Development and characterisation of olive oil based spreads containing different seasonings

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The main objective of the present study was to produce olive oil-based table spreads enriched with different spices by using the organogel technique and to investigate the effect of spices on the oleogel network formed by the self-assembly of natural waxes. For that reason, the oleogels were prepared with sunflower wax, beeswax and shellac wax, and enriched with poppy seeds, thyme, lemon peel, mint, and their physico-chemical, thermal, textural and structural properties were determined. In order to determine the storage stability, peroxide, acid and colour values were monitored during 90 days of storage at +4°C. The sunflower oleogels with and without additives had higher oil binding capacity, firmness and stickiness values than the beeswax and shellac wax oleogels. The sunflower and shellac wax oleogels had peaks around 3.70 and 4.10 Å that indicated presence of beta prime polymorphic form. Both enriched and plain oleogels prepared with sunflower wax remained stable even at the end of the storage period at 5% wax addition level. Similar results were observed in the beeswax and shellac wax oleogels without spices. Particularly, the oil-binding capacity, stability, firmness and stickiness values of the oleogels were influenced by spice addition. In conclusion, sunflower wax was found to be more suitable than beeswax and shellac wax for the formation of oleogels with additives, such as spices, at 5% wax addition level.

Keywords: Oleogels, Olive Oil, Natural waxes, X-ray diffraction, Particle size.

INTRODUCTION

Olive oil is one of the most beneficial oils and an indispensable item of the Mediterranean diet. Olive and olive tree are included in both mythology and the Holy Scriptures such as the Quran, the Bible, and the Torah. Olive oil is obtained from the olive that is the fruit of *Olea europaea* L., and its production dates to 4000 BC according to the archaeological data. Olive oil is obtained through a process which uses only mechanical processes; hence, it consists of fatty acids and triglycerides as well as minor ingredients such as hydrocarbons, sterols, phenolic compounds, volatile compounds, waxes, fatty alcohols, mono- and diglycerides, and pigments. Moreover, the major fatty acid is oleic acid (60-85%; C18:1n-9) which is the monounsaturated fatty acid and is known as omega-9 fatty acid. All these features make it superior, beneficial, healthier and more stable against oxidation compared to refined oils [1 - 3].

Olive oil is mostly consumed by adding seasonings in Aegean- and Mediterranean-type diets, and currently sold commercially in oils with seasonings. Gambacorta et al. [4] reported that the addition of some spices to olive oil was an ancient tradition that had an effect not only on flavour but also on shelf life and nutritional value. For this purpose, thyme, marjoram, rosemary, red pepper, basil, lavender, sage, mint, chili pepper, gooseberry and lemon were used as flavouring agents [5, 6]. Thyme is a good source of bioactive compounds, carvacrol, and thymol which are generally safe for consumption.

These compounds are used in dental applications, and the food and feed industry due to antiseptic, antibacterial and antiviral features [7]. Thyme is one of the most popular spices, particularly in the last decade, and some scientific studies in literature suggest that it could be used to reduce the symptoms of COVID-19 [8 - 10]. Poppy plant is grown for its seeds and opium. Poppy seed contains up to 50% oil as well as various bioactive compounds. Poppy seeds are widely used for confectionary, bakery, and extraction of oil [11, 12]. Dried mint leaves were used in ancient times and found in Egyptian pyramids in 1000 BCE. Mint was effective on the digestive system, and is widely used against the asthma, chest problems, and mouth ulcers [13]. Dried lemon peel contains polyphenols, dietary fibre, and volatile compounds due to its aromatic compounds used as a flavour enhancer in many kinds of food formulations [14].

Organogels have stood out as a popular technique in structuring liquid oils, especially in recent years. Organogels are defined as organic solvents entrapped in a three-dimensional network formed by organic gelators. Organogels are called oleogels when the liquid phase was oil. In recent years, many kinds of oils and gelators have been used for the preparation of oleogels. The most important advantages of oleogels are that they do not change the composition of fatty acids, have low saturated fat content, do not contain *trans*-fat, are spreadable and have plastic properties. These advantages not only meet consumer need but also increase the usage area of liquid oil [15, 16].

In literature, oleogels produced with natural waxes have been reported as being an alternative to spreadable margarine and butter [17 - 23]. However, spice-enriched oleogels (containing additives with different particle sizes and solid particles) were not used in the studies mentioned above, and their stability and structural form were not tested. Recently, Yılmaz and Demirci [24] reported common characteristic properties of virgin olive oil-sunflower oleogels enriched with thyme and cumin spices. Nevertheless, there were no detailed information on how different particle sizes (in the added additives) affected the gel structure and stability in previous studies.

