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Article

PRELIMINARY STUDIES ABOUT SOME FACTORS INFLUENCING THE PROPERTIES OF OLEOGELS

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Abstract: *Nowadays, the use of oleogels offers several advantages in food products. They can mimic the functionality of solid fats, providing structure, stability, and mouthfeel to various food formulations. By replacing solid fats or hydrogenated oils with oleogels, manufacturers can reduce or eliminate trans fats and therefore produce healthier food products. The present study focuses on the formulation of oleogels based on pumpkin seeds and rapeseed oils and the use of beeswax or ethylcellulose as oleogelators. The preliminary results indicate that the pumpkin seeds oil contributes to the formation of oleogels with higher consistency. Furthermore, the mechanical properties of oleogels can be manipulated, by adjusting some parameters, such as the type of oil and the kind and concentration of the oleogelator.*

Keywords: oleogels, pumpkin seed oil, rapeseed oil, texture

INTRODUCTION

Oleogels, also known as structured oils, are semi-solid materials that are formed by solidifying vegetable oils with oleogelators, leading to the formation of three-dimensional micro-structured systems where the structuring molecules network immobilises the fraction of liquid oil (Alongi et al., 2022; Li et al., 2022). Oleogelation is a relatively new food technology, considered innovative due to its potential for creating semi-solid gels from liquid oils with no chemical modifications, thus improving the functional properties of food products while these promising ingredients are incorporated (Jeong et al., 2021).

Oleogelators are important substances for modifying the viscosity of the oil phase, with a variable efficiency in oleogelation processes depending on their chemical structure, used concentration, and formulation conditions, thus being selected by the intended food-application specific requirements. There is a large number of compounds used as oleogelators for obtaining food-grade oleogels that can be divided into two major categories depending on their molecular weight: (i) low-molecular-weight gelators, i.e. lipid components such as fatty acids, esters of fatty acids (especially waxes), glycerolipids (especially monoglycerides), glycerophospholipids (especially lecithins), sterol lipids (especially phytosterols and phytosterol esters), sphingolipids (especially ceramides), and fatty alcohols; (ii) high-molecular-weight or polymeric gelators, i.e. non-lipid components such as proteins (e.g. soy protein, whey protein, zein, casein, gelatin), and polysaccharides (e.g. agar, xanthan gum, pectins, chitin and chitosan, ethylcellulose) (Perta-Crisan et al., 2023). The low-molecular-weight oleogelators form three-dimensional crystal networks or self-assembled fibrillary networks (Wang et al., 2022), while the high-molecular-weight ones develop a three-dimensional network that is stabilized by hydrogen bonds, electrostatic, and hydrophobic interactions between molecules and that traps the liquid oil (Sivakanthan et al., 2022).

Depending on the oleogelator solubility, two categories of methods are used to formulate the oleogels: direct dispersion into the oil heated to the upper temperature of its melting point, which is specific to hydrophobic oleogelators, is simple and therefore is the most frequently used (Martins et al., 2020), and indirect pathways such as foam method, biphasic emulsion method or solvent exchange, which are specific to hydrophilic compounds that cannot be dispersed directly into the oil and consist of the initial structural network formation in a water-continuous system and the subsequent removal of the aqueous phase (Floter et al., 2021; Singh et al., 2017). None of these methods produces chemical or structural changes in the oils, thus preserving their nutritional value (Silva et al., 2021b).

Currently, oleogels are extensively studied for their technological and nutritional advantages in foods development, such as replacing or reducing saturated, trans- or hydrogenated fats, which lead to healthier products with unaffected texture, mouthfeel or stability, in extending food shelf-life, in bioactive compounds delivering, in obtaining low-calorie and functional food products, or in preventing oil leaks in various foodstuffs (Davidovich-Pinhas, 2016; Lupi et al., 2013; Manzoor et al., 2022; Temkov and Muresan, 2021). Applications of oleogels in foods can also be categorized by the intended purpose, as follows: in meat and dairy products as animal fat replacers; in baked products, chocolate and chocolate spreads, or filling creams as shortening replacers; in breakfast spreads; in different foods as edible coatings, delivery systems of bioactive compounds, or as frying medium (Perta-Crisan et al., 2023).

