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Antioxidant effect of natural plant extracts on the microencapsulated high oleic sunflower oil

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Abstract

This study demonstrates that natural plants extract (NPE) such as rosemary, broccoli sprout and citrus can effectively inhibit the lipid oxidation of microencapsulated high oleic sunflower oil (MEHS). By employing a dextrin-coating method with supplements such as milk protein isolates (MPI), soy lecithin and sodium triphosphate emulsifier in the presence of NPE, MEHS with high microencapsulation efficiency was obtained. Similar to that of high oleic sunflower oil (HS) in liquid state, lipid oxidation of MEHS was remarkably reduced under the accelerated storage condition in the presence of a mixture of NPEs rather than a single component of NPE. Specifically, induction period of MEHS was significantly elongated in the presence of NPE when tested by using the Rancimat method, and the peroxide value (POV) and *p*-anisidine value (ASV) were also significantly lowered by addition of NPE even after storage for 30 days at 60 ± 1 °C. Based on the results, it is anticipated that NPEs find wide applications as an antioxidant for the elevated quality of microencapsulated oil products in food industries.

Keywords: Antioxidant; Natural plant extract; Rosemary; Broccoli sprout; Citrus; High oleic sunflower oil; Microencapsulation; Peroxide value; *p*-anisidine value

1. Introduction

Lipids in seed oils are important functional components of foods and have a significant effect on the quality of foods even though they constitute a minor component. They not only contribute to flavor, odor, color and texture, but also confer a feeling of satiety and palatability to foods. However, the major problem in these oils lies in lipid oxidation during storage or food processing (Frankel, 1998), which can lead to the rancidity (Gordon, 1991) and defective nutrition due to degradation products such as reactive oxygen species, resulting in harmful effects on human health (Esterbauer, Schaur, & Zollner, 1991; Guardiola, Dutta, Codony, & Savage, 2002; Sanders, 1983).

Recently, many attempts have been made to prevent the oxidative deterioration of lipids by using natural antioxidants (Chaudiere, 1994; Frankel, 1998; Frankel, Huang, Kanner, & German, 1994; Gordon, 1990). Some components in natural products such as carotenoids, flavonoides, anthocyanins and phenolic compounds are known to function as scavengers in both primary and secondary oxidation process. In particular, it has been reported that potential antioxidants exist in a number of natural plant extract (NPE) including grapes (Lapidot, Harel, Akiri, Granit, & Kanner, 1999), green teas (Frankel, Huang, Aeschbach, & Prior, 1997), berries (Nielsen,

Abbreviations: CO, corn oil; SO, sunflower oil; HS, high oleic sunflower oil; POV, peroxide value; ASV, *p*-anisidine value; MPI, milk protein isolates; NPEs, natural plant extracts; MEE, microencapsulation efficiency; MEY, microencapsulation yield; MEHS, microencapsulated high oleic sunflower oil.

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Haren, Magnussen, Dragsted, & Rasmussen, 2003), tomatoes (Gahler, Otto, & Böhm, 2003) and rosemary (Frankel, Huang, Aeschbach, & Prior, 1996; Richheimer, Bernart, King, Kent, & Bailey, 1996). Although antioxidant effect of rosemary was extensively investigated on various samples (Banias, Oreopoulou, & Thomopoulos, 1992; Wada & Fang, 1992), little information is available about the combined effect with other NPEs on the seed oils. Furthermore, no study on the combined antioxidant effect of natural plant extracts such as citrus, broccoli sprout, and palm oil extract on the various seed oils has been carried out.

Another approach to protect lipid from oxidation is to microencapsulate the lipid products, which has been widely used in manufacturing powder-type oil and fat products (Keogh & O'Kennedy, 1999; Rosenberg & Lee, 1993; Rosenberg & Young, 1993). Microencapsulation can provide more prolonged shelf-life by protecting oils with encapsulating agent such as milk protein or dextrin, etc. By choosing the appropriate materials to enclose small oil particles, core components such as oil can be protected from deterioration due to adverse environmental conditions such as light, moisture and oxygen, resulting in the increase of the shelf-life of the product (Shahidi & Han, 1993). Although microencapsulation can protect seed oils from oxidation, severe lipid oxidation on the surface of the microcapsule could also occur due to high temperature during the spray-drying process and the residual oils on the surface. However, there have been few studies regarding prevention of lipid oxidation of residual free fat on the surface of microcapsules by using natural antioxidants. Shelf-life of edible oils is normally predicted from the accelerated storage tests which are usually conducted at high temperature ranging from 60 °C for the Schaal oven test to 100 °C for the Rancimat test (Frankel, 1998; Makhoul, Ghaddar, & Thoufeili, 2006). While the shelf-life of sunflower oil was well assessed by measuring the rancidity (Makhoul et al., 2006), no study on lipid oxidation of sunflower oil in microcapsules has been reported. In this regard, the use of proper antioxidants is required even for microencapsulation of relatively stable HS.