The actual target of this study was to produce natural wax-olive oil oleogels enriched with spices and to determine the effects of spice addition on oleogel structure and stability. To the best of our knowledge, this study is the first report observing the effects of additives with different particle sizes on oleogel formation. In this context, we think that this study will be a guide for determining the different usage potential and purposes of oleogels.

MATERIALS AND METHODS

Olive oil was purchased from local producers in Ezine, Çanakkale, Turkey. Natural waxes (sunflower, shellac and beeswax) were purchased from KahlWax (Kahl GmbH & Co., Trittau, Germany). The producer provided the melting point for beeswax as 61-66°C, sunflower as 74-80°C, and shellac wax as 78-84°C. All the spices (mint, thyme, poppy seed and lemon peel powder) were purchased from a local herbalist (Çanakkale, Turkey). All other chemicals used in analysis were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, USA), and all chemicals were of an analytical grade.

PREPARATION OF THE OLEOGELS

All the prepared oleogels contained 5% waxes (sunflower, shellac and beeswax) and 1% (mint, thyme, lemon peel powder and poppy seeds) spices with 94% olive oil. The amounts of added waxes and spices were determined by preliminary trials. For the oleogel preparation, the waxes were completely melted at 85-90°C on water bath, and at the same time, the olive oil was heated at the same temperature. Then, the olive oil was added into the wax at isothermal conditions, and the mixture was stirred. After these procedures, spice was added into the mixture and stirred until the first crystal formation was observed. At the end of the process, the mixture was cooled at room temperature overnight and stored at 4°C until the planned analyses were performed. Formulations, sample codes of the oleogels, and particle size of used additives are given in Table I.

PHYSICOCHEMICAL ANALYSES

The crystal formation time (CFT), and oil-binding capacity (OBC) of all the oleogel samples were measured according to Öğütcü et al. [21]. The minimum gel formation (MGF) concentration represents the minimum wax addition level (%) that formed stable gel. The centrifuge stability test (CST) values of the samples were measured 1500 g centrifugation for 10 min at room temperature and results expressed +/- (+; no phase separation/ -; phase separation). Acid value of the samples were measured according to AOCS (Ca 5a-40) method [25]. Approximately 2.5 g samples dissolved in ethanol: diethyl ether (1:1) and titrating with ethanol-KOH solutions against the phenolphthalein indicator and results were expressed mgKOH/g. Peroxide value was measured by AOCS (Cd 8-53) method [25]. For the determination of PV, approximately 2.0 g sample dissolved in acetic acid: chloroform (3:2) and added potassium iodide in darkness, then titrating with a sodium thiosulfate solution for free iodine titration. The PV results were expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg). Iodine value (IV) was measured by AOCS (Cd 1-25) method [25] using with Wijs solutions and results were expressed as gI_2/100g.

The colour measurements of the samples were performed using a colorimeter (Konica, Minolta CR-400, Osaka, Japan) and L (lightness), a (+redness/-greenness) and b (+yellowness/-blueness) values were

Table I - Formulations of the produced oleogels and particle size distribution of seasonings used in oleogels

Samples	VOO (%)	SW (%)	BW (%)	SH (%)	Poppy Seeds (%)	Thyme (%)	Lemon Peel (%)	Mint (%)
SW	95	5.0	-	-	-	-	-	-
SWP	94	5.0	-	-	1.0	-	-	-
SWT	94	5.0	-	-	-	1.0	-	-
SWL	94	5.0	-	-	-	-	1.0	-
SWM	94	5.0	-	-	-	-	-	1.0
BW	95	-	5.0	-	-	-	-	-
BWP	94	-	5.0	-	1.0	-	-	-
BWT	94	-	5.0	-	-	1.0	-	-
BWL	94	-	5.0	-	-	-	1.0	-
BWM	94	-	5.0	-	-	-	-	1.0
SHW	95	-	-	5.0	-	-	-	-
SHP	94	-		5.0	1.0	-	-	-
SHT	94	-		5.0	-	1.0	-	-
SHL	94	-		5.0	-	-	1.0	-
SHM	94	-	-	5.0	-	-	-	1.0

