Assessment Quality of Pumpkin Seed Oil and Natural Wax, as an Alternative for Animal Fats

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Abstract—**Dietary fats are essential for human nutrition, yet excessive intake of saturated and trans fatty acids remains a major health concern. Oleogelation represents a promising approach for structuring liquid vegetable oils into semi-solid systems suitable for replacing conventional solid fats. This study aimed to develop oleogels based on pumpkin seed oil structured with carnauba wax at concentrations ranging from 2 to 10% and to evaluate their chromatic properties, acidity index, and peroxide value. Oleogels were produced by thermal mixing followed by controlled cooling. Increasing wax concentration significantly influenced the color parameters, resulting in higher lightness and yellowness due to the formation of a denser crystalline network. The acidity index showed a slight increase with wax content but remained within regulatory limits. Peroxide values remained low for all samples, indicating that oleogelation did not induce oxidative degradation, while higher wax levels improved oxidative stability by limiting oxygen diffusion. These findings highlight the potential of pumpkin seed oil-carnauba wax oleogels as stable and functional fat alternatives for food applications.**

Keywords—**carnauba wax, fat stability, oleogelators, pumpkin oil, sustainable food**

I. INTRODUCTION

Compared to carbohydrates or proteins, dietary fats constitute an essential macronutrient for good health, serving as the most concentrated source of energy [1]. In processed foods, fats in the form of Saturated Fats (SFA) and Unsaturated Fatty Acids (UFA) significantly improve quality characteristics, primarily affecting organoleptic properties [2].

According to the recommendations of the European Food Safety Authority (EFSA) and the World Health Organization (WHO), dietary fats should account for 20–35% of total energy intake. A minimum intake of 20% is required to ensure adequate energy supply, sufficient intake of essential fatty acids, and effective absorption of fat-soluble vitamins. From a qualitative perspective, trans fatty acids should contribute no more than 1% of total energy intake, while saturated fats should remain below 10% [3].

There are various alternatives for reducing trans fatty acids, such as applying chemical and enzymatic esterification, using traditional fat substitutes of lipid or non-lipid origin, using oleogelation, applying in situ genetic modification of lipids, using algae oil. However, each of the aforementioned approaches has specific limitations [4]. A modern approach to solving this problem is the preparation of structured emulsions called oleogels.

Oleogels are semi-solid systems obtained by entrapping liquid vegetable oils within a three-dimensional network formed by low concentrations of structuring agents. The structural and physical properties of oleogels are influenced by the characteristics of the oil phase (type and polarity), processing conditions (mixing time, shear rate, and cooling profile), as well as storage conditions, including temperature and storage period [5].

Oleogels are considered a distinct alternative to hydrogenated oils or animal fats. Oleogels transform vegetable oils rich in unsaturated fats into solid systems, meeting consumer demands for functional and sensory qualities [6]. Oleogelators, such as natural waxes, glycerides, alcohols, ethylcellulose and phytosterols are incorporated into vegetable oils, creating a gel network in the oil phase and efficiently entrapping liquid oils. Thus, vegetable oils are restructured to obtain a semi-solid, firm, spreadable and viscoelastic state, commonly called oleogel [7].

Natural waxes have been extensively reported as effective structuring agents for lipid systems. From a chemical standpoint, they consist mainly of long-chain fatty acids esterified with long-chain fatty alcohols. Their use as oleogelators is driven by two principal factors: their renewable, bio-derived nature and their compatibility with food-grade applications. Even at low inclusion levels, natural waxes are able to self-assemble into a three-dimensional network capable of physically immobilizing the oil phase. Of particular interest is oleogelators such as natural waxes which have shown a higher storage modulus (i.e. more solid behavior) than other prepared organogels [8, 9]. As structuring agents in oleogel systems, natural waxes have demonstrated considerable potential as oleogelators for industrial applications, particularly as fat replacers. This potential is attributed to their wide availability, effective structuring performance, strong oil-binding capacity, and cost efficiency. The functional properties of wax-based oleogels are governed by the compactness of the crystalline network responsible for immobilizing the liquid oil phase, as well as by the extent of hydrogen bonding within the matrix. The architecture of the three-dimensional network varies according to the type of wax employed and its interaction with different oils, reflecting differences in chemical composition and resulting crystalline morphology. Consequently, oleogels with varying degrees of elasticity and mechanical behavior can be obtained. Moreover, the chemical composition of natural waxes exhibits substantial variability, even within the same wax type, as it is influenced by the source material, cultivation conditions, and the extraction and purification processes [10–12].

