

Improvement of the lipid oxidative stability of soybean oil-in-water emulsion by addition of daraesoon (shoot of *Actinidia arguta*) and samnamul (shoot of *Aruncus dioicus*) extract

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Abstract The effect of 75% ethanol extract of daraesoon and samnamul (200 mg/kg) on the lipid oxidation of soybean oil-in-water (4:6, w/w) emulsion containing iron (5 mg/kg) in dark conditions at 25°C was studied by determining headspace oxygen and hydroperoxide contents. Polyphenol, carotenoid, and chlorophyll contents were also evaluated using spectrophotometry. The headspace oxygen contents were higher and hydroperoxide contents were lower ($p < 0.05$) in the emulsions with added daraesoon and samnamul extracts compared with the control emulsion without the extract. The antioxidant activity of the daraesoon and samnamul extracts in the lipid oxidation of the emulsions was comparable to that of dibutylhydroxytoluene at 200 mg/kg. Polyphenols, carotenoids, and chlorophylls were degraded during oxidation of the emulsions, possibly due to a role of the antioxidants. The results suggest that contribution to the improved lipid oxidative stability of the emulsion with added samnamul and daraesoon would be due to polyphenols and pigments, respectively.

Keywords: daraesoon and samnamul extracts, lipid oxidation, emulsion, polyphenol, pigment

Introduction

An emulsion undergoes lipid oxidation that affects its quality, and the oxidation is accelerated by the presence of transition metals (1). Antioxidants and/or food materials with antioxidant properties are frequently added to emulsions to decrease lipid oxidation. Plant food materials have been known for their high content of antioxidants such as polyphenols. Polyphenols are generally defined as compounds having >12 phenolic hydroxyl groups with moderate water solubility (2); however, the term “polyphenols” is also used to define compounds exclusively derived from the phenylpropanoid and/or polyketide pathway with more than one phenolic unit and deprived of nitrogen-based functions (3). Rosmarinic acid, caffeic acid, catechin, chlorogenic acid, and luteolin are typical examples of polyphenols found in plant food materials. Polyphenols have been reported to have antioxidant activity via radical scavenging, metal chelating, and/or singlet oxygen quenching (4).

Daraesoon (shoot of *Actinidia arguta*) and samnamul (shoot of *Aruncus dioicus*) are rich in pigments as well as nutrients such as dietary fiber, vitamins, and minerals (5,6). Their production in Korea is not as high as that of Chinese bellflower (*Platycodon grandiflorum*); however, their texture, in particular, chewiness, is sufficiently preferable for consumers (7). The extracts of daraesoon and samnamul were

recently reported to provide high activity for α -glucosidase inhibition, blood glucose lowering, and DPPH radical scavenging (5,6,8), possibly due to high content of polyphenols. Daraesoon and samnamul have been consumed as namul (a Korean seasoned vegetable dish), and most research related with foods were confined to how to cook namul to have beneficial health effects (5,9,10), and the efforts to utilize them in the food industry have been scarce. This research was performed to evaluate the effect of daraesoon and samnamul extracts on the lipid oxidation of the oil-in-water (O/W) emulsion to apply these food materials to the food industry, especially in salad dressing by providing antioxidative and coloring effect. Polyphenols of the extracts and the emulsions during oxidation were also determined.

Materials and Methods

Materials and reagents Dried samnamul and daraesoon were purchased from Woolwellbeing Food Inc. (Ulleung, Korea). Rosmarinic acid, caffeic acid, gallic acid, *p*-coumaric acid, catechin, xanthan gum, cumene hydroperoxide (CuOOH), Folin–Ciocalteu’s phenol reagent, chlorophyll *a* and *b*, β -carotene, dibutylhydroxytoluene (BHT), ammonium thiocyanate, activated silicic acid (75–150 μ m), and

alumina (5.8 nm) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Egg yolk lecithin was purchased from Goshen Biotech (Namyangju, Korea). Isooctane, ferrous sulfate, and celite were purchased from Junsei Co. (Tokyo, Japan). Methanol, water, and formic acid at HPLC grade were purchased from Mallinckrodt Baker Co. (Phillipsburg, NJ, USA). All other chemicals were of analytical grade. Refined, bleached, and deodorized soybean oil (Samyang Corp., Seoul, Korea) for the emulsion preparation was further purified by silicic acid-alumina-celite column chromatography according to the method of Kim and Choe (11).