Following the encouraging results reported in the scientific literature and considering the current state of knowledge in the field, the present paper shows the results of preliminary studies on the influence of several factors on some properties of different oleogels. As a relatively new element, pumpkin seed oil was used in their formulation, either alone or combined in various proportions with rapeseed oil. To the best of our knowledge, quite a few studies can be found so far on the formulation and characterization of pumpkin seed oil-based oleogels. In this sense, direct dispersion oleogelation methods have been applied, and several factors influencing the consistency and visual appearance of the obtained oleogels have been evaluated: type and proportion of oils, nature and concentration of the oleogelator. Preliminary conclusions have been formulated, but further studies are imperatively needed to draw more detailed conclusions that are related to the final intended purpose of incorporating formulated oleogels as animal fat substitutes in certain food products.

1.1. Oleogelator characterisation

a. Beeswax (BW)

Food-grade beeswax is obtained by purifying the honeycomb of bees from the genus *Apis mellifera L.* (Tinto et al., 2017). Depending on the purification method of the raw wax, it is obtained yellow beeswax (CAS Registry Number: 8006-40-4), a yellow-brown solid, and white beeswax (CAS Registry Number: 8012-89-3), a yellowish-white solid, obtained by bleaching yellow beeswax (Gupta and Anjali, 2023). Beeswax has no nutritional value because it is not digested in the human body. The physical properties of beeswax vary depending on its geographical origin and its age and are presented in Table 1.

Table 1. The physicochemical characteristics of beeswax (Tinto et al., 2017)

Physical properties	Description
Smell	honey-like odour
Taste	insipid
Melting range	61 - 66°C
Density at 20°C	0.960 – 0.970 g/cm^3
Solubility	insoluble in water; sparingly soluble in alcohol; soluble in organic solvents (chloroform, ether); after warming, soluble in fatty oils

Chemically, beeswax is a mixture of fatty acid esters (also called wax esters), free fatty acids, free fatty alcohols, hydrocarbons, and other minor compounds (Table 2). Due to its composition, beeswax is a gelling agent for various oils, including fish oil, rapeseed oil, olive oil, canola oil, linseed oil sunflower oil (Frolova et al., 2022; Gao et al., 2021; Silva et al., 2021a; Yi et al., 2017; Zhang et al., 2021).

Table 2. Chemical composition of beeswax (Tinto et al., 2017)

Component	Amount (%)	Description
Wax esters	58 - 71	fatty alcohols esterified by fatty acids or hydroxy acids: - monoesters and diesters - hydroxymonoesters and hydroxydiesters eg: saturated alkyl esters of palmitic acid (C_{38}-C_{52}) - unsaturated alkyl esters of oleic acid (C_{46} C_{54})

Hydrocarbons	12 - 26	long chains with odd carbon numbers - n-alkanes (C_{27}- C_{33}) - n-alkenes (C_{21}- C_{41})
Free fatty acids	8 - 18	unbranched saturated acids with even carbon numbers (C_{16} -C_{34}), eg: Palmitic acid (16:0)
Free fatty alcohols	1 - 6	unbranched alcohols with even carbon numbers (C_{24}-C_{34})

b. Ethylcellulose (EC)

Ethylcellulose (CAS Registry Number: 9004-57-3), is a semi-synthetic polymer built from cellulose by partial substitution of hydrogen from the hydroxyl groups with ethyl groups (Puscas et al., 2021). By this etherification, various degrees of substitution (DS) are obtained. Ethylcellulose is characterized by the viscosity value because there is a correlation between the DS, water solubility and viscosity of the polymer (Davidovich-Pinhas et al., 2014). The physicochemical characteristics of ethylcellulose are presented in Table 3.