Of particular importance is the type, degree of purity, fatty

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acid composition (oleic, linoleic and linolenic acids) and minor components (tocopherols and phytosterols) of the vegetable oil chosen for the manufacture of oleogels.

The purpose of this research was to obtain oleogels using pumpkin oil and natural waxes (carnauba wax) and analyze the chromatic parameters, acidity index and peroxide index.

II. MATERIALS AND METHODS

A. Manufacture of Oleogels

As raw materials, locally produced pumpkin oil was used, obtained by cold pressing, refining and deodorization.

Carnauba waxes are characterized by a chemical composition that usually includes wax esters (50–70%, hydrocarbons (1.5–3%), free fatty acid (3–6%), free fatty alcohol (15–30%), with a melting point of 80–85 °C [13].

The process of obtaining oleogels provided for mixing pumpkin oil with 2%, 4%, 6%, 8%, 10% of the respective wax, which were heated to a temperature of 80 °C, continuous mixing until a homogeneous mass was obtained. Subsequently, the mixture was cooled to room temperature and stored under refrigeration conditions. 5 samples of oleogels were obtained.

PO-2-CW—oleogel obtained from pumpkin oil and 2% carnauba wax.

PO-4-CW—oleogel obtained from pumpkin oil and 4% carnauba wax.

PO-6-CW—oleogel obtained from pumpkin oil and 6% carnauba wax.

PO-8-CW—oleogel obtained from pumpkin oil and 8% carnauba wax.

PO-10-CW—oleogel obtained from pumpkin oil and 10% carnauba wax.

B. Color Parameters

The CIELab color parameters (L^*, a^*, and b^*) were determined using a Chroma Meter CR400/410 colorimeter (Konica Minolta, Tokyo, Japan), following the procedure described in Ref. [14]. Measurements were performed at three different locations on each sample to assess variations in lightness (L^*), the red-green coordinate (a^*), and the yellow-blue coordinate (b^*).

The color differences (ΔE^*) between the sample PO-2-CW and the other samples were calculated using Eq. (1) [15].

$$\Delta E* = \sqrt{\left(L^*_{sample} - L^*_{control}\right)^2 + \left(a^*_{sample} - a^*_{control}\right)^2 + \left(b^*_{sample} - b^*_{control}\right)^2} \quad (1)$$

Chroma index (C^*) indicates the degree of the color and is proportional to the intensity of the color and was calculated according to Eq. (2) [15]:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

Hue angle (h^*) is expressed in a 0°–360° range, where 0° = bluish-red, 90° = yellow, 180° = green and 270° = blue. The h^* was calculated using the following Eq. (3) [15]:

$$h^* = arctan\left(\frac{b^*}{a^*}\right) \quad (3)$$

Browning Index (BI) was calculated using the following Eq. (4) [15]:

$$BI = \left(\frac{(a^* + 1.75 \times L^*)}{(5.645 \times L^* + a^* - 3.012 \times b^*)} - 0.31\right) \times \frac{100}{0.17} \quad (4)$$

Yellow Index (YI) was calculated using the following Eq. (5) [16]:

$$YI = 142.86 \times \frac{b^*}{a^*} \quad (5)$$

C. Acidity Index

The acidity index of oleogels was determined by alkaline titration in an alcohol/ether medium following the internationally standardized procedure ISO 660:2020 [17]. The acidity index was expressed as mg KOH/g and calculated using Eq. (6):

$$AV(mg\ KOH/g) = \frac{V \cdot c \cdot 56.11}{m} \quad (6)$$

where:

V—volume of KOH used for titration, mL,
c—molar concentration of KOH, mol/L,
56.11—molar mass of KOH, g/mol,
m—sample mass, g.

