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Physicochemical properties and oxidative stability of oleogels made of carnauba wax with canola oil or beeswax with grapeseed oil

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Abstract Two types of oleogels—made of carnauba wax with canola oil or beeswax with grapeseed oil—were prepared at concentrations from 0 to 15% (w/w) of wax. Physical characterization was done and oxidative stability of the oleogels were evaluated. As the proportion of wax increased from 5 to 15%, the enthalpy of crystallization and melting increased in both oleogels. The carnauba wax-based oleogel (CWO) required greater enthalpy than the beeswax-based oleogel (BWO). Differences in L*, a*, and b* values between control oils and the oleogels significantly decreased as the concentration of wax increased in the oleogels (5–15%; $p<0.05$). Oil-binding capacity of the BWO was higher than that of the CWO. Solid-fat content of the CWO did not change significantly from 10 to 60°C, whereas that of the BWO decreased. In general, oxidative stability of the CWO was better at 60 and 180°C heat treatment in comparison with control oils ($p<0.05$). However, the BWO did not provide high oxidative stability than the control oils.

Keywords: oleogel, carnauba wax, beeswax, physicochemical property, oxidative stability

Introduction

An oleogel is defined as an organic liquid entrapped by a low-molecular-weight oleogelator and/or polymeric gelator. An oleogel is thermally reversible, three-dimensional, self-standing, anhydrous, and viscoelastic materials (1,2).

Oleogelators, which can entrap oils, can be classified into two systems: a self-assembly system and a crystal particle system (3). Oleogelators based on the self-assembly system can self-organize in oils themselves. These oleogelators are sorbitan monostearate, ceramides, monoacylglycerols, and mixtures of a phytosterol and oryzanol. In contrast, oleogelators based on the crystal particle system form nuclei with subsequent growth of the crystals. These oleogelators are fatty acids, fatty alcohols, wax esters, dicarboxylic acids, high-melting-point triacylglycerols, and plant waxes including carnauba wax, candelilla wax, and rice bran wax (4).

Fat sources such as palm kernel oil, palm oil, coconut oil, and hydrogenated oil as well as butter, margarine, and shortening have a high proportion of saturated fat and/or varied concentrations of trans fatty acids (5,6). Saturated and trans fatty acids are known to pose health risks such as cardiovascular diseases (e.g., coronary heart disease) (5,7-9); therefore, avoiding foods that are rich in these fats is strongly recommended (7,10). Oleogelation is one of the promising strategies for lowering the saturated- and trans-fat content in foods (1,6). Oleogels can be applied to many areas because of their solid structure at room temperature and thermally reversible properties. The interest in oleogels has increased in food, cosmetics, and pharmaceutical industries (8).

Jang *et al.* (11) suggested the use of oleogels made of candelilla wax for baking. They reported that cookies made of canola oil oleogels based on candelilla wax have a lower concentration of saturated fatty acids, and the low viscosity of oleogels at the baking temperature contributes to spreadability in baked products. Botega *et al.* (9) stated that rice bran wax oleogels are a possible alternative to milk fats, which are solid fat sources (e.g., in ice cream).

Carnauba wax is obtained from the palm *Copernicia prunifera* (12) and was granted the generally recognized as safe (GRAS) status; this wax can be used for coating fruits and vegetables (4). It comprises a mixture of high-molecular-weight esters of an acid and hydroxyacids (4,13). Beeswax is natural wax from honey bees of the genus *Apis mellifera L.* (14). Its main components are long-chain monohydric alcohol compounds (15,16).

Many studies have been focused on the development of new oleogelators, evaluation of physicochemical properties and stability

of oleogels, and application of oleogels to foods, cosmetics, and pharmaceuticals (10,11,17,18). However, oxidative stability of oleogels based on carnauba wax or beeswax has not been studied yet.

The objectives of this study were to explore the physicochemical properties of oleogels made of carnauba wax or beeswax and to evaluate the oxidative stability of oleogels made of wax at high or low temperatures (180 or 60°C).

Materials and Methods

Materials Carnauba wax (Starlight Co., Fortaleza, Brazil) and beeswax (Hooper pharm GmbH, Hamburg, Germany) were acquired from corporate vendors, whereas canola oil and grapeseed oil were purchased from a local grocery market (Suwon, Korea). Other reagent grade chemicals were obtained from Daejung Chemical Co. (Seoul, Korea).

Sample preparation Canola oil and grapeseed oil in beakers were placed in an oil bath (OB-07; HYSC Co., Ltd., Seoul, Korea) and preheated at 140°C for 5 min. Oleogels were made by dissolving carnauba wax in canola oil or by dissolving beeswax in grapeseed oil at the concentrations of 0, 5, 10, and 15% (w/w) for 5 min and stirring by hand. The mixtures of oil and wax in beakers were cooled down at room temperature for 1 h.

Thermal analysis of the oleogels The physical properties such as onset temperature and the temperature and enthalpy of crystallization and melting were analyzed by differential scanning calorimetry (DSC; DSC4000 with an intracooler, Perkin-Elmer, Waltham, MA, USA). A sealed oleogel sample in a DSC pan (4–8 mg) was heated from room temperature to 140°C at 10°C/min, cooled down to −20°C at 10°C/min, and then maintained at -20°C for 3 min. The sample was then heated to 100°C at 5°C/min.