Preparation of daraesoon and samnamul extract Dried and ground daraesoon and samnamul were mechanically stirred in 75% ethanol solution (1:10, w/v) at 25°C for 12 h and then filtered through a Whatmann filter paper #42 (GE Healthcare Life Sciences, Little Chalfont, UK). The solvent was removed using a rotary evaporator (N-N series; Eyela, Tokyo, Japan) at 65°C.

Preparation of emulsions and oxidation The emulsion system for the experiment was an acidic O/W emulsion with relatively high content of oil, comprising purified soybean oil and citrate buffer solution at pH 4.0 (4:6, w/w). The egg yolk lecithin (350 mg/kg), xanthan gum (350 mg/kg), FeSO₄ (5 mg/kg), and daraesoon or samnamul extract (200 mg/kg) were also added to the emulsion, followed by homogenization using an Ultra-Turrax T25 homogenizer (IKA Instruments, Staufen, Germany) at 10,000 rpm for 6 min according to the method of Kim and Choe (11). The emulsion (7 g) was transferred into 20 mL serum bottles, which were then tightly capped with teflon-coated stoppers and aluminum caps. The bottles were placed in an air-forced incubator (Daihan Labtech Co., Seoul, Korea) at 25°C for 6 days in dark conditions. The control sample was prepared without the daraesoon and samnamul extract, and the positive control was prepared with BHT (200 mg/kg). All samples were prepared in duplicate, and there was no phase separation observed throughout the experimental period.

Analysis of lipid oxidation of the emulsion samples Lipid oxidation of the emulsion was evaluated on the basis of the headspace oxygen and hydroperoxide contents. The headspace oxygen content was determined using a gas chromatograph (YL 6100; Younglin Instrument Co., Ltd., Anyang, Korea) equipped with an autosampler and a stainless steel column (1.83 m×0.32 cm; Alltech, Deerfield, IL, USA) packed with 80/100 mesh molecular sieve 13X (12). Helium was used as the carrier gas at 20 mL/min. The temperatures of the oven, injector, and thermal conductivity detector were 35, 100, and 140°C, respectively. The hydroperoxide content of the emulsion was determined using the ferric thiocyanate method (12). The absorbance was recorded at 510 nm with an HP8453 UV-visible spectrophotometer (Hewlett Packard, Wilmington, DE, USA), and the hydroperoxide content was expressed as mmol of CuOOH in kg of oil using a calibration curve ($r^2=0.9991$).

Analysis of polyphenols in daraesoon and samnamul extracts and emulsion Polyphenols in the daraesoon and samnamul extracts were separated and determined by HPLC (11) using a YL 9100 HPLC (Younglin Instrument Co., Ltd.) equipped with an autosampler and symmetric C18 column (4.6×150 mm, 5 μm; Waters, Milford, MA, USA). The mobile phase was a mixture of 2.5% formic acid and 100% methanol in a gradient system of 5% (0 min), 30% (15 min), 40% (40 min), 50% (60 min), 55% (65 min), and 100% (90–95 min) on methanol basis at 0.8 mL/min. Each peak in the chromatogram was presumably identified by comparing the retention time with those of standard polyphenol compounds. The total polyphenol contents of the extracts were determined by spectrophotometry at 725 nm using the Folin–Ciocalteu reagent and a UV-visible spectrophotometer (HP 8453; Hewlett Packard) (11).

The total polyphenol contents of the emulsion samples were also determined by the Folin–Ciocalteu method after dissolving the emulsion in a mixture of methanol and water (60:40, v/v), followed by centrifugation (Avanti J; Beckman) at 4°C and 11,872×g for 20 min. The concentration of polyphenols was expressed as a caffeic acid equivalent using a calibration curve ($r^2=0.9919$).

Analysis of chlorophylls and carotenoids in daraesoon and samnamul extracts and emulsion Chlorophylls and carotenoids of daraesoon and samnamul extracts were analyzed using HPLC (5). For chlorophyll determination, the extracts were dissolved in dichloromethane and injected into a YL 9100 HPLC (Younglin Instrument Co., Ltd.). The column was a symmetry C18 column (5.0 μm, 4.6×300 mm, Waters) with a mixture of ethyl acetate: methanol:water (50:37.5:12.5, v/v/v) as an eluting solvent at 1.5 mL/min. The UV-Vis detector was set at 438 nm. For carotenoid determination, the extracts were saponified following the AOAC method 970.64 (13) and then dissolved in a mixture of *n*-hexane: acetone: ethanol: toluene (10:7:6:7, v/v/v/v) to inject into a YL 9100 HPLC equipped with a μ-porasil column (300×3.9 mm, 10 μm i.d., Waters) and an UV-Vis detector at 436 nm. The eluting solvent was a mixture of *n*-hexane:isopropanol (97:3, v/v) at 1.0 mL/min. Chlorophylls and carotenoids were identified on the basis of comparison of retention times with standard chlorophylls *a* and *b*, lutein, and β-carotene and quantified using respective calibration curves ($r^2>0.998$).