D. Peroxide Index

The Peroxide Value (PV) of pumpkin seed oil and the corresponding oleogels structured with carnauba wax was determined by iodometric titration according to the international standard ISO 3960:2017 (visual endpoint), which is also consistent with the IUPAC peroxide value methodology widely applied for fats and oils [18]. The value of the PV was determined 5 d after the formulation of the oleogels. They were kept in refrigerated conditions at a temperature of 3±1 °C. The peroxide value was expressed as meq O_2/kg and calculated using Eq. (7):

$$PV(meqO_2/kg) = \frac{(V - V_0) \cdot c \cdot 1000}{m} \quad (7)$$

where:

V—volume of $Na_2S_2O_3$ used for the sample, mL,
V_0—volume used for the blank, mL,
c—molar concentration of $Na_2S_2O_3$, mol/L,
m—sample mass, g.

E. Statistical Analysis

All calculations were performed using Microsoft Office Excel 2007 (Microsoft, USA). Data obtained in this study are presented as mean values ± the standard error of the mean calculated from three parallel experiments.

III. RESULTS AND DISCUSSION

A. Color Parameters

The color was determined by measuring the reflectance of freshly prepared oleogels using CIE L^*, a^* and b^* values. This analysis plays a significant role in consumers' evaluation of the visual characteristics of novel food products. The results regarding the color parameters of the obtained oleogels are presented in Table 1.

Table 1. CIELab parameters of oleogels

Sample	L^*	a^*	b^*	ΔE^*
PO-2-CW	16.02±0.23	5.38±0.07	18.56±0.11	-
PO-4-CW	22.19±0.21	2.38±0.09	26.94±0.32	10.83±0.17
PO-6-CW	24.89±0.18	2.64±0.11	26.42±0.54	12.16±0.12
PO-8-CW	29.42±0.17	−0.26±0.03	28.79±0.56	17.78±0.22
PO-10-CW	39.91±0.21	−3.87±0.06	58.80±0.38	47.70±0.35

The increase in the concentration of carnauba wax in the oleogels obtained from pumpkin seed oil caused significant changes in the CIELab color parameters, highlighting the influence of lipid structuring on the optical properties of the product. The lightness (*L**) increased progressively, increasing from 16.02 in the PO-2-CW sample to 39.91 in the PO-10-CW sample, indicating a pronounced color opening with the formation of a denser and more uniformly organized crystalline network at high wax concentrations.

The values of the *a** coordinate decreased from positive values in the PO-2-CW sample (5.38) to negative values in the samples with 8% and 10% wax (−0.26 and −3.87), suggesting a chromatic shift from reddish to green shades, as a result of the masking of the natural pigments of the pumpkin oil, especially carotenoids.

Regarding the *b** coordinate, a general increase in values was observed with increasing wax concentration, reaching a maximum of 58.80 in the PO-10-CW sample. The intensification of the yellow component is probably determined both by the intrinsic color of the carnauba wax and by the cumulative effect of lipid crystallization on the enhancement of the golden-yellow hues.

The total color difference *ΔE** increased significantly with increasing wax concentration, from 10.83 for the PO-4-CW sample to 47.70 for the PO-10-CW sample, indicating chromatic changes ranging from slightly perceptible to very obvious to the human eye. These results confirm that the addition of carnauba wax is a determining factor in modifying the chromatic characteristics of pumpkin seed oil-based oleogels. Similar results for color parameters were recorded for oleogels obtained from soy wax and rice bran oil. The authors showed that the variation of the parameters is greatly influenced by the color of the oil used [16].

The derived color parameters were markedly affected by increasing carnauba wax concentration in pumpkin seed oil-based oleogels, indicating notable changes in color intensity and hue. The results regarding the respective color parameters for the analyzed oleogels are presented in Table 2.