Samples	5-2 mm (%)	2-1 mm (%)	1000-500 µm (%)	500-100 µm (%)	100-63 µm (%)	63-20 µm (%)	Sample Amount (g)
Mint	5.20	34.20	45.53	15.07	Nd	Nd	16.90
Thyme	21.21	64.57	13.64	0.58	Nd	Nd	16.01
Poppy Seeds	Nd	Nd	Nd	100.00	Nd	Nd	38.55
Lemon peel powder	Nd	Nd	1.89	56.02	16.73	25.37	19.69

VOO: virgin olive oil, SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint, Nd: not detected.

determined. The colour differences (delta E) of the samples were calculated from recorded CIE lab data. The acid, colour and peroxide values of the oleogels samples were measured once a month during the 90-day storage period at 4°C, while the other physicochemical analyses were conducted only with fresh samples. The measurement of the particle size distribution (PSD) of seasonings was performed by sieving (Retsch AS200 Retsch Technology, Haan, Germany).

STRUCTURAL ANALYSES

The textural properties of the oleogel samples were measured using texture analyser (TA-HD Plus, Stable Microsystems, UK), the texture analyser equipped with spreadability rig, and the results were calculated using the instrument software (Texture Exponent v.6.1.1.0, Stable Microsystems, UK). For the spreadability test, the oleogel samples were filled sufficiently into conic spreadability cup and waited for 15 min at room temperature, and the measurements were applied at this temperature. The spreadability test specifications were test mode compression, test speed at 3.0 mm/sec, post-test speed at 10 mm/sec and distance 23.00 mm. The XRD measurements of the oleogels samples were measured using a PANalytical empyrean XRD (PANalytical, Netherlands). The angular scans from 2.0° to 50° 2-theta range were performed by 2°/min scan rate. A Cu source x-ray tube (λ = 1.54056 Å, 45 kV and 40 mA) and an X'Pert Highscore Plus software were used for the data analysis. The macro images of the oleogels were taken with stereomicroscope (Zeiss, Stemi 305, Germany) equipped with a digital camera (Argenit, Kameram image processing system, Turkey).

THERMAL ANALYSES

The thermal properties of the enriched oleogels samples were evaluated using Differential Scanning Calorimeter (DSC7020, Hitachi High-tech Science Corporation, Japan), as explained in detail by Öğütcü et al. [21]. 5-7 mg oleogel samples were weighted into aluminium pan that was also used as standard (without sample). The samples were heated from 30°C to 120°C heating rate at 15°C/min and held at 1 min at this temperature for removal to water, and then the samples were cooled from 120 to -20°C cooling rate at 10°C/min and held at 3 min at this temperature for the crystallisation process of the oleogels to complete. After this section, the samples were heated again from -20°C to 100°C at 5°C/min heating rate, and the crystallisation and melting *onset*, *peak* temperatures and *enthalpy* values of the oleogels were calculated using the DSC software (TA7000 Measurement 10.5v, Hitachi High-Tech Science Corp.).

STATISTICAL ANALYSES

The oleogels prepared in the study were in two replications, and the planned analyses for these samples were performed at least three times. The data collected from the study were evaluated using the MINITAB statistical software programme [26]. The results were presented as mean and standard deviation. The similarities and differences between the samples were evaluated using the Analysis of Variance (ANOVA) with Tukey multiple tests.

RESULTS AND DISCUSSIONS

The particle size distribution and average particle size influenced the rheology, viscosity, texture spreadability, stability, and mouthfeel properties of the emulsion and suspension [27]. The PSD of the seasonings in dry form is presented in Table I. Considering the particle size distribution of the mint, 45.53% consisted of 1000-500µm particles, while 34.20% contained 2-1 mm particles and 15.07% contained 500-100µm particles. The PSD of the thyme was formed mostly by 2-1 mm (64.57%), 5-2 mm (21.21%) and 1000-500 µm (13.64%) particles. Unlike thyme and mint, the PSD of the poppy seed was homogeneous and consisted of 500-100 µm particles. On the other hand, the PSD of the lemon powder was smaller than that of the others, and consisted of 500-100 µm (56.02%), 100-63 µm (16.73%) and 63-20 µm (25.37%) particles. The results showed that the PSD of the seasonings used in the prepared oleogels was quite different from one another.

