Food Control 23 (2012) 528–534

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Effect of natural antioxidants on the lipid oxidation of microencapsulated seed oil

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ARTICLE INFO

Article history:
Received 7 May 2011
Received in revised form
8 August 2011
Accepted 10 August 2011

Keywords:
Natural antioxidant
Microencapsulation
Sunflower oil
Lipid oxidation
Peroxide value

ABSTRACT

Lipid oxidation was effectively prevented by natural antioxidants (NA) for surface free and encapsulated oils in microencapsulated seed oil (MESO) coated with dextrin and milk protein. Three kinds of NA were extracted from rosemary, broccoli sprout, and citrus, respectively. The antioxidant effect was significantly enhanced by the use of a mixture composed of 0.05% (w/w, oil based) rosemary, 1% broccoli sprout, and 1% citrus extract. The peroxide value (POV) of the total oil in the MESO was shown to lower to about 60 meq/kg even under the accelerated storage condition at 60 ± 1 °C for 30 days. The POV of total oil from MESO was 28.1 meq/kg in the presence of antioxidants, whereas the POV of the control sample without antioxidants reached 78.8 meq/kg. The present approach can find widespread use for preventing various microencapsulated seed oils from lipid oxidation.

1. Introduction

Microencapsulation has been widely applied to protect edible oils from oxidation by spray drying using a coating wall such as dextrin and/or milk protein (Calvo, Hernández, Lozano, & González-Gómez, 2010; Rosenberg & Young, 1993; Shahidi & Han, 1993), resulting in a prolonged shelf-life of core oil. In addition, it has been effectively used for manufacturing powder type food ingredients when no food processing system for liquid type ingredients is available. However, lipid oxidation on the surface of the microcapsules is inevitable since fats are necessarily spread wide on the surface of spray-dried microcapsules and resultantly are exposed to the atmosphere according to their schematic model (Buma, 1971; Vega & Roos, 2006). Lipid oxidation of microencapsulated seed oil (MESO) could become more serious in cases of low microencapsulation efficiency (MEE) (Ahn, Kim, Lee et al, 2008), and could be influenced by various storage conditions (Pristouri, Badeka, & Kontominas, 2010).

Control of surface free oil on the microcapsules is hence crucial for a prolonged shelf-life because free oil is widely spread on the surface of microcapsules and exposed to the atmosphere. The amount of oil on the surface of microcapsules is relatively low compared with encapsulated oil, and lipid oxidation patterns between surface free oil and encapsulated oil are different (Velasco, Marmesat, Dobarganes, & Márquez-Ruiz, 2006). Thus, evaluation of lipid oxidation in total oil in MESO particles rather than in free oil could be a comprehensive method for controlling lipid oxidation in the cases of MESO with high MEE.

Lipid oxidation causes defective nutrition due to the reactive oxygen species (Esterbauer, Schaur, & Zollner, 1991; Guardiola, Dutta, Codony, & Savage, 2002), giving rise to a deleterious effect on human health (Frankel, 1998). Since lipid oxidation is a critical factor for food quality and prolonged shelf-life of edible oils, many studies have concentrated on the prevention of lipid oxidation by natural antioxidants (NA) (Baydar, Özkan, & Yasar, 2007; Ebrahimabadi, Djafari-Bidgoli, Mazoochi, Kashi, & Batooli, 2010; Rodríguez Vaquero, Tomassini Serravalle, Manca de Nadra, & Strasser de Saad, 2010). Recently, much effort has been made to prevent the lipid oxidation for sunflower oil in conjunction with some synthesized food additives (Guilleän, Cabo, Ibargoitia, & Ruiz, 2005; Iqbal, & Bhanger, 2007; Makhoul, Ghaddar, & Toufeili, 2006). However, few studies about the application of natural antioxidants to microencapsulated edible oils have been reported. Moreover, lipid oxidation patterns between free and encapsulated oils in NA-supplemented microcapsules have not been investigated. Here we report the details of the inhibitory effect of natural antioxidants on the lipid oxidation of free, encapsulated, and total oils in MESO.