Table 3. The physicochemical characteristics of ethylcellulose (EFSA, 2004; Kim et al., 2014)

Characteristics	Descriptions
Appearance	free-flowing powder
Color	white to off-white
Smell	odourless
Taste	tasteless
Density at 20ºC	1.09 - 1.17 g/cm^3
Solubility	- insoluble in water (hydrophobic character); after warming soluble in fatty oils
Structure	- polymer; semicrystalline

Ethylcellulose is an accepted food additive in the EU, known as E462, and it is used as an emulsifier, stabilizer and thickener or carrier agent for other additives (Kim et al., 2014). Ethylcellulose is non-digestible in the human body, thus it has no nutritional value. Ethylcellulose is representative of polymeric gelling agents of vegetable oils by direct dispersion. The semi-crystalline structure and the hydrophobic character are the arguments for the functioning of ethyl cellulose as an oleogelator (Garcia-Ortega et al., 2021; Giacintucci et al., 2018; Gómez-Estaca et al., 2019a).

1.2. Oil characterization

a. Pumpkin seed oil

Pumpkin seeds oil is achieved by pressing or extraction and further processing like decanting and filtering. The physicochemical characteristics of pumpkin seed oil are shown in Table 4.

Table 4. Physicochemical characteristics of pumpkin seed and rapeseed oil (Encinar et al., 2018; Tsaknis et al., 1997)

Parameter	Rapeseed oil	Pumpkin seed oil
Color	yellow to greenish-yellow	dichromatic - dark brown with a green tint
Smell	pleasant, without foreign, bitter or rancid smell	a mild nutty odour
Taste	pleasant, without foreign, bitter or rancid taste.	pleasant, without foreign, bitter or rancid taste
Density at 20°C	0.909 - 0.925 g/cm^3	0.918 - 0.927 g/cm^3
Viscosity at 2°C, mPa.s	–	70 – 72
Refractive index at 40°C	1.466 - 1.47	1.465 - 1.474
Saponification index	189 - 195 mg KOH/g	185 -203 mg KOH/g
Iodine index	120 - 143 g I/100 g	103 - 134 g I/100 g
Unsaponifiable matter	max 1.5%	0.79 - 1.22%
Peroxide index	max 10 meq/kg	9.04 - 9.20 meq/kg
Free acidity as oleic acid	max 0.1%	max 0.4%

The chemical composition of the pumpkin seed oil may vary depending on the variety, geographical area, etc. The predominant compounds of pumpkin seed oil are triacylglycerols, while diacylglycerols, monoacylglycerols and free fatty acids are practically absent. In the composition of triacylglycerols of pumpkin seed oil, unsaturated fatty acids are found in the greatest proportion, primarily represented by linoleic acid, followed by oleic acid, while saturated fatty acids are represented by palmitic acid and stearic acid, Table 5.

Table 5. Fatty acids composition of pumpkin seed oil (Bardaa et al., 2016; Tsaknis et al., 1997)

Type of fatty acid	Amount (%)	Major representants
Saturated (SFA)	20	palmitic acid (C16:0); stearic acid (C18:0)
Unsaturated (UFA)	80	
Polyunsaturated (PUFA)	42 - 43	linoleic acid (C18:2)
Monounsaturated (MUFA)	37 - 38	oleic acid (C18:1)

In recent years, pumpkin seed oil has attracted a lot of attention due to its high nutritional value. It has been shown that the intake of pumpkin seed oil can have beneficial effects on health, such as preventing prostate enlargement, improving diabetes, and protecting against high blood pressure and carcinogenic diseases. These positive health effects could be attributed not only to the high content of polyunsaturated fatty acids, especially oleic and linoleic acids but also due to the presence of substances such as pigments, phytosterols, vitamins, minerals and phenolic compounds with functional properties.

b. Rapeseed oil

Rapeseed oil is obtained from rapeseed (*Brassica napus var. napus*). It can be prepared through various processes such as pressing (cold or hot) extraction (enzymatic or with hexane), followed necessarily by refining. The processes used for preparation influence the characteristics and composition of the obtained oil (Wu et al., 2019). At room temperature, refined rapeseed oil appears as a clear, suspension-free and sediment-free liquid. The physicochemical characteristics of rapeseed oil are presented in Table 4.

The chemical composition of the rapeseed oil may vary depending on the processes used for preparation, the variety of rapeseed, geographical area, etc. Table 7, contains the fatty acids composition of rapeseed oil.