Solid-fat content (SFC) analysis of the oleogels SFC of oleogel samples was determined according to the American Oil Chemists' Society (AOCS) method (19), Cd 16d-93. Samples were poured into nuclear magnetic resonance (NMR) spectroscopy glass tubes and heated at 100°C for 15 min to obtain the liquid state. The liquid samples were incubated at 60°C for 5 min and at 0°C for 60 min. Furthermore, they were maintained for 30 min at each measurement temperature from 10 to 60°C with 5°C intervals. The SFC values of the samples were determined using a mq20 NMR analyzer (Bruker Co., Rheinstetten, Germany).

Color analysis The color of the oleogels based on wax was determined using a Minolta Chromo Meter CR-400 (Minolta, Tokyo, Japan). The color values were recorded in CIE units: L* (white or brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). The ΔE value was calculated according to Eq. (1):

$$\Delta E=\sqrt{(L^*_{con}-L^*_{sam})^2+(a^*_{con}-a^*_{sam})^2+(b^*_{con}-b^*_{sam})^2} \quad (1)$$

where subscripts, con and sam, indicate control oils and olegel samples, respectively.

Oil-binding capacity (OBC) This property was measured according to a modified method of Yılmaz and Öğütcü (20). Briefly, 1 g of oleogels was placed into weighed (*a*) Eppendorf tubes. The tubes with samples were refrigerated at 4°C for 1 h and then weighed (*b*). Each tube was centrifuged at 9,167×*g* for 15 min at 20°C. The lipid oils were removed from the tubes by decanting, and the remaining sample was weighed (*c*). The OBC was calculated by means of the following formulas:

$$\% \text{ Released oil}=\frac{(b-a)-(c-a)}{(b-a)}\times 100 \quad (2)$$

$$\% \text{ OBC}=100-\% \text{ Released oil} \quad (3)$$

Oxidation of the oleogels made of carnauba wax or beeswax To study the oxidative stability of the oleogels, 1 g of each sample was sealed in a 10 mL headspace vial (Agilent Technologies, Inc., Santa Clara, CA, USA) with a magnetic cap (Agilent Technologies, Inc.). The sample vials were placed in a convection oven (CO-150; HYSC Co., Ltd.) at 180°C for 0, 30, 60, or 90 min and at 60°C for 0, 2, 4, or 6 day. The sample vials were prepared in triplicate at each data point.

To recover the oils from the oleogels, the samples were mixed with 10 mL of a mixture of methanol and 2-propanol (2:1, v/v), shaken using a vertically moving mixer (Taitec Co., Ltd., Saitama-ken, Japan), and centrifuged for 10 min at 100x*g* (VS-5500N; Vision Scientific Co., Ltd., Daejeon, Korea). The upper layer was collected, and the solvent was removed under a stream of nitrogen gas. The samples were prepared in triplicate. Oxidative stability of the oleogel samples was analyzed by means of headspace oxygen content, conjugated dienoic acid (CDA), *p*-anisidine content (*p*-AV), and headspace volatile compounds.

Oxidative stability

***Headspace oxygen analysis*:** The headspace oxygen in the airtight sample vials was analyzed according to the method of Yi *et al.* (21). Headspace gas (30 μL) was removed from each sample vial using an airtight syringe, and oxygen content was determined using a gas chromatograph (GC)-thermal conductivity detector (TCD). A Hewlett-Packard 7890 GC (Agilent Technologies, Inc.) equipped with a 60/80 packed column (3.0 mx2 mm internal diameter [i.d.], Restek Ltd., Bellefonte, PA, USA) and a TCD (Agilent Technologies, Inc.) was used. The flow rate of helium gas was 20 mL/min. Temperatures of the oven, injector, and TCD were 60, 180, and 180°C, respectively.

***CDA and* p-*AV determination*:** CDA value indicates the diene conjugation of the unsaturated bonds present, which are primary