Table 2. Color indices of oleogels

Sample	H	C	YI	BI
PO-2-CW	73.83±1.13	19.32±0.24	165.51±9.26	492.50±12.67
PO-4-CW	84.95±0.87	27.05±0.38	173.44±7.54	521.36±9.65
PO-6-CW	84.30±1.09	26.56±0.25	151.64±11.12	427.50±14.21
PO-8-CW	90.52±1.23	28.80±0.33	139.80±7.95	380.94±14.02
PO-10-CW	93.76±1.42	58.93±0.28	210.48±9.78	875.69±19.48

Chroma (C) generally increased with wax content, reflecting enhanced color saturation. Similar values observed for PO-4-CW, PO-6-CW, and PO-8-CW suggest a stabilization of chromatic intensity at intermediate wax levels, whereas the sharp rise in PO-10-CW indicates a strong effect of lipid structuring on color enhancement.

The Hue angle (H) showed a progressive increase from 73.83 to 93.76, revealing a shift of the dominant color toward the yellow-green region, likely due to interactions between oil pigments and the crystalline network formed by carnauba wax.

The Yellowness Index (YI) increased overall with higher wax concentrations, reaching its maximum in PO-10-CW, which points to an intensification of yellow tones associated with both the intrinsic color of the wax and increased light scattering within the oleogel matrix.

In contrast, the Browning Index (BI) varied markedly with wax level, showing lower values at intermediate concentrations and a pronounced increase at 10% wax, suggesting enhanced golden-brown coloration at high wax contents due to increased opacity and combined chromatic effects of natural pigments and lipid crystallization.

B. *Acidity Index*

The acidity index of wax-based oleogels vary depending on the concentration of wax used. These values, governed by the presence of free fatty acids in the wax, directly influence the oxidative stability, color characteristics, and technological properties of the oil-wax system [19]. The results regarding the acidity index are tabulated in Fig. 1.

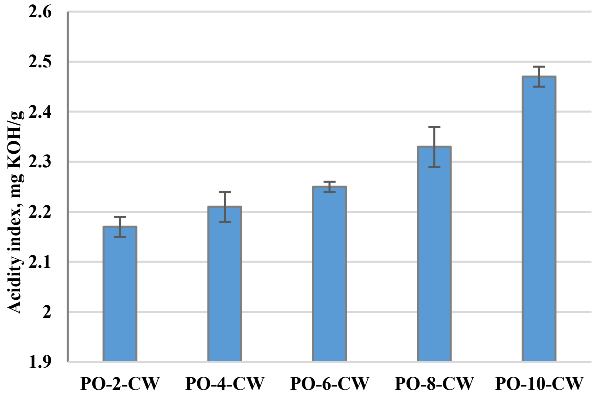

Fig. 1. Acidity index of oleogels.

The acid index values of pumpkin seed oil-based oleogels increased progressively with increasing carnauba wax concentration, from 2.17 mg KOH/g in sample PO-2-CW to 2.47 mg KOH/g in sample PO-10-CW. This trend indicates a direct correlation between the level of added wax and the free fatty acid content of the oleogel system. The increase in acidity index can be attributed to the contribution of free fatty acids from carnauba wax, as well as to the intensification of interactions between the lipid phase and the crystalline structure formed at high wax concentrations. Although the variations are moderate, which suggests that higher wax levels may influence the oxidative stability and technological properties of the oleogels, without indicating a significant degradation of lipid quality. However, these results are within the limits set by the CODEX STAN 210-1999 standard for vegetable oils. Similar results, up to 2.08 mg KOH/g, were obtained for oleogels made from linseed oil and candelilla wax at concentrations of 3% and 6% [20].

C. *Peroxide Index*

The peroxide value is a sensitive indicator of primary lipid oxidation and is therefore essential for evaluating the oxidative stability of pumpkin oil and its oleogels during and immediately after processing. The obtained results are showed in Fig. 2.