Refined rapeseed oil was found to have higher antioxidant activity but significantly lower levels of phenolic acids (0,008 mg/kg oil) than cold-pressed rapeseed oil (0.46 mg/kg oil). In contrast, tocopherol levels are similar in cold-pressed and refined rapeseed oils (Cristea et al., 2018; Wu et al., 2019).

Table 6. Fatty acids composition of rapeseed oil (Encinar et al., 2018; Wu et al., 2019)

Type of fatty acid	Amount (%)	Major representants
Saturated (SFA)	6 -7	palmitic acid (C16:0); stearic acid (C18:0)
Unsaturated (UFA)		
Polyunsaturated (PUFA)	27 - 28	linoleic acid (C18:2); α-linolenic acid (C18:3)
Monounsaturated (MUFA)	63 - 65	oleic acid (C18:1)
Trans fatty acids	2	

MATERIALS AND METHODS

2.1. Materials

Ethylcellulose with 64 cP viscosity and beeswax refined were purchased from Sigma-Aldrich.

Pumpkin seed oil and rapeseed oil were purchased from Arad, Romania's local market, and used for the preparation of the oleogels.

2.2. Oleogel preparation

a. Ethylcellulose oleogels preparation

Ethylcellulose oleogels were obtained using the method described by Gravelle et al (Gravelle, 2017). Mixtures of different proportions of pumpkin seed oil and rapeseed oil (mass ratio of 1:0, 3:1, 1:1 and 1:3) were used to prepare oleogels with 5 and 10% ethylcellulose, Table 8.

Table 7. Ethylcellulose oleogel samples

Sample	Pumpkin seed oil [%]	Rapeseed oil [%]	Ethylcellulose [%]
DR0-5	100	0	5
DR1-5	75	25	5
DR2-5	50	50	5
DR3-5	25	75	5
DR0-10	100	0	10
DR1-10	75	25	10
DR2-10	50	50	10
DR3-10	25	75	10

The oils were previously heated at 100°C and the oleogelator was added by stirring at 200 rpm, using a magnetic stirrer. The mixture was heated, not exceeding 140°C, with continuous stirring until ethylcellulose was completely dissolved in the oil and a clear solution was obtained. After that, the mixture was cooled at room temperature to form the gels and finally stored at 4°C.

b. Beeswax oleogels preparation

Beeswax oleogels were obtained according to the procedure described by Gómez-Estaca et al. (Gómez-Estaca et al., 2019b). Mixtures of different proportions of pumpkin seed oil and rapeseed oil (mass ratio of 1:0, 3:1, 1:1 and 1:3) were used to prepare oleogels with 7 and 10% BW, Table 9. The oils were heated at 62°C, and beeswax was added under continuous stirring (200 rpm) until complete dissolution. After that, the samples were cooled down to ambient temperature until gelification and stored in a refrigerator at 4°C.

Table 8. Beeswax oleogels samples

Sample	Pumpkin seed oil [%]	Rapeseed oil [%]	Beeswax [%]
C7-0	100	0	7
C7-1	75	25	7
C7-2	50	50	7
C7-3	25	75	7
C10-0	100	0	10
C10-1	75	25	10
C10-2	50	50	10
C10-3	25	75	10

2.3. Texture measurement - penetration test

The oleogel texture is an important parameter attribute related to their structure, overall performance and future application. Firmness of the oleogel samples was measured by penetration test using a PCE-PTR 200 texturometer (PCE Instruments UK Ltd. Hampshire, UK). Before the analysis of oleogel, the samples were tempered to about 20°C. Each sample was penetrated by an 8 mm diameter stainless plunger to a depth of 5 mm. Three independent measurements were performed for each sample, and the maximum force (N) was recorded.

2.4. Visual appearance

After the oleogels preparation, they were disposed of in 80 ml containers and maintained at room temperature until gelification. After this period, the containers were turned upside down to observe the stability, including phase separation and liquid oil exudation on the surface. After that, the visual appearance examination started with analyzing the oleogels: texture, color, opacity, shine, and homogeneity.