In this report, the effect of NPEs such as rosemary, broccoli sprout, and citrus on the oxidation of microencapsulated high oleic sunflower oil (MEHS) is demonstrated. High oleic sunflower oil (HS) is increasingly used owing to relatively high stability, but vulnerability of MEHS to oxidation has hampered its widespread use. A single component or a mixture of NPEs were tested regarding their antioxidant effects on the MEHS.

2. Materials and methods

2.1. Materials

Rosemary extract (*Rosmarinus officinalis*) was supplied from FLAVEX (Lehlingen, Germany), broccoli sprout extract (*Brassica oleracea* var. *italica*) was supplied from ORYZA (Ichinomiya, Japan), citrus extract mixture (*Citrus Aurantium dulcis*, *Citrus Aurantium amara* and *Citrus paradisi*) was supplied from BREKO (Bremen, Germany) and palm oil extract (*Elaeis Guineensis*) was supplied from Carotech (Perak, Malaysia). CO (Corn oil of *Zea mays*), SO (Sunflower oil of *Helianthus annuus*) and HS (High oleic sunflower oil of *Helianthus annuus*) was supplied from Tradin Organic Agriculture B.V. (Amsterdam, Netherlands) as certified organic products. Spray-dried dextrin with dextrose equivalent (DE) 8-12 was supplied from Sunrich (Minnesota, USA), spray-dried milk protein isolates (MPI) with 80.6 g/100 g (w/w) protein and 4.9 g/100 g lactose was supplied from Emmi Milch AG (Dagmersellen, Switzerland) by concentration from skimmed milk pasteurized at 72 °C for 15 s. The paste type soybean lecithin, made by drying after degumming of pressed soybean oil, was supplied from Clarkson Soy Products (Cerro Gordo, USA). Dextrin contained 93.7 g/100 g carbohydrates according to product specifications and was produced as part of a full-scale standard production. MPI contained 4.0 g/100 g lactose, 83.0 g/100 g milk protein, 2.0 g/100 g milk fat. Soybean lecithin contained 36.0 g/100 g phospholipids, 10.0 g/100 g phosphatidylcholine. Tocopherol was supplied from DSM (Basel, Switzerland) and trisodium phosphate (Na_3PO_4) was supplied from SDBNI Co., Ltd. (Ansan, Republic of Korea).

Chloroform, petroleum ether and hexane were acquired from Fisher Scientific Korea Ltd. (Seoul, Republic of Korea). Sodium thiosulfate was from Merck (Darmstadt, Germany). Acetic acid, potassium iodide and *p*-anisidine were purchased from Sigma–Aldrich Korea Ltd. (Seoul, Republic of Korea). All solvents and reagents were appropriate grade for chromatographic analysis and purchased from Aldrich (Milwaukee WI).

2.2. Methods

2.2.1. Sample preparation for antioxidant test in liquid oils

A single component or a mixture of NPEs such as rosemary, broccoli sprout, citrus mixture, and palm oil were added to CO, SO and HS. NPEs were prepared by simple grinding, spray drying, or extraction method, respectively. Rosemary extract was prepared as 87% (w/w) final total solid content by extraction from leaves of *Rosmarinus officials* under CO_2 with an extraction solvent composed of essential oil, water, and ethyl alcohol (4:5:4, w/w). Palm oil extract was made by collecting residuals of palm oil after processing the oils of *Elaeis Guineensis* through saponification and washing with hot water. Broccoli extract was prepared by spray drying as 50% (w/w) of grinded brocolli sprout powder and 50% (w/w) of dextrin as a coating agent. Citrus mixture extract was made with three kinds of citrus (40% (w/w) of *Citrus Aurantium dulcis*, 30% (w/w) of *Citrus Aurantium amara,* and 30% (w/w) of *Citrus paradisi*) as powder form with 94% (w/w) total solid by spray drying

after extraction and evaporation with ethyl alcohol (50%, w/w).