Chlorophyll and carotenoid contents of the emulsion samples were determined using a UV-Vis spectrophotometer (HP 8453; Hewlett Packard, DE, USA) according to the method of Chung *et al.* (14). Aliquots of the emulsion dissolved in dichloromethane were separated by centrifugation (Avanti J; Beckman) at 4°C and 11,872×g for 20 min. The absorbance of the bottom layer was read at 486 and 665 nm for total carotenoid and chlorophyll determination, respectively, using respective calibration curves ($r^2>0.996$).

Statistical analysis Samples were prepared in duplicate, and two replicates were in the analysis of each sample. Data were statistically analyzed using SAS/PC (SAS 9.2, SAS Institute Inc., Cary, NC, USA)

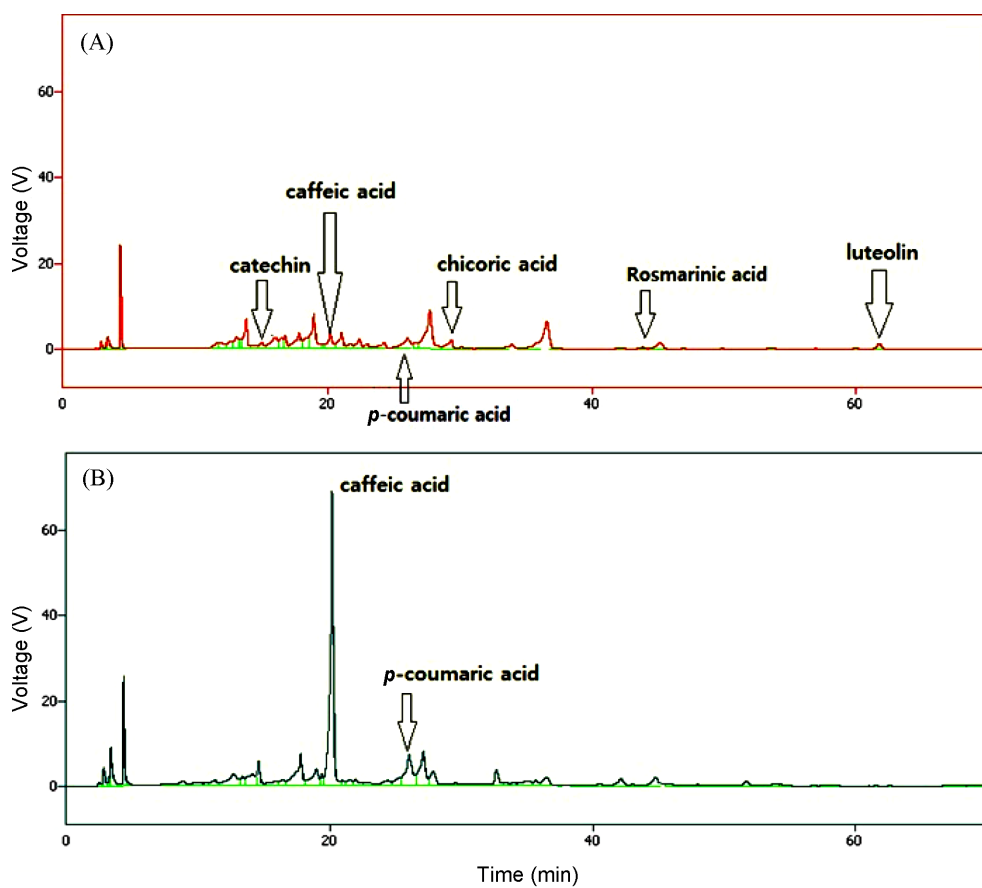


Fig. 1. HPLC chromatogram of daraesoon (A) and samnamul extracts (B) 8,000 mg/kg in 75% ethanol.

including regression analyses and Duncan's multiple range test at a significance level of 5%.