RESULTS AND DISCUSSIONS

3.1. Texture measurement

a. Ethylcellulose oleogels

The firmness is one of the most important physical properties of structured fats as they will be used as substitutes for solid fats in different food products. The firmness of the EC oleogel samples is shown in Figure 1. It can be seen that the texture was significantly higher with the increase in the EC concentration. As the concentration of EC increased from 5 to 10%, the firmness of oleogels increased exponentially for all samples from 0.11N to 24.79N.

On the other hand, the nature of the oil also influences the firmness of oleogels. The highest firmness was found for the pumpkin seed oil oleogels, 2.93 N for DR0-5 and 24.78 for DR0-5. The mixture of pumpkin seed oil with rapeseed oil leads to a linear decrease in the texture of the oleogels, proportional to the amount of substituted pumpkin seed oil. For the 5% EC samples, a 75% replacement of pumpkin seed oil with rapeseed oil resulted in a significant reduction of oleogel firmness by 96.2% while in the case of the 10% EC samples, the replacing of pumpkin seed oil induced a 35.4% decreasing of texture.

b. Beeswax oleogels

In both cases of BW oleogels, the firmness is significantly affected by wax concentration. As the concentration of BW increased from 7 to 10%, the firmness of oleogel samples increased from 0.42 N to 14.16 N. On the other hand, figure 2 shows that the oleogel prepared only from pumpkin seed oil with 10% EC has the highest firmness and the replacement with rapeseed oil decreased the oleogel firmness proportionally with the ratio amount.

The higher firmness for the oleogel prepared only by pumpkin seed oil, in the case of EC and BW, may be attributed to the fatty acid composition of the oils since rich unsaturated fatty acids oils produce stronger gels (Wang et al., 2023; Zetzl et al., 2014).

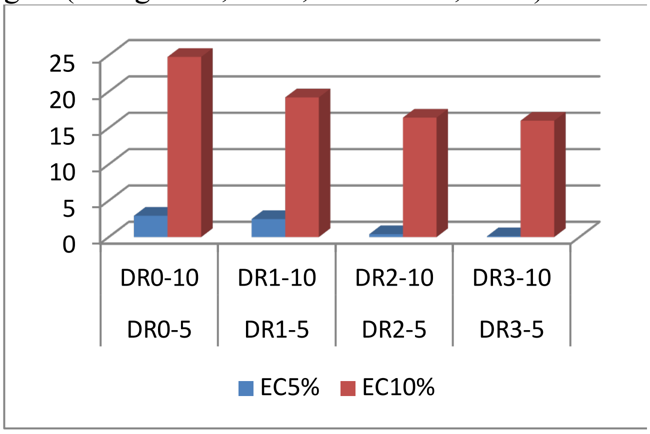

Figure 1. Firmness of oleogels prepared from pumpkin seed oil and rapeseed oil with 5 and 10% EC

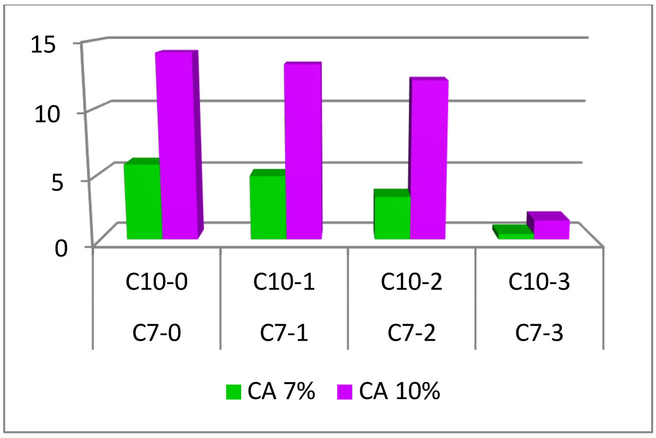

Figure 2. Firmness of oleogels prepared from pumpkin seed oil and rapeseed oil with 7 and 10% BW

3.2. Visual appearance

The visual appearance of the oleogelation phenomenon was monitored for the oleogels obtained with pumpkin and rapeseed oil with ethylcellulose and beeswax at 10% w/w concentration.