2.2.2. Analytical method for lipid oxidation by the rancimat

Induction time to primary oxidation of lipid in seed oils and lipid on the surface of microencapsulated high oleic sunflower oil (MEHS) was measured by the Rancimat method (Läubi & Bruttel, 1986) with Rancimat apparatus (Metrohm, Herisau, Switzerland). A flow of air (20 L/h) was bubbled through 5.0 g of oil heated to 98 °C. The volatile oxidation products were stripped from the oil and dissolved in cold water, increasing its conductivity. The time taken to reach an inflection point on the induction curve was measured for lipid in seed oils and lipid on the surface of MEHS.

2.2.3. Analytical method for lipid oxidation under accelerated storage condition

Changes in the Peroxide value (POV) and *p*-anisidine value (ASV) of lipid on the surface of MEHS prepared in the presence of NPEs were measured. Extraction of fat from microcapsules was conducted according to the method described elsewhere (Dieffenbacher & Lüthi, 1986). Briefly, 15 g of microcapsules were mixed with 60 mL pentane in 125 mL Erlenmeyer flask with a stopper. The mixture was then shaken for 2.5 h at 25 °C in the dark. The organic solution was passed through a Büchner funnel with a Whatman No. 4 filter and was collected in a round-bottom flask to evaporate using a rotary evaporator in a water bath at less than 30 °C to minimize influence of heating on lipid oxidation.

Hydroperoxides, primary oxidation products, were measured and represented as the peroxide value (POV) as described in the AOCS (AOCS method Cd 8-53, 1996). The POV was expressed as milliequivalents (mequiv) of active oxygen per kilogram of oil. Automatic titration was performed by using a potentiometric titlatoin system (Model 799 GPT Titrino, 685 Dosimat, Pt Titrode) equipped with a sample changer (Metrohm, Herisau, Switzerland). Secondary oxidation products were presented as the *p*-anisidine (ASV) value described in the AOCS (AOCS method Cd 18-90, 1996). The ASV was expressed as 100 times the optical density measured at 350 nm in a cuvette with 1 cm path-length. The solution contained 1.0 g of the oil in 100 mL of a mixture of solvent and reagent.

2.2.4. Sample preparation for antioxidant test in microcapsule under accelerated storage condition

Microencapsulation was carried out using the similar method as described elsewhere (Keogh & O'Kennedy, 1999). A suspension of 9.7% (w/w) MPI in deionized water was prepared by mixing with a homomixer (Ultra Turrax T-50, Janke & Kunkel Ika-Labortechnik, Germany) for 20 min at 6000 rpm at 65 °C after HS was added at the ratio of 3.5:1 (w/w), followed by addition of soybean lecithin at the ratio of 0.1:1 (w/w) with respect to the MPI solid. The resulting MEHS was composed of HS (33.2%) (w/w), dextrin (56.3%), MPI (9.7%), and soybean lecithin (0.8%). Components for microencapsulation of seed oil (core material) were optimized in terms of the microencapsulated efficiency (MEE). As a result, the compositional portions of MPI and dextrin were determined to be 9.7% and 56.3%, respectively. In other words, we measured the microencapsulated efficiency (MEE) at various molar ratios of two components, and found little change in MEE under the conditions of more than 9.7% MPI. Although it has been reported that the balance between MPI and dextrin affects the efficiency of microencapsulation, it was sufficient to construct the microencapsules with minimal amount of MPI, when considering high cost of MPI. Moreover, in the present study, there was no difference in the antioxidant effect of NPEs on microencapsulation even at varied ratios of two additives.