2. Materials and methods

2.1. Materials

Typical seed oil, certified organic sunflower oil of *Helianthus annuus*, was supplied from Tradin Organic Agriculture B.V. (Amste-

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doi:10.1016/j.foodcont.2011.08.026

rdam,Netherlands). Acid and peroxide values of the seed oil were estimated to be 0.02 and 0.32, respectively, and the levels of residual minor components such as pigments and phospholipids were negligible according to the supplier's specification. Spray-dried dextrin was purchased from Sunrich (Hope, MN, USA). Dextrose equivalent (DE) was 8–12, and carbohydrate content was 93.7% (w/w) according to the supplier's specification. Spray-dried milk protein isolate (MPI) was obtained from Emmi Milch AG (Dagmersellen, Switzerland). MPI was concentrated from skimmed milk pasteurized at 72 °C for 15 s, and its protein and lactose contents were 80.6% (w/w) and 4.9% (w/w), respectively, according to the supplier's specification. Paste type soybean lecithin was purchased from Clarkson Soy Products (Cerro Gordo, IL, USA), and was manufactured by drying after degumming pressed soybean oil. It contained 36.0% (w/w) phospholipids and 10.0% (w/w) phosphatidylcholine according to the supplier's specification.

Rosemary extract from leaf of *Rosmarinus officinalis* was from FLAVEX (Lehlingen, Germany). Contents of total phenolics and carnosolic acid, which were shown to be active ingredients for the antioxidant effect of rosemary extract, were 25.0 g/100 g and 16.0 g/100 g, respectively. Broccoli extract from sprout of *Brassica oleracea var. italica* was purchased from ORYZA (Ichinomiya, Japan). Contents of total phenolics and sulforaphane, active ingredients for antioxidant effect in broccoli extract, were 3.8 g/100 g and 2.0 g/100 g, respectively, according to the supplier's specification. The total solid content was 94%. Citrus extract, a mixture of *Citrus Aurantium dulcis*, *Citrus Aurantium amara* and *Citrus paradisi*, was from BREKO (Bremen, Germany). Total phenolics and flavonones, active ingredients for the antioxidant effect in the citrus mixture extract, were 72.1 g/100 g and 17.5 g/100 g, respectively, according to the supplier's specification.

2.2. Chemicals

Butanol, chloroform, petroleum ether, and hexane were acquired from Fisher Scientific (Pittsburgh, PA, USA). Sodium thiosulphate was from Merck (Darmstadt, Germany). Acetic acid, potassium iodide, and the other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents and reagents were appropriate grade for chromatographic analysis and purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Methods

2.3.1. Sample preparation for microencapsulation of sunflower oil

From the preliminary test on the antioxidant effects of each antioxidant component, we first determined the effective concentrations of rosemary, broccoli sprout, and citrus extract in oil. For each single test, NA was added to sunflower oil as the following composition: 0.05% (w/w) rosemary, 1% broccoli sprout, and 1% citrus extract. This composition was shown to have a maximum antioxidant effect on MESO in our previous study (Ahn, Kim, Seo et al, 2008).The resulting suspension was mixed using a homomixer (Ultra Turrax T-50, Janke & Kunkel Ika-Labortechnik, Staufen, Germany) for 10 min at room temperature. Each NA was simultaneously added to sunflower oil with those contents for the test of synergistic effect. The NA was prepared by simple grinding, spray drying, or extraction according to the suppliers. Rosemary extract contained 87% (w/w) total solid, and was extracted from leaves of *Rosmarinus officials* by using a solvent composed of medium chain triglyceride (MCT) oil, water, and ethyl alcohol (4:5:4, w/w) under a CO_2 atmosphere. Broccoli extract was composed of 50% (w/w) of grinded brocolli sprout powder and 50% (w/w) of dextrin as a coating agent by spray drying. The citrus extract was a mixture of 40% (w/w) *Citrus Aurantium dulcis*, 30% *Citrus Aurantium amara*, and 30% *Citrus paradisi* and was prepared by spray drying after extraction and evaporation with ethyl alcohol (50%, w/w).

2.3.2. Spray-dried microencapsulation

Microencapsulation was carried out according to the method as described elsewhere (Keogh & O'Kennedy, 1999; Sankarikutty, Sreekumar, Narayanan, & Mathew, 1998). Briefly, dextrin and MPI were dissolved in deionized water using a homomixer (Ultra Turrax T-50, Janke & Kunkel Ika-Labortechnik, Staufen, Germany) for 20 min at 6000 g and 65 °C. The suspension was then added to the previously prepared sunflower oil containing NA and soybean lecithin at the optimized ratio as reported in the previous study (Ahn, Kim, Lee et al, 2008; Ahn, Kim, Seo et al, 2008). The optimized composition for MESO by response surface methodology was 23.7% (w/w) sunflower oil, 19.0% MPI, 54.83% dextrin, and 2.5% soy lecithin. The emulsion was then homogenized at a feeding rate of 1 L/min at 50–250 kg/cm^2 for 3 cycles in a homogenizer (APV RANIE, Albertslund, Denmark). The resulting homogenized emulsion was immediately fed into a pilot-scale spray dryer using a disk type nozzle (Niro Atomizer, Niro, Søborg, Denmark) equipped with a spray drying chamber that was 160 cm in height and 90 cm in diameter. The emulsion was fed into the chamber at a feeding rate of 1.6 L/h, atomized by the hot air (air velocity of 2 m/s). Inlet and outlet temperatures of the spray dryer were 160 ± 5 °C and 95 ± 5 °C, respectively.