In the present study, pumpkin oil exhibited a PV of 3.28 meq O_2/kg, confirming a low initial oxidation level typical for high-quality cold-pressed oils. After oleogelation with carnauba wax, the values remained in a narrow and low range (2.73–3.59 meq O_2/kg), demonstrating that the applied thermal treatment and structuring step did not trigger relevant oxidative deterioration. The oleogel containing 2% of carnauba wax showed the highest peroxide indices (3.59±0.06 meq O_2/kg), while further wax enrichment produced a progressive reduction in peroxide formation: PO-4-CW—3.44, PO-6-CW—3.20, PO-8-CW—2.94, and the lowest value at PO-10-CW—2.73 meq O_2/kg. Importantly, all samples were far below the regulatory threshold of 15 meq O_2/kg for unrefined cold-pressed pumpkin seed oil, indicating full compliance and confirming that the produced oleogels are oxidatively safe at the initial stage [21]. From a quality perspective, these results are also well within internationally accepted ranges frequently used for edible oils (often <10 meq O_2/kg as a benchmark for low primary oxidation), and align with the general observation that properly processed oleogels exhibit low PV immediately after preparation due to limited formation of primary oxidation products. The slightly elevated peroxide index at 2% carnauba wax likely reflects a system where the wax concentration is still insufficient to form a fully continuous and compact crystalline network, leaving a larger fraction of the oil phase relatively mobile and more accessible to oxygen diffusion during cooling and early stabilization. As the wax content increases, the increasingly dense crystal network physically immobilizes the oil, reduces molecular mobility, and limits oxygen transport pathways, thereby suppressing the hydroperoxide formation. This "diffusion-barrier" concept is consistent with oxidative behavior reported for structured oil systems, where stronger gel networks restrict exposure to pro-oxidizing factors (oxygen, heat, light, metal ions) and reduce peroxide accumulation by limiting oxygen penetration into the bulk lipid phase [22].

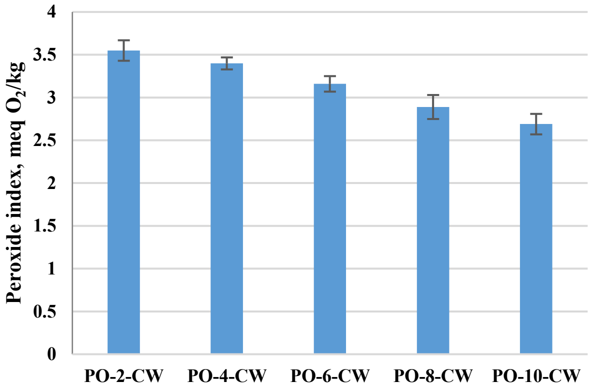

Fig. 2. Peroxide index of oleogels.

Most importantly, the decreasing peroxide indices trend with rising carnauba wax concentration observed in this work is in agreement with carnauba wax oleogel literature, where higher wax levels often correlate with improved oxidative stability due to tighter structuring and reduced oxygen diffusion; for instance, carnauba wax oleogels in other vegetable oil matrices have been reported to reach very low peroxide index values at higher wax concentrations (e.g., ~2.30 meq O_2/kg at 10% carnauba wax), reflecting enhanced protection against early oxidation [23].

IV. CONCLUSION

The results of this study demonstrate that carnauba wax is an effective structuring agent for pumpkin seed oil-based oleogels, enabling the formation of stable semi-solid lipid systems with favorable physicochemical properties. Increasing wax concentration significantly influenced the chromatic characteristics, acidity, and oxidative behavior of the oleogels. All samples exhibited low peroxide values and acidity index within acceptable regulatory limits, confirming their oxidative stability and quality. The improved stability observed at higher wax levels highlights the protective role of a dense crystalline network. Overall, these oleogels show strong potential as functional fat replacers in food formulations, so confectionery and frozen desserts, such as ice cream.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Ropciuc S. and Bulgaru V. conducted the research; Netreba N. and Pauliuc D. analyzed the data, Bulgaru V., Netreba N. and Ropciuc S. wrote the paper and approved the final version. All authors had approved the final version.

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