Results and Discussion

Characteristics of daraesoon and samnamul extracts The yield of 75% ethanol extracts of daraesoon and samnamul were 12.26 ± 0.43 and 13.23 ± 0.33 %, respectively, on a dry basis. The total polyphenol contents of the daraesoon (111.39 ± 0.00 g/kg) and samnamul (114.44 ± 2.01 g/kg) extract were not significantly different ($p > 0.05$). These values were higher than the values (35–38 g/kg) of herb extracts, such as rosemary, thyme, and oregano (11). Despite the insignificantly different contents of polyphenols between daraesoon and samnamul extracts, they showed different polyphenol composition (Fig. 1), presumably identified by the retention time of standard polyphenol compounds. The samnamul extract showed a sharp and significant peak of caffeic acid with traces of catechin, *p*-coumaric acid, chicoric acid, and rosmarinic acid. The daraesoon extract showed a luteolin peak in addition to these peaks but very small peak of caffeic acid. To the best of our knowledge, the polyphenol composition of samnamul and daraesoon has never been reported previously, and detailed polyphenol composition of daraesoon and

samnamul is still under investigation.

Both extracts contained chlorophylls and carotenoids that might affect lipid oxidation. The daraesoon extract contained chlorophyll *a*, β -carotene, and lutein at 145.68 ± 0.13 , 138.14 ± 0.67 , and 1.25 ± 0.02 mg/kg, respectively. The contents of chlorophyll *a*, β -carotene, and lutein of the samnamul extract were significantly ($p < 0.05$) lower (71.42 ± 0.06 , 57.15 ± 2.01 , and 0.73 ± 0.13 mg/kg, respectively) than those of the daraesoon extract. Difference in the polyphenol composition and contents of chlorophylls and carotenoids between daraesoon and samnamul extracts might result in different behavior in decreasing the lipid oxidation of the emulsions.

Effect of daraesoon and samnamul extracts on the iron-catalyzed autoxidation of lipids in the O/W emulsion

Degree of lipid oxidation of the soybean O/W emulsions, as measured on the basis of the headspace oxygen and hydroperoxide contents, is shown in Fig. 2. The headspace oxygen contents of the control emulsion without any added extract decreased with time, due to oxygen consumption for the lipid oxidation, at a rate of $0.4961 \mu\text{mol O}_2/\text{mL}/\text{day}$ (Table 1). Addition of the daraesoon and samnamul extracts or BHT significantly ($p < 0.05$) decreased the oxygen consumption in the headspace of the samples with an oxygen decreasing rate of 0.3374, 0.3432, and $0.3694 \mu\text{mol O}_2/\text{mL}/\text{day}$, respectively, as shown in Table 1, resulting

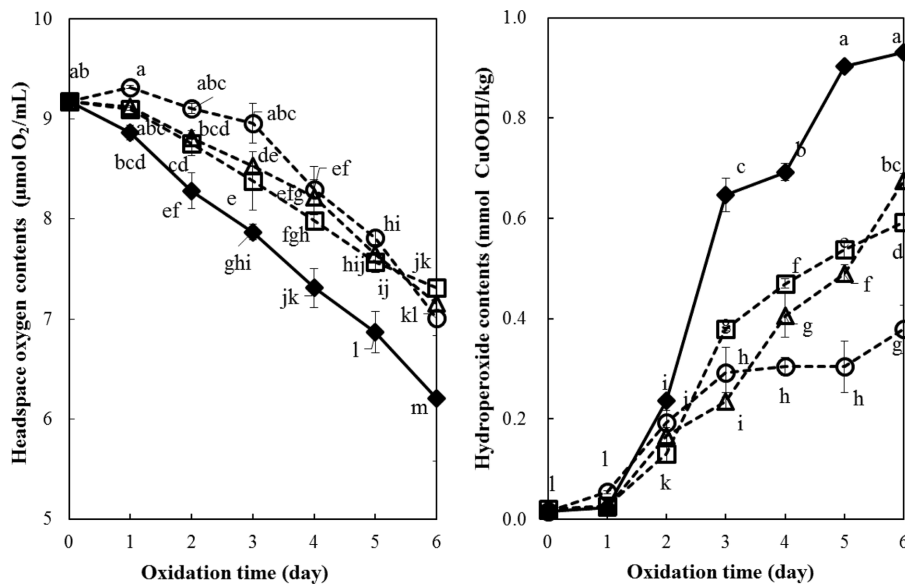


Fig. 2. Headspace oxygen and hydroperoxide contents of soybean oil-in-water (O/W) (4:6, w/w) emulsion with added daraesoon or samnamul extract (200 mg/kg) during iron-catalyzed oxidation at 25°C (◆; control without extract, □; daraesoon extract, △; samnamul extract, ○; dibutylhydroxytoluene as positive control). Different letters on the line represent significantly different values at 5%.