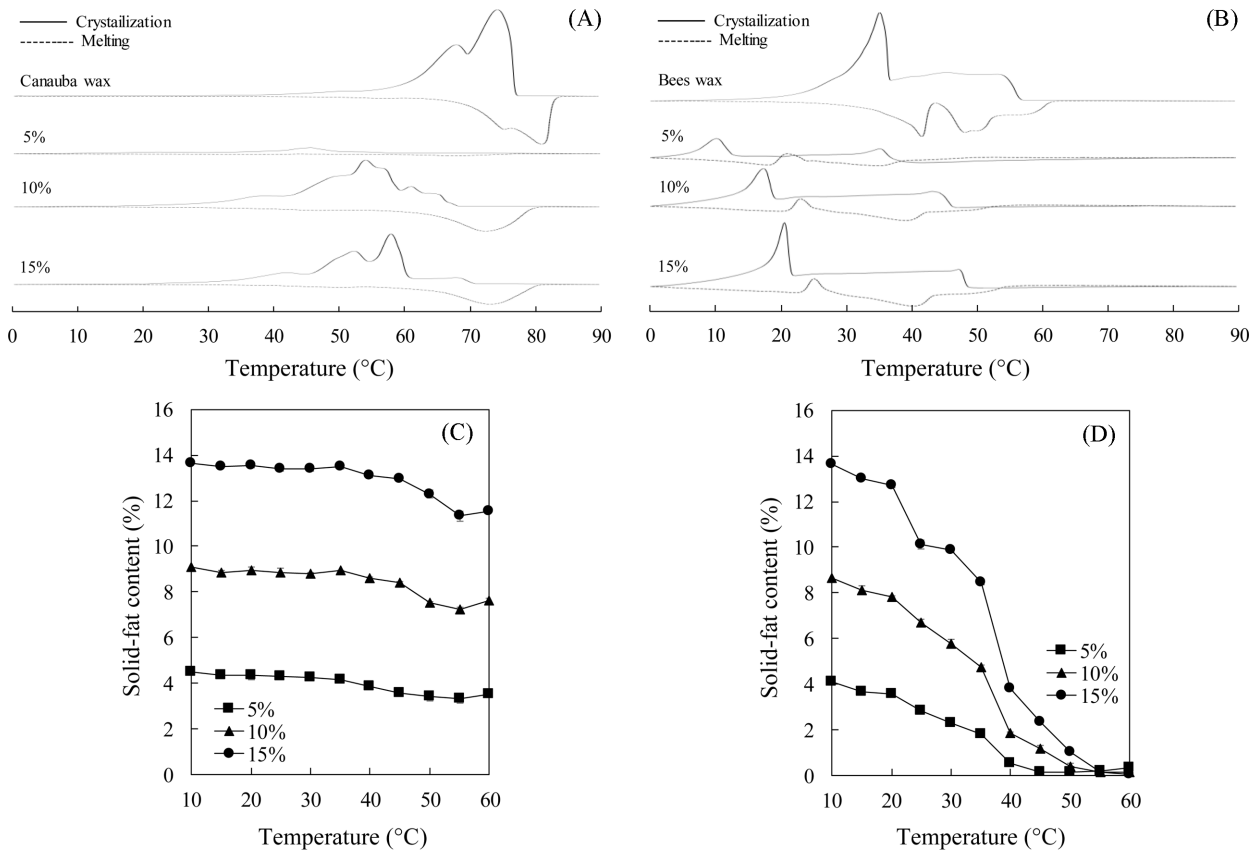

Fig. 1. Differential scanning calorimetry (DSC) pattern of crystallization and melting and solid fat content (SFC) for oleogels based on canola oil made of carnauba wax and on grapeseed oil made of beeswax. (A) and (B) are DSC patterns of oleogels made of carnauba wax and beeswax, respectively. (C) and (D) are SFC in oleogels made of carnauba wax and beeswax, respectively.

oxidation products. *p*-AV is a measure of the α-β oils' unsaturated aldehydes, which are secondary oxidation products. CDA and *p*-AV of samples were analyzed according to the AOCS methods (19), Ti 1a-64 and Cd 18-90, respectively.

***Headspace volatile compound analysis*:** Headspace volatiles in samples were quantified using a Hewlett-Packard 6890 GC with a 5971A mass-selective detector (MS; Agilent Technologies) and a MultiPurpose sampler (MPS; Gerstel, Mülheim, Germany). The solid phase was a 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane solid-phase microextraction fiber. Sample vials were incubated for 15 min at 30°C in the MPS agitator to equilibrate the headspace volatiles. Then the volatiles were extracted for 10 min at 30°C. Volatiles in the fiber were desorbed in the GC injection port for 2 min at 250°C and separated on a DB-5 ms column (30 m×0.25 mm i.d., 0.25-μm film thickness) with gradient temperatures. Oven temperature started at 40°C for 2 min, increased at the rate of 5.5°C/min to 160°C and at 10°C/min to 220°C, and remained stable for 1 min. The flow rate of helium carrier gas was 1.0 mL/min, and the GC system was operated in splitless mode (22). All mass spectra were obtained at 70 eV and 220°C ion source temperature. The compounds were identified by means of a combination of the NIST mass spectra and Kovat index.

Statistical analysis Samples were analyzed in triplicate at each sampling time point. Data on color, headspace oxygen content, CDA, *p*-AV, and headspace volatiles were subjected to analysis of variance (ANOVA) and least square difference method using the SPSS software (SPSS Inc., Chicago, IL, USA). Differences with a p value <0.05 were considered significant.

Results and Discussion

Thermal analysis and SFC of the oleogels made of carnauba wax or beeswax The DSC patterns and SFC of the oleogel samples are shown in Fig. 1. As the proportion of wax increased in the oleogels, peaks for crystallization and melting became more clear-cut. Crystallization and melting temperatures of the CWO were higher than those of the BWO. In addition, peak patterns of crystallization and melting of the oleogels started to resemble those of the corresponding waxes as the proportion of wax increased from 5 to 15%.

Onset and peak temperatures and peak enthalpy of crystallization and melting are shown in Table 1. The peak points of crystallization and melting of carnauba wax were 77.13 and 69.75°C, respectively,

Table 1. Thermal parameters by DSC, CIE L*, a*, and b* values, and oil-binding capacity of oleogels with carnauba wax or beeswax

Thermal parameters by DSC		Carnauba wax				Beeswax			
		Wax	5%[1)]	10%	15%	Wax	5%	10%	15%
Crystallization	Onset T_c (°C)	77.13±0.12[2)]	54.31±2.96	60.34±0.45	62.93±2.50	37.30±0.03	15.08±1.85	19.78±0.29	21.73±0.18
	T_c (°C)	74.44±0.29	51.53±5.12	56.74±2.30	59.92±1.76	35.61±0.29	12.43±1.84	18.22±0.49	20.45±0.35
	ΔH (J/g)	173.56±4.63	7.72±4.10	22.09±3.22	33.05±5.91	175.57±5.48	3.48±2.52	11.00±1.21	15.52±4.17
Melting	Onset T_m (°C)	69.75±0.09	61.84±1.57	63.88±1.13	64.21±1.24	38.15±0.07	20.58±0.41	22.46±0.24	24.11±0.21
	T_m (°C)	81.30±0.18	69.60±2.15	73.22±0.70	74.30±1.05	42.25±0.22	36.30±1.00	39.59±0.05	40.52±0.17
	ΔH (J/g)	172.04±3.76	4.48±2.54	20.25±2.81	30.26±4.94	179.06±4.24	2.90±1.37	7.41±1.49	16.78±3.96