The minimum gel concentrations (MGC), centrifuge stability test (CST), crystal formation time (CFT), oil-binding capacity (OBC) and iodine values (IV) of the oleogels are given in Table II. The MGC values of the sunflower (SW) beeswax (BW) and shellac waxes (SHW) were 1.0, 3.5 and 4.0%, respectively. In the literature, it was reported that, depending on the wax compositions, the critical gel concentrations of the SW, BW and SHW were 0.5, 2.0-3.0 and 5.0%, respectively [28, 29]. The CFT values of the plain SW, BW and SHW oleogels were 1.38, 7.42 and 16.50 min: s, respectively. Additionally, the plain oleogels had lower CFT values compared to the oleogels with additives (Table II). The CST results showed that the plain BW, SW and SHW oleogels were stable (no phase separation) at 5% wax addition level at room temperature. On the other hand, a phase separation was observed in the enriched oleogels prepared with BW and SHW (SHT and SHM) at 5% added wax concentration at room temperature, except the enriched SW-based oleogels. Öğütcü et al. [21] reported that the CFT values of the enriched and aromatised virgin olive oil prepared with 5% of SW and BW oleogels were 3.50 and 6.50 min:s, respectively. Fayaz et al. [30] reported that the gelling time of oleogels containing 5-15% of BW ranged between 3.3-4.8 min. The OBC values of the BW, SW and SHW oleogels enriched with spices ranged from 10.42 to 96.03%, while the OBC values of the oleogels without additives were > 97%. Like our findings, previous research reported that the OBC values of the 5% SW and BW oleogels prepared with olive oil were ≥ 97% [21, 22]. The enriched SW oleogels had higher OBC values compared to the enriched BW and SHW-based oleogels. These results showed that solid particles added to oleogels affected the OBC, CFT and CST values of the oleogels and decreased OBC and CST and increased CFT values (Table II). Furthermore, it also showed that enriched shellac and beeswax may have higher OBC and lower CFT value at higher concentration level than 5%. In other words, when it is desired

Table II - Physico-chemical parameters of oleogels prepared with different waxes and spices

Samples	MGC (%)	CST	CFT (min:s)	OBC (%)	IV (gI$_2$/100g)
SW	1.0	(+:+)	1:38	99.70±0.26Aa	46.58±3.75b
SWP	1.0	(+:+)	1:42	93.00±0.81b	
SWT	1.0	(+:+)	1:44	94.20±1.45b	
SWL	1.0	(+:+)	1:40	96.03±1.47ab	
SWM	1.0	(+:+)	1:40	93.05±1.44b	
BW	3.5	(+:+)	7:42	99.83±0.07Aa	58.15±3.74a
BWP	3.5	(-:-)	8:00	10.42±1.22b	
BWT	3.5	(-:-)	8:00	15.99±0.55b	
BWL	3.5	(-:-)	8:00	12.89±2.20b	
BWM	3.5	(-:-)	8:00	17.56±3.40b	
SHW	4.0	(+:+)	16:50	97.58±0.21Ba	60.87±6.82a
SHP	4.0	(+:+)	17:00	53.20±0.48c	
SHT	4.0	(+:-)	17:00	63.07±3.33b	
SHL	4.0	(+:+)	17:00	47.70±1.08c	
SHM	4.0	(-:-)	17:00	61.66±1.36b	

MGC: minimum gel concentration, CST: centrifuge stability test, CFT: crystal formation time, OBC: oil-binding capacity, IV: iodine value, SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

*The capital letters show differences among used waxes and the lower case letters show differences among the samples prepared with the same wax (p≤0.05).

Table III - Acid (AV), peroxide (PV), delta E values of the fresh and 90 days stored oleogels prepared with different waxes and spices.

Samples	AV (mgKOH/g)		PV (meqO_2/kg)		Delta E
	Fresh	Stored	Fresh	Stored	
SW	1.98±0.10a*	1.78±0.03ab	14.08±0.16cd	17.45±0.22a	3.83±0.04a
SWP	1.57±0.06ab	1.46±0.01b	15.44±0.26bc	16.13±0.16ab	2.92±0.02b
SWT	1.77±0.19ab	1.48±0.10b	13.86±1.16cd	15.01±0.07bc	3.05±0.03b
SWL	1.78±0.04ab	1.63±0.02ab	15.18±0.25bc	15.07±0.11bc	3.85±0.08a
SWM	1.40±0.26b	1.55±0.02ab	12.45±0.42d	17.42±0.14a	4.08±0.17a
BW	2.03±0.09a	1.93±0.01ab	17.51±0.34bc	17.36±0.49bc	2.44±0.01c
BWP	1.63±0.01c	1.67±0.01bc	14.92±0.27cd	17.04±0.05bcd	5.83±0.03a
BWT	1.66±0.08c	1.76±0.09bc	14.66±0.70cd	21.41±0.67a	5.16±0.02b
BWL	1.57±0.04c	1.67±0.02bc	14.08±0.58d	20.54±0.39a	5.10±0.07b
BWM	1.81±0.13abc	1.60±0.04c	14.73±1.98cd	18.78±0.02ab	2.00±0.08d
SHW	1.93±0.05a	1.72±0.13ab	19.74±1.11cde	21.43±0.56bc	6.37±0.06c
SHP	1.76±0.04ab	1.82±0.06ab	17.46±0.04def	19.80±0.37cd	6.18±0.01c
SHT	1.60±0.19b	1.82±0.03ab	17.20±1.06def	21.39±0.85bc	6.08±0.31c
SHL	1.65±0.08ab	1.85±0.01ab	17.08±0.55ef	23.07±0.15b	7.43±0.05b
SHM	1.59±0.03b	1.77±0.03ab	16.22±0.75f	26.03±0.39a	9.06±0.01a
OO**	1.97±0.14	1.94±0.01	18.67±0.17	21.82±0.13	Nd