A single or a mixture of NPEs were added to the solution for microencapsulation of HS, and their antioxidant effect was tested. The concentrations of added NPEs were; 1% (w/w) citrus extract (MEHS-C), 0.05% rosemary extract (MEHS-R), and a mixture of 1% citrus mixture extract and 0.05% rosemary extract (MEHS-CR). To investigate the effect of emulsifier, 1% sodium triphosphate was added to the solution for microencapsulation of HS in the presence of respective NPE, and the resulting microcapsules were named MEHS-CRE. Tocopherol (0.03%) was added to the solution for MEHS-CRE, and represented as MEHS-CRET. MEHS-CRBET indicates MEHS-CR containing 0.03% tocopherol and 0.33% broccoli extract. The mixture was homogenized with a homogenizer (APV RANIE, Denmark) at the feeding rate of 1 L/min at 150 kg/cm^2 by a cycle. The homogenized emulsions were immediately fed into a pilot-scale spray drier using a disk type nozzle (Niro Atomizer, Niro, Denmark) equipped with a spray drying chamber (160 cm height and 90 cm diameter). The emulsion was fed into the chamber at the feeding rate of 1.6 L/h, atomized by the hot air (air velocity of 2 m/s) from blower. Temperatures at inlet and outlet of spray dryer were 160 ± 5 °C and 95 ± 5 °C, respectively.

The prepared microcapsules were stored at 60 °C for 30 days. This condition was previously used to test the synergistic effect of rosemary extract on the stability of sunflower oil in the presence of tocopherol, ascobyl palmitate, and citric acid (Hras, Hadolin, Knez, & Bauman, 2000). Aliquots (250.0 ± 0.1 g) of each sample were poured into PYREX glass vessels (500 mL, 80 mm i.d.) with a cap in the incubator at 60 ± 1 °C. Initial water content and water activity (a_w) of the test samples were measured to investigate their effects on the lipid oxidation. Samples in glass vessels were taken at intervals, and the peroxide value (POV) and *p*-anisidine value (ASV) were determined. The remaining samples were stored in the freezer (−20 °C) under a nitrogen blanket to determine fatty acid composition, tocopherols and minerals such as Fe, Cu and Zn.

2.2.5. Microencapsulation efficiency (MEE)

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

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

The total oil content of the powder was determined by the Röse–Gottlib method (IDF, 1993). The extractable oil was measured after gently shaking according to the methods described elsewhere (Sankarikutty, Sreekumar, Narayanan, & Mathew, 1998; Velasco, Marmesat, Dobarganes, & Márques-Ruiz, 2006). MEE was used as a key factor to evaluate the coating quality of microcapsules and defined as the portion of major unsaturated fatty acid (C18:1$n-9$, C18:2$n-6$, C18:3$n-3$) in microcapsules of HS that was neither extracted by solvent and nor exposed to environment.

2.2.6. Microencapsulation yield (MEY)

The microencapsulation yield is defined as the ratio of core material in the final dried microcapsules to that in the emulsion (Zilberboim, Kopelman, & Talmon, 1986) and calculated as follows:

$$\text{MEY} = \frac{\text{core material in microcapsules (g/100 g solids)}}{\text{core material in emulsion (g/100 g solids)}}$$

Core materials in this study were major unsaturated fatty acids such as oleic acid (C18:1$n-9$), linoleic acid (C18:2$n-6$), and linolenic acid (C18:3$n-3$) in HS.

2.2.7. Stabilization factor

The effectiveness of all tested NPEs and their mixtures as an antioxidant was expressed as the stabilization factor (Yanishilieva & Marinova, 1996):

$$F = \frac{(\text{induction period with inhibitor}) \times 100}{\text{induction period without inhibitor}}$$

2.2.8. Analytical method for fatty acids

The fatty acid composition of oils was determined by using a capillary gas chromatograph (Agilent, 6890A Plus, USA) with a flame ionization detector and a DB-225 column (30 m × 0.25 mm i.d, 0.25 μm film thickness, J&W Scientific, USA), according to the standard methodology (AOCS, 1983). T temperature program was from 140 °C to 220 °C for 5 min with a 4 °C/min gradient. The carrier gas was nitrogen, flowing at 0.8 mL/min with a split ratio of 100:1. The injector temperature was 250 °C and detector temperature was 260 °C with air flow 300 mL/min and nitrogen flow 30 mL/min, respectively. Determination of each fatty acids content was verified by comparison of retention times of test samples with those of reference standards.

2.2.9. Analytical method for tocopherols

Content of tocopherols in NPE was determined by using a high performance liquid chromatograph (HPLC, WATERS, Allience system, USA) with a μ-porasil column (250 × 4.6 mm, WATERS, USA); excitation was at 325 nm and detection was at 298 nm with a fluorescence detector (ISO, 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.

2.2.10. Analytical method for minerals such as Fe, Cu, Zn

Minerals such as Fe, Cu and Zn, which are known to affect lipid oxidation, were determined by using inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Jovin-Ivon, ULTMA, France) according to the method for powder type infant formula (AOAC method 984.27, 2005).