	Carnauba wax				Beeswax			
	Wax	5%	10%	15%	Wax	5%	10%	15%
L*	58.79±1.49a[3)]	41.64±0.53d	48.10±0.41c	51.07±1.23b	54.45±0.88a	34.66±0.27d	46.56±1.48c	51.67±1.17b
a*	3.05±0.49a	2.99±0.28a	3.54±0.17a	3.10±0.49a	7.13±0.37c	2.87±0.29a	4.38±0.20b	4.68±0.32b
b*	10.62±1.74b	5.85±0.16c	10.12±0.47b	13.81±0.57a	19.75±0.87a	4.93±0.15b	4.52±0.19b	4.35±0.22b
ΔE[4)]	-	17.80±0.54	10.72±0.41	8.40±0.97	-	25.08±0.19	17.40±0.85	15.86±0.42
OBC[5)] (%)	-	85.08±4.16	99.00±0.20	99.37±0.15	-	99.54±0.06	99.67±0.46	99.47±0.15

[1)]Content of wax in oleogel.
[2)]Mean±standard deviation (*n*=3).
[3)]Different letters are significantly different in the same column of the each oleogel sample at 0.05.
[4)]Difference of L*, a*, and b* between the control oil and oleogels with wax.
[5)]Oil-binding capacity.

whereas those of beeswax were 35.70 and 42.05°C, respectively. The CWO had the crystallization points from 51.53°C (at 5% of carnauba wax) to 59.92°C (at 15% of carnauba wax) and melting points from 69.60°C (at 5% of carnauba wax) to 74.30°C (at 15% of carnauba wax). In contrast, crystallization temperatures of the BWOs with 5, 10, and 15% of beeswax were 12.43, 18.22, and 20.45°C, respectively, and melting points were 36.30, 39.59, and 40.52°C, respectively. As the concentration of wax increased in the oleogels, enthalpy and temperatures of crystallization and melting increased in agreement with the results of other reports (2,20,23). Öğütcü and Yılmaz (2) compared oleogels made of virgin olive oil with carnauba wax or monoglycerols, and Yılmaz and Öğütcü (20) used hazelnut oil oleogels with beeswax or monoglycerols. Öğütcü *et al*. (23) reported that oleogels with carnauba wax have higher enthalpy and temperature of crystallization and melting than those based on beeswax, in line with our results. Thermal properties of oleogels may be affected by the type of wax. Therefore, the differences in thermal properties between our oleogels may be attributed to the properties of wax, such as the type and concentration.

Increasing the concentration of either carnauba wax or beeswax increased SFC in both oleogels (Fig. 1C and 1D). SFCs of oleogels with 5, 10, and 15% of carnauba wax at 10°C were 4.48, 9.08, and 13.63%, respectively (Fig. 1C) and SFCs of oleogels with 5, 10, and 15% of beeswax were 4.08, 8.66, and 13.66%, respectively (Fig. 1D). These results showed that SFCs of the oleogels made of carnauba wax and beeswax are similar at 10°C.

In case of the CWO, SFC did not change at temperatures from 10 to 35°C. SFCs of oleogels with 5, 10, and 15% of carnauba wax at 60°C were 3.51, 7.64, and 11.55% respectively. The CWO showed a melting point between 67.12 and 73.38°C (Table 1), which can explain the high SFC of the CWO from 40 to 60°C. In contrast, SFC of the BWO decreased substantially as the temperature increased from 10 to 60°C. SFCs of oleogels with 5, 10, and 15% of beeswax at 40°C were 0.54, 1.82, and 3.78%, respectively. Based on melting temperatures (Table 1), the BWO was in the liquid-oil phase between 35.14 and 40.71°C. Therefore, the BWO is more sensitive to temperature changes than the CWO.

Color data and OBC Color parameters and OBC of oleogels with carnauba wax or beeswax are shown in Table 1. The luminosity values (L*) of control oils were higher than those of the oleogels containing wax. The L* values of the CWO and BWO significantly increased in the order of 5, 10, and 15% of wax (p<0.05), although these values were lower than those in control oils (p<0.05). Yellowness (b*) of the oleogel samples with carnauba wax significantly increased with the increasing proportion of wax (p<0.05), whereas redness (a*) did not change significantly (p>0.05). By contrast, a* values increased in the BWO with the proportion of wax, whereas b* values did not change significantly (p>0.05). Furthermore, differences (ΔE) in L*, a*, and b* values between the control oils, which were oils without the addition of wax, and oleogels significantly decreased in oleogels with the increasing proportion of wax (5–15%; p<0.05). The color parameters of oleogels with 15% of wax were similar to control oils.

Öğütcü and Yılmaz (2) reported that L*, a*, and b* values increase in virgin olive oil oleogels as the proportion of carnauba wax increases from 3 to 10%. Nonetheless, as reported by Yılmaz and Öğütcü (20), a* values are not significantly different (p>0.05), whereas L* and b* values increase in hazelnut oil in a concentration-dependent manner (3–10% carnauba wax). Therefore, color values