The MESOs manufactured with sunflower oil containing NA were designated as MESO-R, MESO-B, MESO-C, and MESO-RBC, respectively. The control MESO did not contain any NA. The MESOs manufactured with sunflower oil containing different NAs were designated as follows: 0.05% (w/w, oil base) rosemary extract as MESO-R, 1% (w/w) broccoli extract as MESO-B, 1% (w/w) citrus extract as MESO-C, and all of the NAs together as MESO-RBC. The NA content was determined because the inhibitory effect of NA on hydroperoxide formation in sunflower oil duo to the addition of 0.05% (w/w) rosemary extract was similar to the effects of 1% broccoli extract and 1% citrus extract. Samples were tested for MEE and evaluated at the accelerated storage condition at 60 ± 1 °C for 30 days. The remaining samples, except for those in the accelerated storage test, were stored in a freezer (−20 °C) under a nitrogen atmosphere to determine fatty acid and tocopherol compositions.

2.3.3. Extraction of free oil, encapsulated oil and total oil

Extraction of pure oil was carried out to measure the peroxide value (POV) for lipid oxidation levels of MESO. Surface free oil on MESO particles was extracted by pentane according to the method described elsewhere (Dieffenbacher & Lüthi, 1986). Briefly, 100 g of MESO was mixed with 200 mL pentane in a 500 mL Erlenmeyer flask with a stopper. The mixture was shaken for 2.5 h at 25 °C in the dark and then passed through a Büchner funnel with a Whatman No. 4 filter. Solvent was collected in a round-bottom flask followed by rotary evaporation in a water bath at 30 °C to minimize lipid oxidation while evaporating.

Extraction of encapsulated oil and total oil was performed by the Pont method (Pont, 1955; Newstead & Headifen, 1981) because it was required to destroy the coating wall of MESO and the emulsion properties. Total oil was extracted from the original MESO. To obtain encapsulated oil, the oil was extracted from the residual dried MESO. The residual dried MESO was prepared by simply preserving the free oil-removed MESO into the desiccator for 6 h at room temperature after free oil was extracted from the original MESO by pentane as described above. To obtain the pure encapsulated oil containing no free oil, the Pont method was used on MESO which was dried to a constant weight at room temperature after the free oil was extracted. A de-emulsification reagent was used to release encapsulated oil effectively. For the preparation of

de-emulsification reagent, 10 g of sodium salicylate and 10 g of sodium citrate were dissolved separately in double-distilled water and then mixed with 18 mL n-butanol. The solution was brought to a total volume of 90 mL with double-distilled water. Ten grams of MESO were mixed with 20 mL of water in a 125 mL Erlenmeyer flask with a stopper. The mixture was shaken vigorously and left to stand in the water bath at 30 °C for 6 min after adding 15 mL of the de-emulsification reagent. The resulting mixture was centrifuged at 3000*g* for 10 min followed by evaporation of the solvent in a water bath at 30 °C.

2.3.4. Analysis of fat content

Fat content was analyzed according to the pentane method (Dieffenbacher & Lüthi, 1986) with six different solvents. Approximately 10 g of the sample was added to 100 mL of each solvent. The mixture was then shaken for 10 min at 280 rpm on an orbital shaker (LAB-LINE, plateform 3524-49). The extract was filtered through Whatman No.4 paper and collected. The filtrates were evaporated on a hot plate at 40 °C until dryness. The total content of fat was quantified by gravimetric method.

2.3.5. Analysis of protein content

Protein content in the final residue was determined according to the Kjeldahl method of AOAC (AOAC Official Method 991.20, 2005) with slight modification. The final residue was digested in H_2SO_4 with catalyst (Kjeldahl tablets, Merck), and protein content was determined using Distillation unit (BÜCHI 339).