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint. OO: Olive oil: Nd: Not detected.
* The lower case letters show differences among the samples prepared with same wax on the same column and show the differences between fresh and stored samples on the same line ($p \leq 0.05$).
** Free fatty acid values of the fresh and stored olive oil were 0.99 and 0.97% oleic acid, respectively.

to form a gel with particle-containing additives, more than 5% level of beeswax and shellac wax should be added. The results indicated that sunflower wax compared with beeswax and shellac wax could be effective to form oleogels with spices or like additives particularly at 5% addition level and even at lower concentrations. Like the CFT, CST and MGC values, the OBC values mostly changed depending on the composition, addition level and type of the waxes [29, 31]. Additionally, shear and cooling rate were effective on the OBC values of the oleogels, as similarly reported by Blake and Marangoni, [32, 33].

Iodine value (IV) is defined as a parameter indicating the degree of saturation/unsaturation of oils and fats [34]. The IV value of the olive oil was 84 gI_2/100g, and our findings were within legal limit (75-94 gI_2/100g) according to Codex Stan., 33 [34]. The IV values of the oleogels samples ranged from to 46.58-60.87 gI_2/100g. There were no specifications about iodine limit for butter and margarine in Codex Alimentarius, although in literature, the IV values of butter and margarine were reported as 29.70 and 57.85, respectively [23]. These results showed that the addition of 5% wax reduced the iodine values of the gels to the iodine values level of margarine and butter.

The acid (AV), peroxide (PV) and delta E values of the fresh and 90-day stored oleogels samples and olive oil are given in Table III. The FFA value is an important parameter, particularly for olive oil and olive oil-based products, because the FFA value is used in pricing and classification [35]. The FFA values of the fresh and stored olive oil were 0.99 and 0.97% oleic acid, respectively. The FFA values are given as 0.8, 2.0 and 3.3 for extra virgin olive oil, virgin olive oil and ordinary virgin olive oil in Codex Stan 33, respectively [34]. The olive oil used in this study was classified as virgin olive oil according to Codex Stan [34]. The limits of the acid value for the virgin and cold-pressed fats and oils were up to 4.0 mg KOH/g according to Codex Stan. [35]. The AV values of the fresh and stored samples ranged between 1.40-2.03 and 1.55-1.93 mg KOH/g, respectively. The acid values of the samples were found within legal limits according to Codex Alimentarius Standard [35]. The used additives affected the acid values of the samples (Table III). On the other hand, the statistical evaluations indicated that storage time and wax types were effective on the acid values of the samples ($p \leq 0.05$). The peroxide value is a parameter that shows the degree of oxidation in oils and is an indicator of whether the oil is stored under suitable conditions [36]. The PV values of the fresh samples ranged from 14.08 to 19.74 meqO_2/kg, while the stored samples ranged from 15.01 to 26.03 meqO_2/kg. The PV limits of the Codex Alimentarius Standard for olive oil (33-1981) and cold-pressed fats and oils subjected to modification and fractionation (19-1981) were up to 20 meqO_2/kg and 15 meqO_2/kg,