2.2.11. Analytical method for Vitamin C

Vitamin C content in NPE was determined by using a rapid HPLC method (Thompson & Trenerry, 1995) after high performance liquid chromatograph (HPLC, WATERS, Allience system, USA) separation with a Capcellpak ACR C_{18} column (250 × 4.6 mm, 5 μm, WATERS, USA); detection was at 254 nm by UV detector. The elution solvent used was a mixture of 0.05 M K_2HPO_4 and acetonitrile (60:40, v/v), and the flow rate was 1.0 mL/min.

2.2.12. Analytical method for total phenolics and carnosolic acid

Total phenolics were determined using a spectrophotometric method at 725 nm (Swan & Hillis, 1959). Carnosolic acid was determined by using HPLC method (Thorsen & Hildebrandt, 2003).

2.2.13. Scanning electron microscope (SEM)

Field emission scanning electron microscope (FE-SEM, FEI, Sirion, USA) was used to examine the morphology and surface appearance of microcapsules. Microcapsule samples were attached with a two-sided adhesive tape to specimen stubs and then Pt-coated in a sputter coater (BAL-TEC, SCD 005, Germany) at 30 mA for 150 s. The coated microcapsules were examined in a Sirion SEM at 10 kV with 1.5 nm resolutions (Rosenberg, Kopelman, & Talmon, 1985).

2.2.14. Particle size analysis and water activity

Particle size analyzer (Mastersizer, 2000; MALVERN, UK) was used to determine the mean volume size of microcapsules. Measurement time and snap for microemulsions were 12 s and 12,000, respectively. For microcapsules, measurement time and snap were 10 s and 10,000, respectively. The background snap was 5000.

2.2.15. Moisture content and water activity (a_w)

Moisture content of the microcapsule was determined by using a thermogravimetric analyzer (MA50, Sartorius, Germany) at 105 °C. Water activity was measured using

a water activity analyzer (AW SPRINT TH500, Novasina, Switzerland).

2.2.16. Statistical analysis

All experiments were performed in triplicate, and differences between experiments were analyzed using Student's *t*-test in Microsoft Excel 2000 (Microsoft Corporation, USA).

3. Results and discussion

High oleic sunflower oil (HS) was microencapsulated by using a dextrin-coating method with supplements such as milk protein isolates (MPI), soy lecithin, and sodium triphosphate emulsifier. It was well documented that milk protein and dextrin are a good wall material for microencapsulation of oils (Keogh & O'Kennedy, 1999) and light core material such as flavors (Trubiano & Lacourse, 1988), respectively. In this study, dextrin was employed as a main coating agent by taking into consideration economic merit. Especially, by the addition of sodium triphosphate emulsifier during the microencapsulating process, microencapsulation efficiency (MEE) was enhanced up to 89–92% (Table 1). It was reported that the property of emulsion is an important factor affecting the quality of microencapsulated oils (Fuchs et al., 2006). In this study, sodium triphosphate was used as additional emulsifier since it has a good emulsion power for HS, dextrin, and MPI. When compared to previous methods, the current approach showed a comparable or improved MEE. For example, in the case of sunflower oil, the relatively low MEE (71–74%) was reported with the use of sodium caseinate and lactose by freeze-drying (Velasco et al., 2006). Jimenez et al. (Jimenez, Garcia, & Beristain, 2004) obtained the MEE of 89.6% with the use of conjugated linoleic acid (CLA). In the microencapsulation of carotene, the use of sodium caseinate and gum acasia increased MEE up to 94% (Habi Mat Dian, Sudin, & Affandi, 1996).

With high MEE, the antioxidant effect of NPE on the microencapsulated high oleic sunflower oil was investigated. As can be seen in the image of scanning electron microscope (SEM) for MEHS (Fig. 1), the MEHS had no cracks and holes on the surface, showing smooth shape, and reduced indentation. As the particle size decreased, the MEE increased as shown in Table 1.