2.3.6. Analytical method for lipid oxidation under accelerated storage condition

The prepared MESO samples were stored at 60 °C for 30 days by the accelerated storage method referencing a method for antioxidant effects of rosemary extract on sunflower oil (Hraś, Hadolin, Knez, & Bauman, 2000). A similar method for an accelerated storage test for edible oil was conducted at high temperatures (over 60 °C) in the Schaal oven test (Wanasundara & Shahidi, 1998). Aliquots (250.0 g) of each sample were poured into PYREX glass vessels (500 mL, 80 mm i.d.) with caps followed by incubation at 60 ± 1 °C. Samples in the glass vessels were taken after 10 days, 20 days, and 30 days for POV test.

Hydroperoxides were measured by peroxide value (POV) as described in the AOCS (AOCS Official Method, Cd 8–53, 1993). POV has been generally used as one of simple and rapid methods for testing lipid oxidation (Lin, C. C. & Lin C. S., 2005), and was expressed as milli-equivalents (meq) of active oxygen per kg oil. Automatic titration was performed using a potentiometric titration system (Model 799 GPT Titrino, 685 Dosimat, Pt Titrode) equipped with a sample changer (Metrohm, Herisau, Switzerland).

2.3.7. Microencapsulation efficiency (MEE)

Microencapsulation efficiency (MEE) was calculated according to the method described elsewhere (Pauletti & Amestoy, 1999):

$$\text{MEE} = \frac{(\text{total oil} - \text{extractable oil}) \times 100}{\text{total oil}}$$

2.3.8. Analytical method for fatty acids

The fatty acid composition of sunflower oil was determined using the standard method (AOCS Official Method, 1983). A capillary gas chromatograph (Agilent, 6890 A Plus, Santa Clara, CA, USA) with a flame ionization detector (FID) and a DB-225 column (30 m × 0.25 mm i.d, 0.25 μm film thickness, J&W Scientific Agilent, Wilmington, DE, USA) was used. Temperature was programmed to increase from 140 to 220 °C with a 4 °C/min gradient, and flow rate of carrier gas (nitrogen) was 0.8 mL/min. Injector temperature was 250 °C with air flow of 300 mL/min, and detector temperature was 260 °C with nitrogen flow of 30 mL/min. Each fatty acid was verified by comparison of retention times between test samples and reference compounds.

2.3.9. Analytical method for tocopherols

Content of tocopherols in sunflower oil was determined by using a high performance liquid chromatograph (HPLC, WATERS, Allience system, U.S.A) with a μ-porasil column (250 × 4.6 mm, WATERS, U.S.A); excitation was at 325 nm and detection was at 298 nm with a fluorescence detector (ISO 9936, 1997). The flow program was hexane: isopropyl alcohol (98:2) with 0.5 mL/min. Tocopherols in test samples were verified by comparison of retention times with those of reference standards of alpha, beta, gamma, and delta forms.

2.3.10. Scanning electron microscope (SEM)

A Field Emission Scanning Electron Microscope (FE-SEM, FEI, Sirion, Hillsboro, OR, USA) was used to examine the morphology and surface appearance of MESO particles. MESO particles were attached with two-sided adhesive tape to specimen stubs and then Pt-coated in a sputter coater at 30 mA for 150 s (BAL-TEC, SCD 005, Witten, Germany). The coated microcapsules were examined in a Sirion SEM at 10 kV with 1.5 nm resolution similar to the method described elsewhere (Rosenberg, Kopelman, & Talmon, 1985).

2.3.11. Particle size analysis

A particle size analyzer (Mastersizer 2000, MALVERN, UK) was used to determine the sizes of MESO particles. Measurement time was 10 s, and snap was set to 10,000. The background snap was set to 5000.

2.3.12. Moisture content and water activity (a_w)

Moisture content of the microcapsule was determined using a thermogravimetric analyzer (MA50, Sartorius, Germany) at 105 °C. Water activity was measured using a water activity analyzer (AW SPRINT TH500, Novasina, Switzerland).

2.3.13. Statistical analysis

All experiments were performed in triplicate, and the data were analyzed by ANOVA in the SAS version 9.1 statistical software (SAS Institute Inc., 2004).