Table 1. Regression equation¹⁾ between oxidation time and headspace oxygen and hydroperoxide contents of soybean oil emulsion with added daraesoon and samnamul extracts or dibutylhydroxytoluene (200 mg/kg) during iron-catalyzed oxidation at 25°C for 6 days

Additives	Headspace oxygen content			Hydroperoxide content		
	a	b	r ²	a	b	r ²
No(control)	0.4961 ^{a2)}	9.2849	0.9947	0.1772 ^a	0.0389	0.9376
Daraesoon extract	0.3374 ^b	9.3386	0.9833	0.1099 ^b	0.0214	0.9444
Samnamul extract	0.3432 ^b	9.4099	0.9544	0.1121 ^b	0.0485	0.9673
Dibutylhydroxytoluene (positive control)	0.3694 ^b	9.6331	0.8591	0.0607 ^c	0.0385	0.9007

¹⁾Headspace oxygen contents (µmol O₂/mL)/hydroperoxide contents (mmol CuOOH/kg)=a×oxidation time (day)+b, r²=determination coefficient

²⁾Different superscripts mean significantly different values in each attribute among the emulsion samples by dummy variable regression analysis (p<0.05).

in a higher remaining headspace oxygen content. There was no significant ($p>0.05$) difference among samples with added daraesoon and samnamul extracts and BHT. This suggests that the antioxidant activity of daraesoon and samnamul extracts at 200 mg/kg to decrease the oxygen consumption is comparable to that of BHT.

In contrast to headspace oxygen content, the hydroperoxide content of the control emulsion increased with time at a rate of 0.1772 mmol/kg/day (Table 1), due to the production of primary oxidation products such as hydroperoxides, during iron-catalyzed lipid oxidation. The hydroperoxide content of the control emulsion without added extract was 0.02 mmol/kg before oxidation and significantly ($p<0.05$) increased to 0.65 and 0.93 mmol/kg after 3 and 6 days, respectively. Addition of the daraesoon and samnamul extracts and BHT significantly ($p<0.05$) decreased the hydroperoxide content compared to the control emulsion; the emulsion with added daraesoon and samnamul extract and BHT at 200 mg/kg showed hydroperoxide contents of 0.38, 0.24, and 0.29 mmol/kg, respectively, after 3 days and 0.59, 0.67, and 0.38 mmol/kg, respectively, after 6

days. This clearly indicates an antioxidant activity of daraesoon and samnamul extracts as well as BHT by lowering hydroperoxide production. Decreased production of hydroperoxides in the emulsions with added daraesoon and samnamul extracts could be due to polyphenols, carotenoids, and chlorophylls derived from the extracts. The antioxidant activity of these compounds has been reported to be through radical scavenging and metal chelating (15-17). The rates of hydroperoxide production were the lowest ($p<0.05$) in the emulsion with added BHT (0.0607 mmol/kg/day), and there was no significant difference ($p>0.05$) between the samples with added daraesoon (0.1099 mmol/kg/day) and samnamul (0.1121 mmol/kg/day) extracts. The results suggest the possible utilization of daraesoon and samnamul in salad dressing to improve the lipid oxidative stability. Despite many studies on the antioxidant activity of herbs (11,18-22), there was no report regarding the application of daraesoon and samnamul to emulsion foods. This represents the first report regarding the antioxidant activity of daraesoon and samnamul extracts.