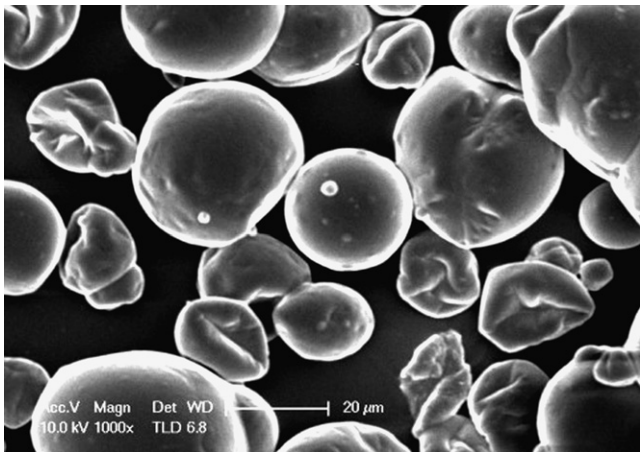

Fig. 1. Scanning electron microscopic (SEM) image of microencapsulated high oleic sunflower oil (MEHS) in the presence of natural plant extracts (NPEs).

To find effective NPE composition for reduction of lipid oxidation, various NPEs were first tested on liquid CO, SO and HS, prior to the antioxidant test on the MEHS. Antioxidant test was conducted according to the Rancimat method (Läubi & Bruttel, 1986) by comparing induction time and stabilization factor of liquid seed oils (CO, SO and HS) in the presence and absence of NPE (Table 2).

Table 2
Induction time and stabilization factor of different seed oils (CO/SO/HS)

Oils and NPE	Induction time (h)	Stabilization factor
CO	20.55	–
CO-P	21.80	106.08
CO-R	23.40	113.87
CO-B	23.72	115.43
CO-C	23.61	119.89
CO-PR	24.07	117.13
CO-PRB	29.07	141.46
CO-PRC	32.24	156.88
CO-RBC	35.32	171.87
CO-PRBC	35.54	172.94
SO	7.03	–
SO-PRBC	16.26	231.29
HS	66.01	–
HS-PRBC	115.20	174.52

The concentrations (w/w) of palm oil extract (P), rosemary extract (R), broccoli extract (B), and citrus mixture extract (C) in seed oils were 0.1%, 0.05%, 1.0%, and 1.0%, respectively.

Table 1
Characteristics of microcapsules mixed with different compositions of NPEs in terms of average particle size, moisture content, MEY, and MEE

Measured factors	Microcapsules						
	MEHS	MEHS-C	MEHS-R	MEHS-CR	MEHS-CRE	MEHS-CRET	MEHS-CRBET
Average particle size (μm)	162.0	138.2	79.33	65.3	57.9	41.4	41.2
Moisture (g/100 g)	5.22 ± 0.03	5.53 ± 0.03	5.54 ± 0.03	5.52 ± 0.02	3.61 ± 0.02	3.43 ± 0.03	3.52 ± 0.02
Water activity (a_w)	0.292	0.278	0.262	0.266	0.265	0.293	0.304
MEY (%)	94.65	94.20	94.26	94.35	96.52	96.80	96.44
MEE (%)	45.08	45.02	49.62	51.25	89.03	89.36	92.10

Although all NPEs tested showed effective antioxidant activity on the three seed oils, combination of NPEs resulted in higher antioxidant effect than individual NPE, showing a combined effect. When various combinations of four different NPEs were tested for CO which is easy to monitor lipid oxidation, induction time and stabilization factor mostly increased in the presence of NPE, indicating significant antioxidant effect of NPE on CO. Maximum antioxidant effect was observed when 0.05% rosemary extract was combined with 1.0% broccoli extract and 1.0% citrus extract. Similarly, antioxidant effects by a mixture of NPEs were also observed for HS and SO. Differences in induction time between CO, SO, and HS seem to result from the difference in fatty acid composition; content of linoleic acid (C18:2$n-6$) as an unsaturated fatty acid in SO is much higher than in HS (Table 3). Lipid oxidation of unsaturated fatty acid easily occurred with the increase of lipid hydroperoxides in the presence of initiators such as free radicals (Frankel, 1998). Despite the different antioxidant effects on three liquid oils, this result indicates that NPE was effective for inhibition of lipid oxidation in liquid oils.