3. Results and discussion

3.1. Analysis of oil components

Fatty acid composition of the sunflower oil was determined as follows: 0.08% (w/w) myristic acid (C14:0), 5.86% palmitic acid (C16:0), 3.28% stearic acid (C18:0), 37.84% oleic acid (C18:1*n*-9), 51.40% linoleic acid (C18:2*n*-6), 0.26% linolenic acid (C18:3*n*-3), 0.20% arachidic acid (C20:0), 0.14% eicosenoic acid (C20:1), 0.53% behenic acid (C22:0), and 0.17% lignoseric acid (C24:0). Total tocopherols content in sunflower oil was estimated to be 33.81 mg/100 g.

3.2. Physical evaluation of MESO

Important factors for improving MEE include encapsulating materials, ratio of coating wall to core oil, proper emulsification levels, and spray drying conditions (Keogh & O'Kennedy, 1999). Five kinds of MESO were prepared based on previously optimized conditions (Ahn, Kim, Lee et al, 2008; Ahn, Kim, Seo et al, 2008). The morphology of MESO was found to be similar regardless of the presence of a single antioxidant or a mixture of antioxidants. The

addition of a NA had no significant effect on the shape, color, and surface condition of the MESO. No holes or cracks on the surface of each MESO, which would occur under poor manufacturing conditions, were observed. The coating quality was shown be good as represented by the 96.4–96.8% MEE from the analysis of fat as shown in the Method section. Extractable oil after pentane extraction from the MESO was relatively small as shown in Table 1. The shape was smooth, which was similar to those prepared by the spray-dried microencapsulation method (Rosenberg & Lee, 1993; Rosenberg & Young, 1993) and our previous approach (Ahn, Kim, Lee et al, 2008; Ahn, Kim, Seo et al, 2008).

Average particle size, moisture content, and water activity (a_w) of each MESO are summarized in Table 1. There were no significant differences in the physical and morphological properties among MESOs. Each MESO could be identified as a microcapsule because the average particle size was as small as 37.3–65.4 μm. As a representative MESO, MESO-RBE containing a mixture of NAs is shown in Fig. 1, and the particle size distribution is shown also in Fig. 1.

Moisture content and a_w of MESOs were estimated to be 3.3–3.6 (w/w) and 0.298–0.306, respectively. Water content of MESOs was above the range known to be susceptible to lipid oxidation. It was reported that when foods are dried to less than 2–3% moisture content, they could become susceptible to oxidation (Labuza, 1971). The effect of a_w on lipid oxidation was negligible even though the MESO was not equilibrated at the initial a_w level under constant humidity. The initial a_w was estimated to be within the ideal range of 0.2–0.4 for storage stability in lipid oxidation as reported elsewhere (Rockland & Beuchat, 1987; Rückold, Grobecker, & Isengard, 2001). In addition, the effect of a_w on lipid oxidation was negligible compared to the subsequent result of the NA effect, since there were no significant differences in the level of a_w.

3.3. Antioxidant effect of NA on MESO

Fig. 2 shows the changes in both surface free and encapsulated oils on MESO in the presence of a single antioxidant or a mixture of NAs under accelerated storage condition at 60 ± 1 °C for 30 days. When no NA was added to MESO (optimized MESO), POV of the surface oil increased from 8.7 meq/kg to 417.0 meq/kg (Fig. 2a), while POV of encapsulated oil was enhanced from 8.5 to 58.5 meq/kg (Fig. 2b). This result is similar to that of spray-dried samples containing a low amount of free oil (Hardas, Danviriyakul, Foley, Narwar, & Chinachoti, 2000) in which free oil was more rapidly oxidized than encapsulated oil. It is generally known that difference in the POV levels between outer free and inner encapsulated oils in MESO is caused by different oxidation speeds. In a recent study (Velasco et al., 2006) using HPSEC (High Performance Size Exclusion Chromatography) method, encapsulated oil was shown to have a different lipid oxidation pattern than free oil on the surface of the microencapsulated sunflower oil. High content of polymerized oxidation compounds was detected in encapsulated oil when the antioxidant was present at high levels, whereas the free oil displayed a clear induction period in which hydroperoxides accumulated and marked at the end of the induction period when the initiation of polymerization occurred with the loss of the antioxidant. It is likely that difference in oxygen availability due to varied size in particle and oil droplet causes the formation of pores and air vacuoles in the solid matrix as well as distribution of the oil globules inside the matrix, resulting in different lipid oxidation patterns.

Two different types of lipid oxidation were also observed in this study, and no significant change in POV of the encapsulated oil was observed for 30 days at 60 °C, while POV of the free oil rapidly increased in 10 days. Initial POV (8.6 meq/kg) of free oil drastically increased up to 135.9 meq/kg after 10 days (Fig. 2a), which corresponds to fifteen-fold enhancement. Meanwhile, increment in POV after 10 days was found to be less than twice the initial POV. No drastic change in POV was observed in encapsulated oil for 30 days. This result indicates that encapsulated oil follows different lipid oxidation pattern than surface oil during the induction period.