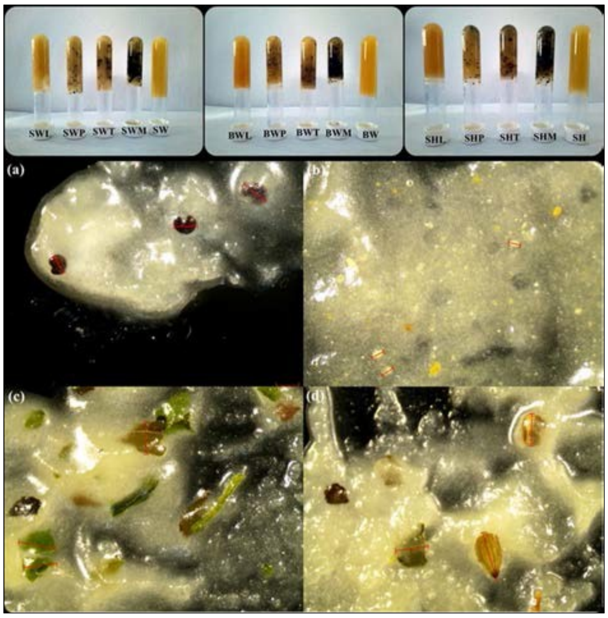

Figure 1 - Oleogels and stereomicroscope images (a) poppy seeds (b) lemon powder (c) mint and (d) thyme leaves in SW-based oleogels.

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

respectively [34, 35]. The fresh SW, SWT, SWM and the BW oleogels with spices were within legal limits according to Codex Stan., [35], and all the fresh oleogels were found within legal limits according to Codex Stan. [34]. On the other hand, all the stored oleogel samples were over legal limit as set by Codex Stan. [35], while the stored BW, BWP, BWM and the SW-based oleogels were within limits as determined by Codex Stan [34]. Additionally, the PV values of the oleogels increased depending on the storage time, and the SW-based oleogels had lower PV values than the BW and SHW oleogels at the end of the storage (Table III). The results indicated that storage time, used waxes and spices affected the PV and AV values of the oleogels. The differences among the PV and AV values of the oleogels may be explained with the differences among the PV and AV values of the used waxes. Delta E (colour differences) was used to determine the overall colour changes between the fresh and stored oleogels. The delta E values of the sunflower-, beeswax-, and shellac wax-based oleogels were 2.92-4.08, 2.00-5.83 and 6.08-9.06, respectively. The oleogels prepared with shellac wax had higher delta E values, while the oleogels with sunflower wax had lower delta E values at the end of the 90-day storage period.

The SW, BW and SHW based oleogels with different seasonings, and stereomicroscope images of SW-based oleogels are given in Figure 1. According to stereomicroscope images, the particle sizes of the lemon peel powder, poppy seeds, mint and thyme leaves used as additives were an average of 90.54, 162.16, 236.13 and 259.52 µm, respectively.

Similar to sieving results, the thyme leaves had the highest particle size, while the lemon peel had the lowest particle size than the other additives. Genovese et al. [37] indicated that food dispersion containing 10-100 µm solid particle size was classified as microscopic/non-colloidal, while food dispersions containing solid particle size over 100 µm were classified as macroscopic non-colloidal. The oleogels enriched with lemon peel were an example of microscopic dispersion, while the other oleogels with spices was an example of macroscopic dispersion, according to the definition of Genovese et al. [37]. The XRD patterns, firmness and stickiness values of the oleogels are shown in Table IV. The oleogels prepared with sunflower and shellac waxes had short spacing peaks around 3.70, 4.10 and 4.50 Å (Table IV). Additionally, the oleogels prepared with beeswax showed short spacing peaks around 4.50 and 4.10 Å. The oleogels prepared with beeswax had no peaks around 3.70 Å. The XRD peaks around 4.10 and 3.70 Å indicated the presence of β' prime polymorphic form [31, 38]. β' is characterised by melting at body temperature and providing a stable and smooth texture; hence, it provides to create the desired texture in margarines and various spreads [31]. On the other hand, the short spacing around 4.50 Å indicated the presence of β-form, while the peak around 4.10 Å indicated presence of α-form [38]. The XRD results of the shellac, sunflower and plain beeswax (without spices) oleogels showed that the oleogels had three polymorphic forms (α, β and β') characterized with presence of short spacing around 3.70, 4.10 and 4.50 Å. On the other hand, the spice-added beeswax oleogels were associated with α polymorphic form according to XRD patterns. The melting point, crystalline structure, and stability of polymorphic forms are different from each other, and unit cells of the alpha, beta and beta prime polymorphic forms have been associated with hexagonal, orthorhombic and triclinic packing, respectively [31, 38]. It has been pointed that α polymorphic form has a low melting point and is less stable than the β and β' forms. Additionally, the β-form has been reported to be an undesirable form for margarine and table spreads due to the rough and uneven texture [38]. Furthermore, like our findings, Da pieve et al. [39] reported that the broadness of the peak of the monoglyceride oleogels (around 24.20 and 4.50 Å) was due to amorphous scattering related to liquid-state triacylglycerol molecules that consisted of approximately 95% of the gel.