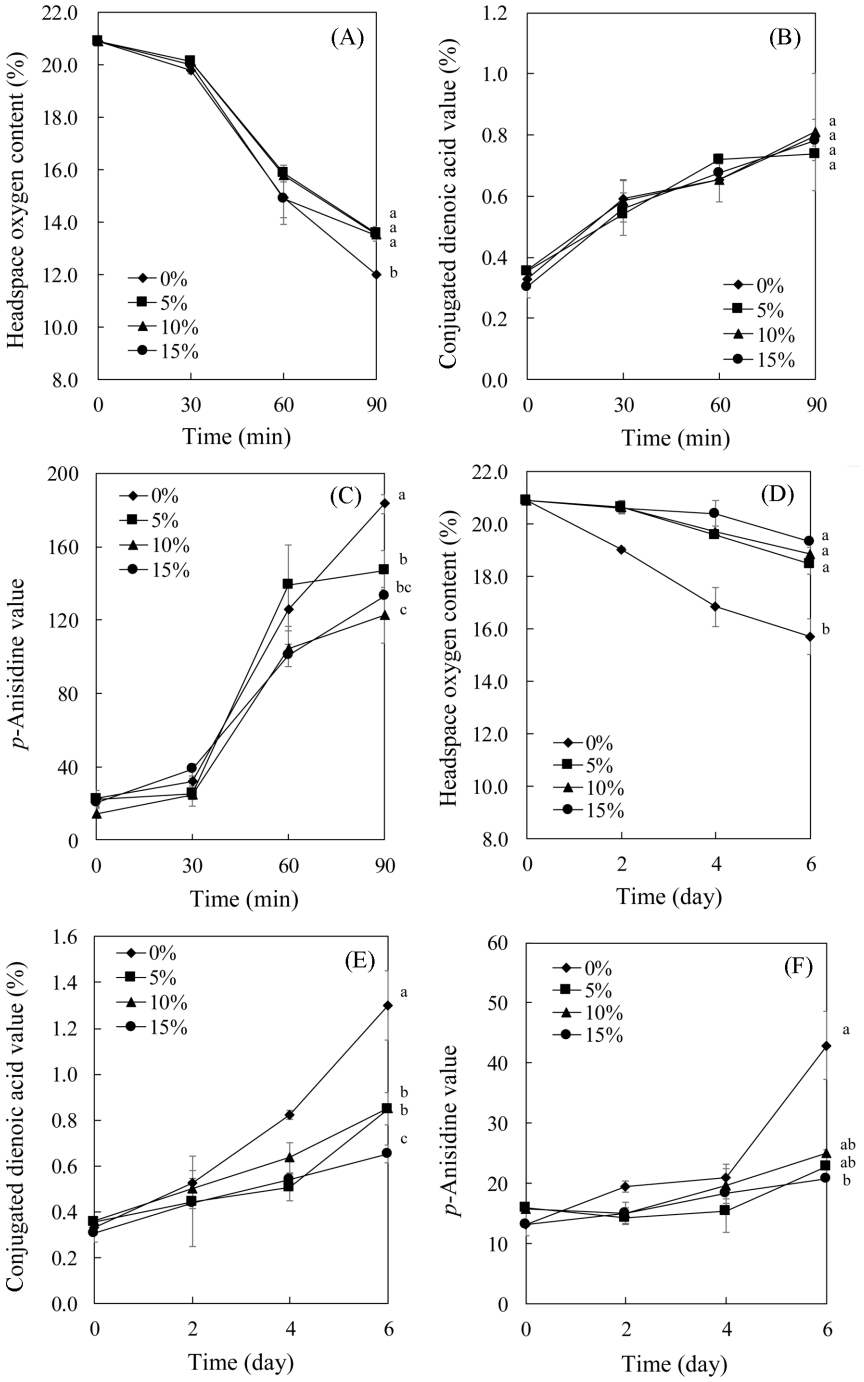

Fig. 2. Headspace oxygen content, conjugated dienoic acid (CDA), and *p*-anisidine value (*p*-AV) in canola oil based oleogels made of carnauba wax at 180 and 60°C. (A) and (D) are the headspace oxygen content in samples at 180 and 60°C, respectively. (B) and (E) are the CDA values in samples at 180 and 60°C, respectively. (C) and (F) and *p*-AV in samples at 180 and 60°C, respectively. Different letters are significantly different at 0.05.

of oleogels are dependent on the type of constituent oil.

The OBC values of oleogels with 5, 10, and 15% of carnauba wax were 85.08, 99.00, and 99.37%, respectively (Table 1). The OBC values of the BWO did not significantly change with the wax concentration ($p>0.05$).

These results are consistent with the findings in other studies (2,20,23). The OBC of oleogels with carnauba wax is lower than that of oleogels based on other oleogelators, and this property depends on the type of oil (2,23). Nevertheless, 3% beeswax can form a stable oleogel without being affected by the type of oil (2,23).

Oxidative stability of the oleogels Changes in headspace oxygen content, the CDA value, and *p*-AV in the oleogels based on canola oil and carnauba wax at 180°C and 60°C are shown in Fig. 2A–2C and