Addition of NA to sunflower oil was reported to be very effective for prevention of lipid oxidation during processing and storage of MESO because oil in various foods is generally known to be vulnerable to lipid oxidation (Bou, Codony, Baucells, & Guardiola, 2005). Increase in POV during the spray drying microencapsulation process was inhibited by the addition of NAs. As shown in Fig. 2, initial POV of total oil in control MESO was 8.5 meq/kg, and decreased insignificantly by addition of a single NA. The POV levels induced by each NA were not significantly different ($n = 3$, $p > 0.05$): 6.5 meq/kg by rosemary extract (MESO-R in Fig. 2c), 7.2 meq/kg by broccoli sprout extract (MESO-B in Fig. 2c), 6.3 meq/kg by citrus extract (MESO-C in Fig. 2c) and by the NA mixture (MESO-RBC in Fig. 2c).

However, antioxidant activity in free oil on the surface of MESO was observed during accelerated storage test at 60 °C for 30 days, and significantly enhanced by addition of a single NA. The POV level was lowered to 329.4 meq/kg when rosemary extract was added, while the POV level of the control MESO was 417.0 meq/kg after 30 days (MESO-R in Fig. 2a). As for broccoli extract, POV level was about 316.5 meq/kg (MESO-B in Fig. 2a). Addition of citrus extract resulted in a POV of 157.9 meq/kg (MESO-C in Fig. 2a). This result seems to be attributed to a higher content of active antioxidant ingredients such as total phenolics in citrus extract (72.1%, w/w) compared to rosemary extract (25%, w/w) and broccoli extract (3.8%, w/w). The antioxidant effect of a NA was synergistically enhanced by the addition of a mixture of these combinations. POV of free oil was reduced to levels as low as 74.1 meq/kg on the surface of MESO (MESO-RBC in Fig. 2a).

The antioxidant effect of a single NA on the encapsulated oil (MESO-R, MESO-B & MESO-C in Fig. 2b) was not evident because lipid oxidation of encapsulated oil itself was not significant. The main purpose of microencapsulation lies in protection of oil from air contacting with a coating wall, but lipid oxidation occurred even in encapsulated oil (Hardas, Danviriyakul, Foley, Narwar, &

Table 1
Characteristics of MESO.

Measured factor	Microcapsules				
	MESO	MESO-R	MESO-B	MESO-C	MESO-RBC
Average particle size (um)	37.30	65.44	44.26	44.75	57.47
Moisture (g/100 g)	3.26 ± 0.03[a]	3.48 ± 0.03	3.42 ± 0.03	3.43 ± 0.02	3.51 ± 0.02
Water activity (a_w)	0.292	0.274	0.298	0.296	0.302
MEE (%)	96.61	96.52	96.38	96.70	96.83
Total oil (%, w/w)	22.98 ± 0.05[a]	22.72 ± 0.06	22.68 ± 0.05	22.76 ± 0.06	22.70 ± 0.06
Free oil (%, w/w)	0.78 ± 0.04[a]	0.79 ± 0.05	0.82 ± 0.04	0.75 ± 0.05	0.72 ± 0.04
Encapsulated oil (%, w/w)	22.16 ± 0.06[a]	21.88 ± 0.05	21.96 ± 0.06	22.04 ± 0.05	21.92 ± 0.05

[a] Mean ± standard deviation ($n = 3$).