The firmness values of the plain oleogels prepared with BW, SW and SHW were 161.35, 885.20 and 70.22 g, respectively. The firmness values of the enriched oleogels formed with BW, SW and SHW were 34.82-78.02, 327.22-569.70 and 69.46-55.09 g, respectively. Furthermore, the stickiness values of the plain BW, SW and SHW gels were 214.03, 1100.30 and 73.43 g, respectively, while those of the enriched oleogels were 46.34-101.69, 363.83-575.60 and 60.98-66.10 g, respectively. Both plain and enriched SW gels had higher firmness and stickiness values than the oleogels formed with BW and SHW (Table IV). In terms of the firmness and stickiness values of the oleogels, there were statistically significant differences among the plain BW, SW and SHW-based oleogels, and similar results were observed in the enriched oleogels ($p \leq 0.05$). These results proved that addition of macroscopic and microscopic solid particles, such as spices, was effective on the textural features of the oleogels. It was concluded that 5% wax addition level was enough to form a stable plain oleogel structure for BW, although it was not enough to form SHW-gels with and without spices. As a result, SW was the most effective gel agent among the waxes to provide a stable gel structure with an additive containing macroscopic and microscopic solid particles at 5% addition level.

On the other hand, additive levels of 5% or more should be preferred for BW and SHW, particularly for the oleogels prepared with additives containing both macroscopic and microscopic solid particles. Similar to our findings, Fayaz et al. [30] reported that firmness of oleogels with 5% BW were 0.74 N. Similar results observed in the firmness and stickiness values of the enriched and aromatized hazelnut and olive oil oleogels prepared with 5% of beeswax and sunflower wax [21, 22]. Additionally, many of the previous research indicated that oleogels prepared with different oils and natural waxes may replace commercial breakfast margarine, spreads or hydrogenated fats in terms of firmness and stickiness values [18, 19, 23, 29].

Melting point is an important parameter, particularly together with spreadability for fat-based spreads. The desired and preferred melting points of fat-based spreads were body temperature (35-36°C). The melting point of these types of products is important for flavour release and consumer acceptance [40, 41]. The thermal properties of the oleogels prepared with BW, SW, SHW waxes and the addition of thyme, mint, lemon peel and poppy seeds are given in Table V. The crystallization point of the control group of the BW oleogel was 24.20°C, whereas that of the SW oleogel was 53.00°C and the SHW oleogel was 51.90°C. The crystallization points of the BW, SW and SHW oleogels prepared with spices were 18.20-31.50, 46.20-54.10 and 44.00-47.30°C, respectively. The SW and SHW oleogels had a higher crystallization point than the BW oleogels ($p \leq 0.05$). On the other hand, the melting point of the BW, SW, and SHW oleogels without spices was 28.80, 59.90 and 69.30°C, respectively. The melting points of the SW, BW and SHW oleogels enriched with seasonings ranged between 54.40-60.90°C, BW - 22.30-38.80°C and SHW - 62.00-66.30°C, respectively. Similar findings were reported by Patel et al. [42] for shellac wax oleogels and by Öğütcü et al. [21] for SW oleogels. Furthermore, the SHW oleogels had a higher melting point than the BW and SW oleogels at 5% added wax concentration. Previous research indicated that there was a linear relationship between the melting points of oleogels and the melt-

Table IV - XRD patterns and textural features of the oleogels.