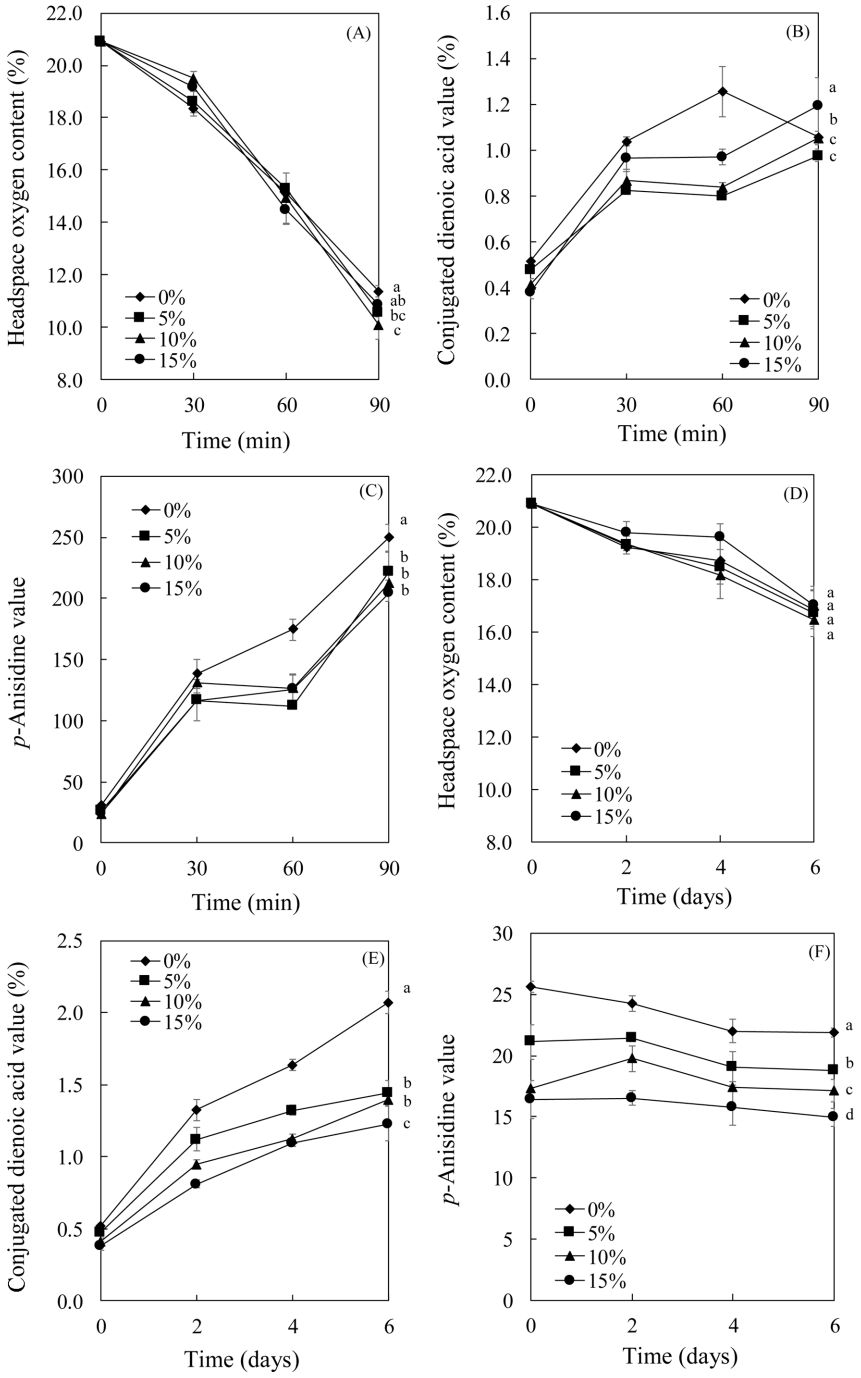

Fig. 3. Headspace oxygen content, conjugated dienoic acid (CDA), and *p*-anisidine value (*p*-AV) in grapeseed oil based oleogels made of beeswax at 180 and 60°C. Different letters are significantly different at 0.05. (A) and (D) are the headspace oxygen content in samples at 180 and 60°C, respectively. (B) and (E) are the CDA values in samples at 180 and 60°C, respectively. (C) and (F) are the *p*-AV in samples at 180 and 60°C, respectively.

2D–2F, respectively. Headspace oxygen content of the CWO was higher than that of the control oil, whereas addition of wax from 5 to 15% did not make a significant difference at 180°C ($p>0.05$; Fig. 2A). The CDA values were not significantly different among the samples treated at 180°C ($p>0.05$; Fig. 2B). *p*-AV in the CWO increased in the order of 0, 5, 15, and 10% of carnauba wax, suggesting that carnauba wax increased the oxidative stability of oleogels compared to the control oil samples at 180°C (Fig. 2C). A relatively high temperature of sample preparation (140°C) may already induce formation of conjugated dienes in oils, which did not significantly affect CDA values in the oleogels (Fig. 2B).

Consumption of headspace oxygen in control oils was higher than that in the CWO (Fig. 2D) at 60°C; these data are consistent with the results at 180°C (Fig. 2A). According to results on CDA (Fig. 2E) and *p*-

AV (Fig. 2F), the oxidative stability of CWO is significantly higher than that of the corresponding oil at 60°C (p<0.05). Therefore, the CWO has a lower rate of lipid oxidation than the control oil, whereas the oxidative stability is not significantly different among oleogels with different proportions of carnauba wax.

Changes in headspace oxygen content, CDA, and p-AV at 180 and 60°C in the oleogels based on grapeseed oil and beeswax are shown in Fig. 3. Although the oxidative stability was not significantly different among the samples according to the results on headspace oxygen content at both 180 and 60°C (Fig. 3A and 3D), the amounts of oxidation products in the BWO were significantly lower than those in the control oil according to CDA (Fig. 3B and 3E) and p-AV (p<0.05; Fig. 3C and 3F).