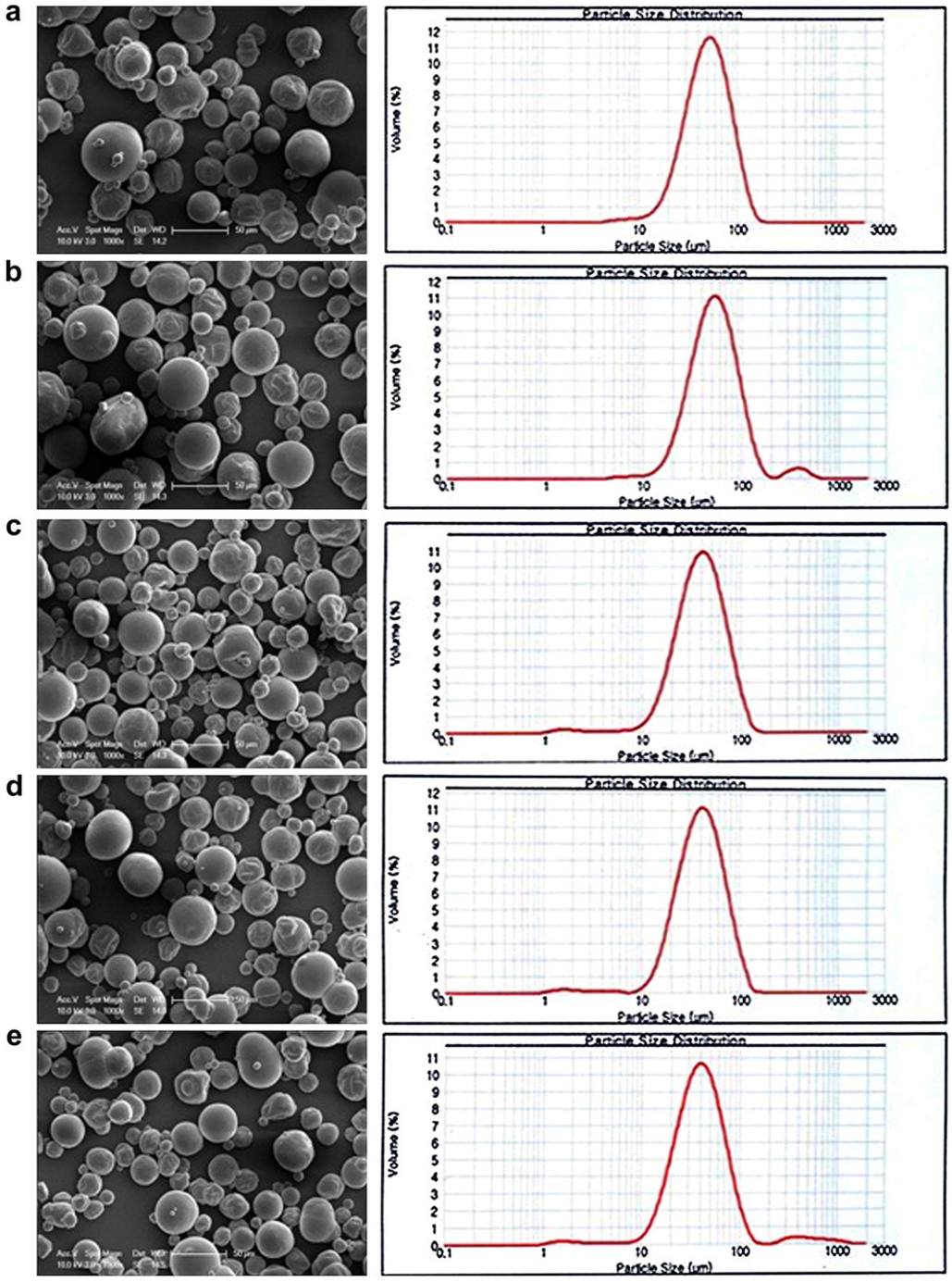

Fig. 1. Scanning electron micrographs and particle size distribution of MESO (a) MESO, (b) MESO-R, (c) MESO-B, (d) MESO-C, (e) MESO-RBC.

Chinachoti, 2000; Velasco et al., 2006).However, the POV level was significantly reduced to 29.7 meq/kg from 58.5 by the addition of a mixture of NAs compared to control sample without NA (MESO-RBC in Fig. 2b). The antioxidant effect was most distinct with a mixture of NAs composed of 0.05% (w/w, oil based) rosemary, 1% broccoli sprout, and 1% citrus extract. The effect of rosemary extract seems to be mainly due to a high content of total phenolics (25.0 g/100 g) and carnosolic acid (16.0 g/100 g). In the case of broccoli extract, antioxidant effect seems to come from 3.8 g/100 g content of total phenolic compounds including flavonoids. Total phenolics and flavonones in citrus extract were estimated to be 72.1 g/100 g and 17.5 g/100 g, respectively, and this is likely to play a crucial role in the antioxidant effect.