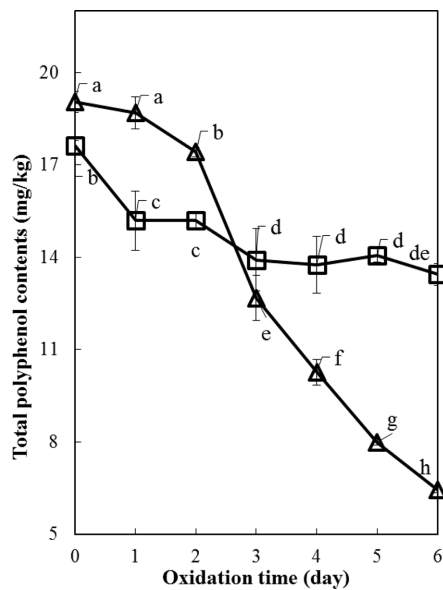


Fig. 3. Polyphenol contents of soybean O/W (4:6, w/w) emulsion with added daraesoon or samnamul extract (200 mg/kg) during iron-catalyzed oxidation at 25°C (□; daraesoon extract, △; samnamul extract). Different letters on the line represent significantly different values at 5%.

Table 2. Regression equation¹⁾ between oxidation time and polyphenol contents of soybean oil emulsion with added daraesoon and samnamul extracts (200 mg/kg) during iron-catalyzed oxidation at 25°C for 6 days

Additives	a	b	r ²
Daraesoon extract	0.583 ^{b2)}	16.48	0.7536
Samnamul extract	2.369 ^a	20.32	0.9602

¹⁾Polyphenol contents (mg/kg) = $-a \times \text{oxidation time (day)} + b$, r^2 = determination coefficient

²⁾Different superscripts mean significantly different values between emulsion samples by dummy variable regression analysis ($p < 0.05$).

Polyphenol contents during oxidation of the emulsion with added extracts

The total content of polyphenols in the emulsions with added daraesoon and samnamul extracts decreased during iron-catalyzed oxidation, as shown in Fig. 3. Before oxidation, the total content of polyphenols in the emulsion with added daraesoon and samnamul extracts at 200 mg/kg was 17.63 and 19.04 mg/kg, respectively, and decreased to 13.91 and 12.66 mg/kg, respectively, after 3 days and to 13.43 and 6.44 mg/kg, respectively, after 6 days. These results clearly indicate degradation of polyphenols derived from daraesoon and samnamul extracts through self-sacrifice during the emulsion oxidation. Polyphenols are oxidized by donating hydroxyl hydrogens to radicals such as peroxy radicals of lipids and hydroxyl radicals, resulting in degradation. They can also directly form a linkage with peroxy radicals (23). Degradation of polyphenols during the oxidation of the soybean O/W (4:6, w/w) emulsion showed relatively high correlation with time ($r^2 > 0.75$). The rate of polyphenol degradation was significantly ($p < 0.05$) higher in the emulsion with added samnamul extract (2.369 mg/kg/day) than in the emulsion with daraesoon extract (0.583 mg/kg/day), as shown in

Table 2. This suggests that polyphenols in the emulsion with added samnamul extract acted as antioxidants faster than those in the emulsion with daraesoon extract. Since improvement in the lipid oxidative stability of the emulsions and polyphenol content by addition of both extracts was not significantly different ($p > 0.05$), factors other than total polyphenol content might have worked on the oxidative stability of the emulsion. Polyphenol composition is thought to be one of the possible factors, as suggested by other studies (11,20,24).

Carotenoid and chlorophyll contents during the oxidation of the emulsion

Carotenoids and chlorophylls have been known as antioxidants in the autoxidation of lipids via radical scavenging (15,16), and contents of these pigments were determined during oxidation of the emulsion. The contents of carotenoids and chlorophylls in the emulsions with added daraesoon and samnamul extracts decreased during iron-catalyzed oxidation, as shown in Fig. 4. Carotenoid contents in the emulsions with added daraesoon and samnamul extracts at 200 mg/kg were 8.65 and 1.52 mg/kg, respectively, before oxidation and decreased to 2.38 and 1.25 mg/kg, respectively, after 3 days and to 1.78 and 0.57 mg/kg, respectively, after 6 days. This indicates carotenoid degradation during the emulsion oxidation, possibly due to their antioxidant role to decrease lipid oxidation of the emulsions. Chlorophyll contents in the emulsions with added daraesoon and samnamul extracts at 200 mg/kg were 2.10 and 0.99 mg/kg, respectively, before oxidation and decreased to 0.54 and 0.70 mg/kg, respectively, after 3 days. Most of the chlorophylls were degraded and thus not detected in the emulsion with added samnamul extract after 6 days, but 0.08 mg/kg chlorophylls still remained in the emulsion with added daraesoon extract. Degradation of these pigments was suggested to be attributed to their antioxidant role in the autoxidation of lipids (25).