Table 2. Changes of headspace volatile compounds (1 × 10^7, ion counts) in oleogels made of carnauba wax and beeswax at 180°C

Oleogels with carnauba wax	0 min				30 min			
	0%[1)]	5%	10%	15%	0%	5%	10%	15%
2-Butenal	ND[2)]	ND	ND	ND	2.28±0.15[3)]	1.66±0.10	1.26±0.36	1.38±0.20
1-Penten-3-ol	ND	ND	ND	ND	1.03±0.19	0.82±0.09	0.70±0.19	0.79±0.13
2-Pentenal	ND	ND	ND	ND	0.60±0.26	0.42±0.03	0.39±0.10	0.52±0.01
Hexanal	ND	ND	ND	ND	0.78±0.15	0.73±0.12	0.77±0.27	1.15±0.22
Heptanal	ND	ND	ND	ND	0.04±0.01	0.03±0.01	0.03±0.01	0.04±0.01
2-Heptenal	ND	ND	ND	ND	0.11±0.09	0.14±0.01	0.16±0.08	0.20±0.08
2,4-Heptadienal	ND	ND	ND	ND	0.23±0.05	0.20±0.01	0.21±0.06	0.25±0.05
Total peak area (×10^8, ion counts)	0.29±0.01	0.18±0.05	0.24±0.02	0.20±0.01	4.23±0.39	3.05±0.23	2.54±0.56	2.84±0.39
Oleogels with carnauba wax	60 min				90 min			
	0%	5%	10%	15%	0%	5%	10%	15%
2-Butenal	12.11±0.43	9.03±0.34	8.68±0.49	6.62±0.44	12.18±0.37	10.20±0.37	8.55±1.26	6.13±0.38
1-Penten-3-ol	10.02±0.79	7.77±0.40	7.94±0.43	7.17±0.48	9.68±1.51	8.85±0.35	8.23±0.75	6.80±0.09
2-Pentenal	5.87±1.24	4.27±0.37	4.47±0.32	3.40±0.22	6.93±0.29	5.69±0.34	5.13±0.78	3.04±0.12
Hexanal	14.23±6.29	10.62±0.45	12.64±0.92	10.27±0.68	21.62±0.27	18.48±0.77	14.26±1.54	12.24±4.17
Heptanal	1.16±0.59	0.82±0.03	1.45±0.73	0.98±0.05	1.84±0.10	1.55±0.15	1.46±0.24	0.93±0.06
2-Heptenal	4.30±2.21	2.38±0.07	3.25±1.66	4.05±0.14	7.31±0.43	6.61±0.21	6.02±0.70	4.26±0.15
2,4-Heptadienal	2.74±0.69	3.31±0.08	3.71±0.36	4.12±0.12	7.01±0.31	6.19±0.27	6.04±0.65	4.68±0.12
Total peak area (×10^8, ion counts)	14.52±0.53	12.65±0.48	12.60±0.57	11.31±0.53	17.25±0.30	14.76 ±0.46	13.62±0.90	10.20±0.21
Oleogels with beeswax	0 min				30 min			
	0%[4)]	5%	10%	15%	0%	5%	10%	15%
Hexanal	0.09±0.01	0.09±0.02	0.09±0.03	0.07±0.01	7.18±1.23	6.44±0.99	2.71±0.35	3.15±0.57
2-Hexenal	ND	ND	ND	ND	0.50±0.12	0.51±0.13	0.17±0.04	0.22±0.05
2-Heptenal	ND	ND	ND	ND	2.54±0.63	2.62±0.41	1.65±0.12	1.71±0.20
2-Pentylfuran	ND	ND	ND	ND	0.31±0.05	0.28±0.04	0.20±0.01	0.24±0.03
2,4-Heptadienal	ND	ND	ND	ND	ND	0.13±0.02	0.03±0.02	0.02±0.01
Total peak area (×10^8, ion counts)	0.37±0.04	0.23±0.05	0.18±0.01	0.25±0.02	3.28±0.48	2.94±0.42	1.86±0.07	2.09±0.18
Oleogels with beeswax	60 min				90 min			
	0%	5%	10%	15%	0%	5%	10%	15%
Hexanal	15.64±0.43	12.57±5.43	13.71±1.73	13.35±0.37	13.39±0.81	12.58±0.36	14.84±1.94	15.65±1.26
2-Hexenal	2.52±0.18	2.49±0.38	2.04±0.20	1.97±0.08	4.73±0.28	4.23±0.17	3.84±0.14	3.63±0.29
2-Heptenal	7.96±0.75	9.13±0.61	9.27±0.74	8.72±0.29	14.90±0.91	15.92±0.63	15.56±0.37	14.13±0.26
2-Pentylfuran	0.98±0.10	1.40±0.16	2.15±0.36	2.44±0.18	3.47±0.20	5.38±0.87	8.27±0.47	8.90±0.64
2,4-Heptadienal	0.44±0.05	0.45±0.03	0.48±0.04	0.46±0.01	0.74±0.04	0.81±0.03	0.97±0.16	0.77±0.02
Total peak area (×10^8, ion counts)	6.67±0.22	6.81±0.32	6.68±0.43	6.39±0.19	9.92±0.35	10.40±0.40	10.46±0.27	9.96±0.29

[1)]The content of carnauba wax in Oleogels.
[2)]ND, Not detected.
[3)]Mean±standard deviation (n=3).
[4)]The content of beeswax in Oleogels.