With the antioxidant effect of NPE on liquid seed oils, the MEHS was tested with NPEs. For this, oxidation degree on the surface of MEHS was determined by measuring the peroxide value (POV) and *p*-anisidine value (ASV) in the absence and presence of NPE. When NPE-free MEHS was exposed to high temperature under accelerated storage condition after microencapsulation process (Inlet and outlet temperatures of spray dryer were 160 °C and 95 °C, respectively), the POV increased with the time course (HS in Fig. 2), indicating that lipid oxidation took place on the surface of MEHS. On the other hand, addition of NPEs did not induce the increase in the POV from the MEHS (MEHS-C, -R, -CR, -CRE, -CRET, -CRBET in Fig. 2). More importantly, MEHS treated with a mixture of NPEs showed much lower change in the POV than that with individual NPE. This combined effect was similar to the results shown in Table 3. Generation of secondary oxidation products, which was measured through ASV, was also effectively inhibited by NPEs (Fig. 3) as observed for POV shown in Fig. 2. This result indicates that the NPEs such as rosemary, broccoli sprout, and citrus extract can effectively prevent the surface lipid oxidation of MEHS.

It is well known that the moisture content of the samples as well as porosity has a significant effect on the lipid stability. Initial water activities (a_w) of tested microcapsules were estimated to be in the range of 0.26–0.30. This level is within the ideal range of 0.2–0.4 for storage stability in lipid oxidation as reported elsewhere (Rockland et al., 1987; Rückold, Grobecker, & Isengard, 2001). In addition, since a_w levels of tested microcapsules were very similar and there were no significant difference in a_w between microcapsules, it is likely that the effect of a_w on the lipid

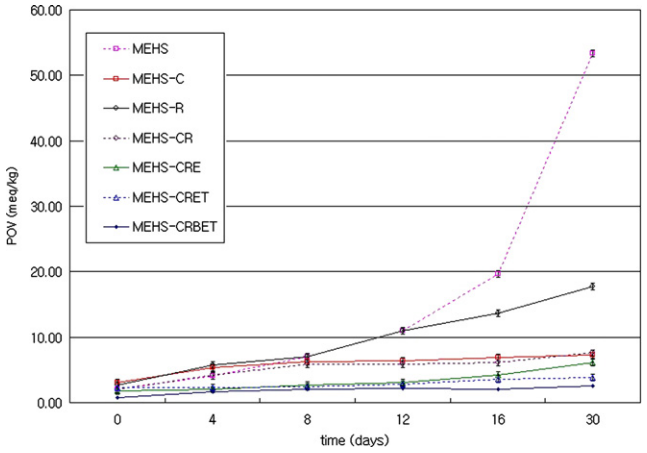

Fig. 2. Change in the POV caused by lipid oxidation on the surface of MEHS. The microcapsules with different compositions were subjected to exposure at 60 °C, and the POV was determined as a function of time.

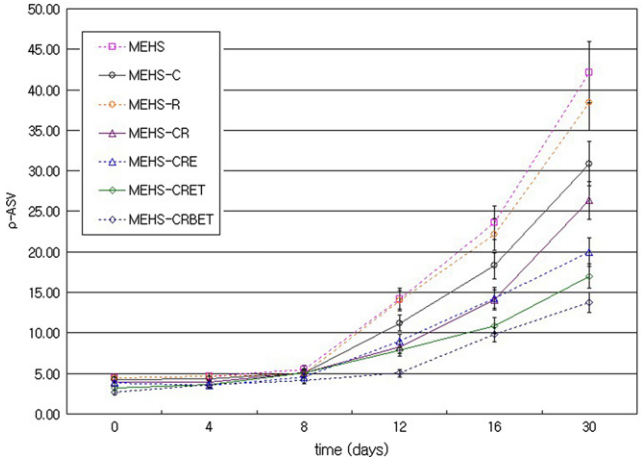

Fig. 3. Change in the ASV caused by lipid oxidation on the surface of MEHS. The microcapsules with different compositions were subjected to exposure at 60 °C, and the ASV was determined as a function of time.

Table 3
Fatty acids compositions of CO, SO, and HS

Seed oils	Fatty acid contents (%, w/w)									
	Myristic C14:0	Palmitic C16:0	Stearic C18:0	Oleic C18:1$n-9$	Linoleic C18:2$n-6$	Linolenic C18:3$n-3$	Arachidic C20:0	Eicosenoic C20:1	Behenic C22:0	Lignoceric C24:0
Corn oil	0.03	11.75	2.31	29.98	54.45	0.38	0.20	0.10	0.51	0.16
Sunflower oil	0.08	5.86	3.28	37.94	51.40	0.26	0.20	0.14	0.53	0.17
High oleic sunflower oil	0.04	3.67	2.85	86.14	5.50	0.16	0.24	0.38	0.64	0.21

oxidation was negligible compared to the effect of natural plant extracts (NPEs). Also, water content of the microcapsules were found to be in the range of 3.4–5.5%, which is above the range known to be susceptible to lipid oxidation. It was well documented that when foods are dried to a lower level of moisture content (less than about 2–3%), they may become susceptible to oxidation (Labuza, 1971). Although the tested microcapsules were not equilibrated at the initial a_w level under the constant humidity condition, its effect on the lipid oxidation seems negligible.