Degradation of carotenoids and chlorophylls during oxidation of the soybean O/W (4:6, w/w) emulsion showed a high correlation with time ($r^2 > 0.76$ and $r^2 > 0.90$, respectively). The rate of carotenoid and chlorophyll degradation was significantly ($p < 0.05$) higher in the emulsion with added daraesoon extract (1.001 and 0.346 mg/kg/day, respectively) than with samnamul extract (0.170 and 0.164 mg/kg/day, respectively), as shown in Table 3. This indicates that chlorophylls and carotenoids acted as antioxidants faster in the emulsion with added daraesoon extract than in the emulsion with samnamul extract by self-sacrifice and suggests that carotenoids and chlorophylls could contribute toward decreasing the iron-catalyzed oxidation more in the emulsion with added daraesoon extract than in the emulsion with samnamul extract. This is contrary to polyphenol degradation and suggests a possible interaction between polyphenols and pigments (chlorophylls and carotenoids). Further research on the degree of contribution of polyphenols, carotenoids, and chlorophylls with their interaction to decrease lipid oxidation in the emulsion is required.

In conclusion, the ethanol extracts of samnamul and daraesoon

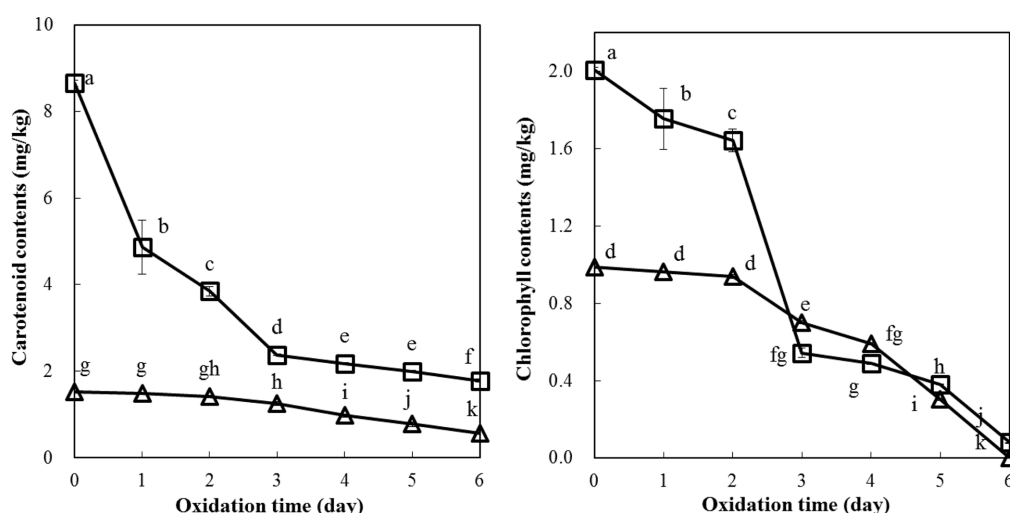


Fig. 4. Carotenoid and chlorophyll contents of soybean O/W (4:6, w/w) emulsion with added darasoon or samnamul extract (200 mg/kg) during iron-catalyzed oxidation at 25°C (□; darasoon extract, △; samnamul extract). Different letters on the line represent significantly different values at 5%.

Table 3. Regression equation¹⁾ between oxidation time and carotenoid and chlorophyll contents of soybean oil emulsion with added darasoon and samnamul extracts (200 mg/kg) during iron-catalyzed oxidation at 25°C for 6 days

Additives	Carotenoids			Chlorophylls		
	a	b	r ²	a	b	r ²
Darasoon extract	1.001 ^{a2)}	6.67	0.7673	0.346 ^a	2.02	0.9060
Samnamul extract	0.170 ^b	1.65	0.9477	0.164 ^b	1.13	0.9088

¹⁾Carotenoid/chlorophyll contents, mg/kg = -a × oxidation time (day) + b, r² = determination coefficient

²⁾Different superscripts mean significantly different values in each pigment between emulsion samples by dummy variable regression analysis (p < 0.05).

significantly improved the oxidative stability of lipid in soybean O/W (4:6, w/w) emulsion, and polyphenols, carotenoids, and chlorophylls could contribute toward decreasing lipid oxidation of the emulsion by self-sacrifice.

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Disclosure The authors declare no conflict of interest.

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