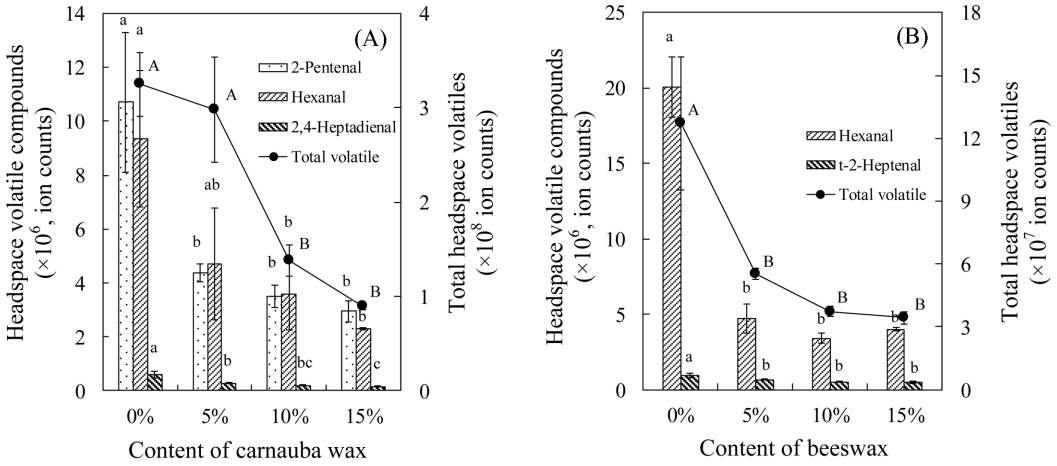

Fig. 4. Headspace volatile compounds in canola oil based oleogels made of carnauba wax (A) and in grapeseed oil based oleogels made of beeswax (B) at 60°C after 6 days of storage. Different capital and small letters were significantly different (p<0.05) among the same volatiles.

Headspace volatile compounds of oleogels made of carnauba wax or beeswax Changes in headspace volatile compounds at 180°C for 90 min in oleogels made of carnauba wax and beeswax wax are shown in Table 2. The headspace volatiles selected for analysis in the CWO are 2-butenal, 1-penten-3-ol, 2-pentenal, hexanal, heptanal, and 2,4-heptadienal. The amounts of these volatile compounds increased with the increasing oxidation duration and decreased with the increasing proportion of carnauba wax. Differences in peak areas at 180°C for 90 min between oleogels with 0 and 15% carnauba wax were in the order of hexanal, 2-butenal, 2-pentenal, 2-heptenal, 1-penten-3-ol, 2,4-heptadienal, and heptanal. In addition, total volatile peak areas in oleogels with 0, 5, 10, and 15% of wax were 17.25×10^8, 14.76×10^8, 13.62×10^8, and 10.20×10^8 (ion counts), respectively. The results on volatile compounds indicate that the CWO has higher oxidative stability than the control oil.

Hexanal, 2-hexenal, 2-heptenal, 2-pentylfuran, and 2,4-heptadienal showed different patterns in the BWO during oxidation. Hexanal content decreased when the beeswax proportion increased from 0 to 5% and increased with a further increase in beeswax concentration to 15%, whereas the amounts of 2-heptenal and 2,4-heptadienal increased and then decreased at these data points. Formation of 2-hexenal decreased as the proportion of beeswax increased. Similarly, 2-pentylfuran formed less in oleogels with a higher proportion of beeswax. Total peak areas were not significantly different among the samples (p>0.05). These results on volatile compounds are not consistent with the oxidative stability of the BWO (Fig. 2).

Changes in headspace volatile compounds at 60°C for 6 days in oleogels made of carnauba wax and beeswax are shown in Fig. 4A and 4B, respectively. In CWO, the peak that corresponds to 2-pentenal was the highest followed by hexanal and 2,4-heptadienal among headspace volatiles, and the amounts of these selected volatile compounds and total volatiles decreased with the increasing proportion of carnauba wax (Fig. 4A), in line with the results on oxidative stability at 60°C (Fig. 2).

The amounts of hexanal, 2-heptenal, and total volatiles were significantly higher in the control oil compared to the BWO (p<0.05), although significant differences were not observed among oleogels with different proportions of beeswax (p>0.05; Fig. 4B). Therefore, oleogels with wax show a lower rate of lipid oxidation as compared to the control oil samples, whereas the proportion of wax may not have a strong effect on oxidative stability. Solid oleogels may have higher oxidative stability as compared to liquid oils partly because of the slower diffusion of oxygen molecules in the gel state or smaller chances of collision between unsaturated fat molecules and oxygen molecules.

Pieve *et al.* (24) prepared cod liver oil oleogels with 5, 7, and 9% of monoacylglycerol and tested the peroxide content and headspace propenal. They reported that the differences in peroxide content (which is an indicator of primary oxidation) among the samples are not significant (p>0.05), whereas the formation of headspace propenal, which is the main secondary oxidation product, is lower in oleogels with monoacylglycerol than in control oil (24). The temperature of 80°C was needed to prepare oleogels made of monoacylglycerol. Monoacylglycerol networks seem to have a limited effect on primary oxidation such as formation of peroxides. Nonetheless, these networks decrease the rate of secondary oxidation via a decrease in molecular mobility by acting as hurdles within oleogels.

On the other hand, Kim *et al.* (6) reported that oleogels based on ethylcellulose accelerate lipid oxidation and that oxidation stability is affected by fatty-acid composition, concentration of ethylcellulose, and heating temperature.

As reported by Öğütcü *et al.* (23), the type of wax can influence lipid oxidation, whereas the concentration of wax does not (p>0.05). In addition, they claimed that the effects of the preparation process, for example, 90°C incubation for more than 10 min, are key factors of lipid oxidation.

In conclusion, oleogels based on carnauba wax have higher temperature and enthalpy of crystallization and melting in comparison

with oleogels based on beeswax. Oleogels made of beeswax are in the solid phase at room temperature but can turn into a liquid phase above 40°C. Thus, oleogels based on beeswax can change from solid phase to liquid-oil phase by means of temperature control. Oleogels based on carnauba wax show enhanced oxidative stability, whereas beeswax is an efficient oleogelator. Beeswax oleogels may be suitable for food applications, which need suitable flow characteristics at 40–50°C.

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