Control of surface free oil on the microcapsule is known to be critical for a prolonged shelf-life since free oil is widely spread on the surface of microcapsules and exposed to the atmosphere. However, stability of total oil in MESO was shown to be also influenced by extractable oil because the amount of oil on the surface was relatively low compared to the encapsulated oil as in the case of MESO with high MEE. In this case, it was reported that the quality of MESO should be evaluated by total oil rather than by free oil (Ahn, Kim, Lee et al, 2008). Furthermore, it is important to evaluate different lipid oxidation patterns between free and encapsulated oils because lipid oxidation of the oils showed different changes in MESO (Velasco et al., 2006). Prediction of shelf-life from the accelerated tests is not easy, and the experimental

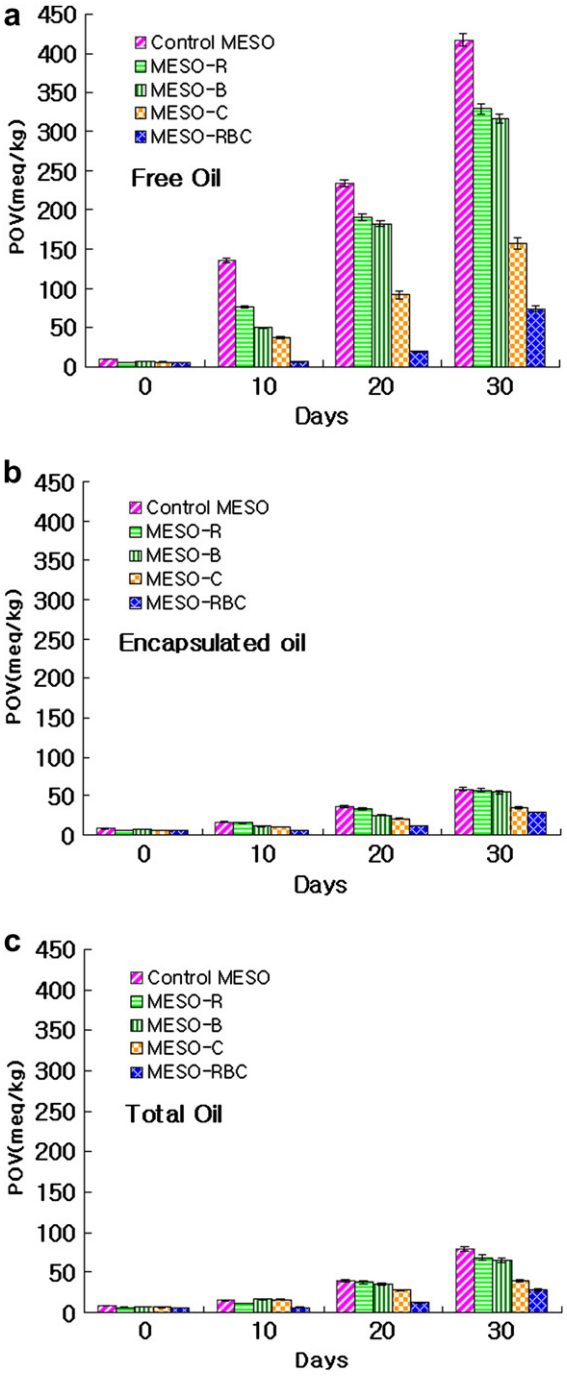

Fig. 2. Changes in POV by lipid oxidation in (a) free, (b) encapsulated, and (c) total oil in MESO at 60 °C as a function of time.

result does not well correlate with stability at ambient temperature. Nonetheless, storage test at high temperature such as 60 °C as in our study was shown to be very useful for investigating the effect of NAs on the sunflower oils in MESO within a short period.

In conclusion, we have demonstrated the preventive effect of different natural antioxidants at high temperature to assess their distinct effect in a simply way. Addition of the NAs mixture effectively lowered the lipid oxidation of total oil in MESO to 35.6% level compared to control MESO. This corresponds to a reduction of POV from 78.8 meq/kg to 28.1 meq/kg. Even though the detailed relation between the shelf-life and PV in MESO remains to be demonstrated, it is clear that lipid oxidation was effectively controlled by NA for MESO. Our result strongly suggests that the lipid oxidation of the MESO should be evaluated in terms of total lipid in the MESO rather than surface oil on the MESO since the amount of surface oil was very low compared with that of encapsulated oil for well coated MESO. This is the first study to report the antioxidant effect of NAs on MESO with two different phases of lipid oxidation. The present approach is expected to provide a crucial guideline to use various antioxidants for preventing other microencapsulated oils from oxidation.

Acknowledgments

This research was supported by World Class University program (Grant R31-2010-000-10071), Brain Korea 21, Advanced Biomass R&D Center (ABC) of Korea Grant funded by MEST (Grant ABC-2010-0029800).

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