Samples	2-theta	d (Å)	Firmness (g)	Stickiness (g)
SW	3.92:19.44:21.38:23.93	22.49:4.56:4.15:3.71	885.20±24.20*Aa	1100.30±86.00Ac
SWP	3.92: 19.23:21.58:23.72	22.49:4.61:4.11:3.74	327.22±12.92c	368.83±13.16a
SWT	4.12:19.64:21.98:24.13	21.38:4.51:4.09:3.68	500.30±28.00b	528.32±7.87ab
SWL	3.97:19.47:21.51:23.76	22.23:4.55:4.12:3.74	505.24±2.75b	530.54±12.33ab
SWM	4.74:19.23:21.27:23.62	18.62:4.61:4.17:3.76	569.70±25.30b	575.60±60.60b
BW	19.13:20.66	4.63:4.29	161.35±8.32Ba	214.03±9.53Bd
BWP	4.53:19.54:21.48	19.46:4.53:4.13	34.82±0.04d	46.34±0.31a
BWT	19.74:21:78	4.49:4.07	48.37±4.32cd	63.13±7.00ab
BWL	4.12:19.13:21.27	21.38:4.63:4.17	65.51±7.84bc	85.61±13.12bc
BWM	4.59:19.23:21.27	19.45:4.61:4.17	78.02±6.79b	101.69±8.76c
SHW	3.31:19.54:21.58:23.83	26.65:4.53:4.11:3.73	70.22±2.36Ba	73.43±13.16Ba
SHP	19.23:20.46:21.78:24.13	4.61:4.33:4.07:3.68	60.89±0.57a	66.10±1.44a
SHT	4.02:19.54:21.38:23.83	21.92:4.53:4.15:3.73	69.46±12.87a	68.74±3.70a
SHL	19.74:21.68:23.93	4.49:4.09:3.71	55.09±5.85a	60.98±6.15a
SHM	19.49:21.40:23.75	4.55:4.14:3.74	56.35±2.21a	62.67±3.14a

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

*The capital letters shows differences among the used waxes and the lower case letters shows differences among the samples prepared with same wax on the same column ($p \leq 0.05$).

Table V - Thermal properties of oleogels prepared with different waxes and spices

	Crystallization			Melting		
Samples	**Onset*c* (°C)**	**Peak *Tc* (°C)**	**Delta H*c* (j/g)**	**Onset*m* (°C)**	**Peak *Tm* (°C)**	**Delta H*m* (j/g)**
SW	58.60	53.00	-2.59	47.40	59.90	1.38
SWT	60.70	54.10	-7.44	46.50	60.90	2.51
SWP	58.00	51.90	-2.99	52.70	59.80	1.10
SWL	57.40	52.00	-1.73	48.90	59.70	0.61
SWM	51.90	46.20	-0.71	46.00	54.40	0.13
Mean±Sd	57.32±2.93a	51.44±2.74a	-3.09±2.31a	48.30±2.41b	58.94±2.31a	1.15±0.80a
BW	32.20	24.20	-0.30	19.60	28.80	0.15
BWT	31.90	18.20	-0.26	15.40	22.30	0.13
BWP	36.00	27.40	-0.39	20.00	31.70	0.04
BWL	34.60	30.50	-0.30	23.50	38.00	0.16
BWM	37.50	31.50	-0.34	25.20	38.80	0.25
Mean±Sd	34.44±2.16c	26.36±4.81b	-0.20±0.25b	20.74±3.40c	31.92±6.11b	0.15±0.07b
SHW	55.30	51.90	-1.91	60.20	69.30	1.22
SHP	52.50	46.50	-0.53	59.00	66.00	0.47
SHT	50.70	47.10	-0.63	67.40	62.00	0.48
SHL	49.30	44.00	-0.38	59.70	65.80	0.21
SHM	53.00	47.30	-0.48	59.20	66.30	0.27
Mean±Sd	52.16±2.05b	47.36±2.56a	-0.79±0.57ab	61.10±3.18a	65.88±2.32a	0.53±0.36ab

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.
*The small letters shows differences among the oleogels on the same column in terms of thermal properties ($p \leq 0.05$).

ing points of the waxes used [18, 29, 42]. Karabulut and Turan [43] showed that the slip melting point of margarines ranged between 31.2-34.9°C. It was determined that the oleogels produced with BW had the closest melting point to margarine, while the SW and SHW oleogels had higher melting points than margarine.

CONCLUSION

Different studies have been conducted on oil structuring, particularly on organogelation, in recent years. The present study focused on the effect of spices with different particle sizes on the structure and stability of oleogels. The results showed that both the used waxes and additives affected the oil-binding capacity, crystal formation time and centrifuge stability values of the oleogels and that the additives decreased oil binding capacity and stability and increased crystal formation time. Not only the additives and waxes but also the storage time affected the acid, peroxide, and colour values of the oleogels. The sunflower and shellac wax oleogels with and without additives had a beta prime polymorphic form according to the XRD patterns of the oleogels. The firmness and stickiness values of the prepared oleogels indicated that sunflower formed stable oleogels with spices at 5% addition level.
The thermal results showed that beeswax had a lower melting point than sunflower and shellac wax at the same wax concentration. As a result of the study, when it is desired to create a stable oleogel structure with additives containing flavouring substances and/or similar particles, 5% or less addition rates are sufficient for sunflower wax, while this ratio is recommended to be higher than 5% for BW and SHW waxes.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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