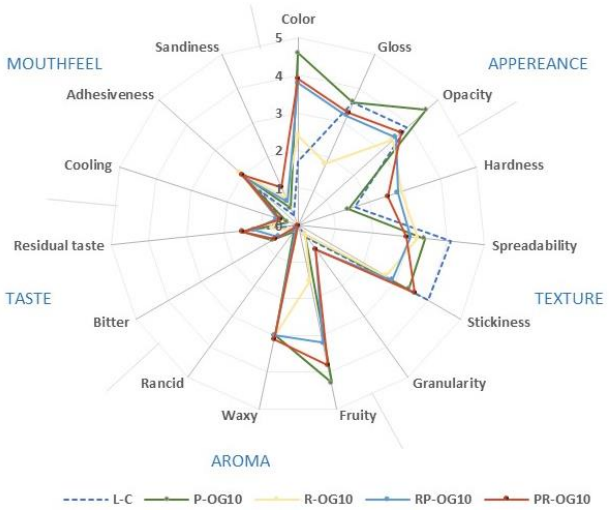



**Figure 8.** Sensory profile of oleogels formulated with 10% BW

According to Figure 9, the oleogel based on pumpkin seed oil was the most appreciated by consumers, with a score close to that of the control sample (pork lard). The Tukey's

comparison test revealed significant differences (95% confidence level) between the hedonic scores of the oleogel based on rapeseed oil (R-OG) and the samples where pumpkin seed oil was predominant (P-OG and PR-OG), respectively, between the score of the control (L-C) and that of the samples where rapeseed oil was found in a larger quantity (R-OG and RP-OG). It should be noted that all the oleogels exceeded the acceptability value limit of 5.

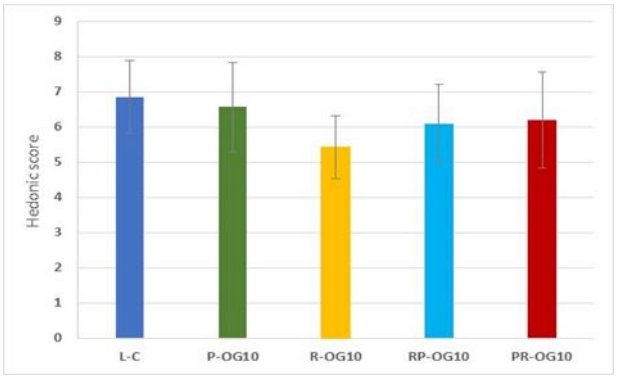



**Figure 9.** Hedonic scores of oleogels formulated with 10% BW

However, the hedonic scores were low, a situation explained by the fact that the formulated oleogels were not food products by themselves but food components to be incorporated as substitutes for animal fats in foodstuff.

## CONCLUSIONS

Beeswax-based oleogels formulated with cold-pressed pumpkin seed oil, rapeseed oil, and combinations were successfully prepared and characterized. Considering the next objective of our research, namely the incorporation of these oleogels in various meat products, it was concluded that the oleogels prepared with 10% BW and based on pumpkin seed oil, respectively, on the mixture of pumpkin seed oil and rapeseed oil in a ratio of 3:1, represent the optimal formulations to obtain stable oleogels with suitable textural profiles, high OBC, adequate oxidative stability, and good consumer acceptability.

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