Tabel 9. Visual aparence of pumpkin and rapseed oil oleogels with 10% BW and 10% EC

Characteristics	**Pumpkin and rapeseed oil oleogels with 10% BW**	**Pumpkin and rapeseed oil oleogels with 10% EC**
Texture	the oleogel have typically a smooth and creamy texture due to the presence of beeswax. The texture may be slightly firm, but still spreadable.	the oleogels made with ethylcellulose have a smooth and homogeneous texture. The gel formed by ethylcellulose helps to distribute the oil uniformly, creating a consistent and cohesive appearance
Color	the color of the oleogel depends on the natural color of the pumpkin and rapeseed oil. Pumpkin oil typically has a rich, golden, or dark green color, while rapeseed oil is often yellow. The combination of these oils results in a blend of colors ranging from golden yellow to deeper green.	the pumpkin oil and rapeseed oil possess their characteristic colors, and the addition of ethylcellulose in the oleogel formulation does not significantly alter the natural color of these oils. Therefore, the oleogels retain the original color of the oils used.
Opacity	the oleogels made with beeswax at a 10% concentration have a translucent appearance. The presence of beeswax can create a certain level of opacity, but it should still allow some light to pass through.	the ethylcellulose based oleogels tend to be transparent or translucent, allowing light to pass through, and in the concentration used the translucent presents homogeneity.
Shine	the beeswax can provide a natural shine to the oleogels surface, giving it a glossy or satin-like finish. This can enhance the visual appeal of the product.	the ethylcellulose oleogels exhibit a certain level of glossiness on the surface. The presence of the gelling agent contributes to the reflective properties of the gel, resulting in a shiny appearance.
Homogeneity	the oleogel has a uniform appearance, without any visible separation or clumps. Proper formulation and thorough mixing of the ingredients is essential to achieve a consistent texture throughout the product.	although oleogels are technically semi-solids, they can resemble solid fats in their visual appearance. The addition of ethylcellulose allows the oil to take on a solid-like form, giving the oleogel a more rigid and structured appearance.

a. Pumpkin and rapeseed oil oleogels with 10% BW

The visual appearance of oleogels made from a mixture of pumpkin and rapeseed oil with 10% BW (Figure 3) can vary depending on several factors such as the formulation, preparation, and storage conditions. Table 10 shows the main visual characteristics of the samples.

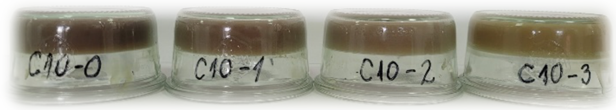

Figure 3. The visual appearance of pumpkin and rapeseed oil oleogels with 10% BW

b. Pumpkin and rapeseed oil oleogels with 10% EC

The method of visual appearance for oleogels made with pumpkin and rapeseed oil using 10% EC (Figure 4) involves observing the physical characteristics of the resulting gel. Table 10 shows the main visual characteristics of the samples.

Figure 4. The visual appearance of pumpkin and rapeseed oil oleogels with 10% EC

CONCLUSIONS

The present results are in good agreement with the ones previously published by other authors proving that EC and BW are efficient oleogelators for obtaining oleogels. Moreover, the mechanical properties of EC and BW oleogels can be manipulated, by adjusting some parameters, such as the type of oil and oleogelator concentration. An important aspect that should be considered when oleogels are used in food products is that for developing the right texture, it is essential to optimize the properties of the formulated oleogel to meet the desired expectations for consumers' satisfaction.

Our preliminary results indicate that pumpkin seed oil, due to its higher content of polyunsaturated fatty acids (≈ 61%) compared to rapeseed oil (≈28%), forms oleogels with a higher consistency, which might be considered an advantage for future applications.

For choosing the optimal oleogelator, additional studies are needed as the results indicate that each of the oleogelators used in the present study has advantages and disadvantages. Moreover, oleogels obtained with BW are processed at lower temperatures, which can positively influence the oxidative stability of the oleogel, while oleogels obtained with EC present a higher consistency and a more pleasant appearance.

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