The antioxidant effect of NPEs on the MEHS was tested by analyzing induction time that is dependent on primary oxidation of lipid in microcapsules. Prolonged induction time of MEHS in the presence of NPEs indicates high stability against lipid oxidation (Fig. 4). The use of a mixture of NPEs was also very effective as observed above. In the case of MEHS with low MEE (MEHS, MEHS-CI, MEHS-RM and MEHS-CIRM in Table 1), NPE did not function as a significantly effective antioxidant as reported elsewhere (Frankel, 1998). In contrast, high antioxidant effect by rosemary extract and citrus extract was observed for MEHS with high MEE (MEHS-CRE, MEHS-CRET and MEHS-CRBET), resulting in longer induction time (185.3 h) when compared with a control experiment (123.8 h). But, addition of tocopherol (MEHS-CRET and MEHS-CRBET) reduced the antioxidant ability of NPE. Thus, the use of tocopherol should be carefully considered when NPEs are employed to elongate the induction time in microcapsule system.

Based on these results, it is evident that NPEs have a combined effect as an antioxidant on MEHS. From the viewpoint of chemical components, it is likely that the antioxidant effect of NPEs on the MEHS mainly resulted from the combined effect of polyphenols, carnosolic acid, flavonones, and some minerals. It is noteworthy that antioxidant effect of rosemary extract is mainly due to phenolic diterpenes such as carnosolic acid (Frankel et al., 1996; Richheimer et al., 1996). Contents of total phenolics and carnosolic acid in rosemary extract were estimated to be 25.0 g/100 g and and 16.0 g/100 g, respectively. The levels of Fe, Cu, and Zn in rosemary extract as mineral sources

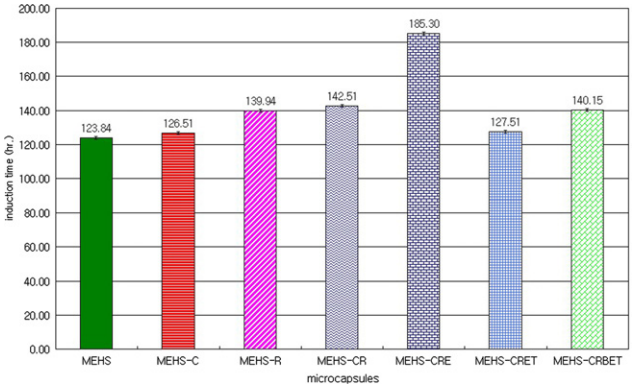

Fig. 4. Induction time of lipid for MEHS.

were about 1.22 mg/100 g, 28.1 μg/100 g, and 0.08 mg/100 g, respectively. Likewise, broccoli extract contained 3.8 g/100 g of total phenolics and 2.0 g/100 g of sulforaphane in addition to 0.5 mg Fe, 236 mg Na, 263 mg Ca, and 526 mg vitamin C per 100 g. Antioxidant effect of citrus mixture extract also seems to be attributed to total phenolics (72.1 g/100 g), flavonones (17.5 g/100 g), and other minerals such as Fe, Cu, Zn, and vitamin C. It is evident that these chemicals in NPE played an important role in blocking severe lipid oxidation of MEHS.

In conclusion, we have demonstrated that natural plant extracts (NPE) such as rosemary, broccoli sprout, and citrus can effectively prevent MEMS from the lipid oxidation. By simply mixing and adding NPEs at proper combination during the microencapsulating process of HS, lipid oxidation was significantly reduced. Although HS contained large amount of oleic acid (C18:1$n-9$) which is less vulnerable to lipid oxidation, extended storage of MEHS at high temperature also led to severe lipid oxidation. In this regard, the use of NPEs will ensure the prolonged stability of miroencapsulated oil products as an effective antioxidant.

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