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Effects of a natural antioxidant, polyphenol-rich rosemary (*Rosmarinus officinalis* L.) extract, on lipid stability of plant-derived omega-3 fatty-acid rich oil

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ABSTRACT

Omega-3 fatty acids are often attempted to incorporate into foods due to their beneficial effects on health. However, their susceptibility to autoxidation limits their utilization. After analyses, rosemary extracts (REs) contained abundant antioxidant phenolics, flavonoids, and condensed tannin. Therefore, the aims of this study were to (1) determine the optimal extraction condition of a natural antioxidant from rosemary leaves; (2) investigate an lipid stability of plant-derived omega-3 fatty-acid rich oil (flaxseed oil) with an RE addition. Rosemary extracts obtained by using 80% ethanol solution extraction for 30 min had higher ($p < 0.05$) phenolic recoveries and better ($p < 0.05$) *in vitro* antioxidant abilities. Furthermore, REs can retard ($p < 0.05$) lipid oxidation of flaxseed oil under a Schaal oven test condition (60 °C) compared to α-tocopherol (α-TOCO) and butylated hydroxytoluene (BHT). In conclusion, REs have a positive efficacy to stabilize flaxseed oil against oxidation, posing as a potential application of omega-3 fatty acids in foods.

1. Introduction

As we know, a dietary consumption of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) has been reported to be prophylactic and therapeutic for several chronic diseases nowadays. Due to the physiological functions of ω-3 PUFAs and the demand to meet the balanced ω-6/ω-3 PUFA ratio, food products incorporated these functional fatty acids have been of great interest in the field (Dellarosa, Laghi, Martinsdóttir, Jónsdóttir, & Sveinsdóttir, 2013). Therefore, augmenting the presence of ω-3 PUFAs in diverse frequently-consumed food products may improve the nutritional values in the western diet, and meanwhile, enable the public to get more use to the recommended intake without drastically changing the habitual diet (Elkin, Ying, & Harvatine, 2015).

In comparison with marine derived ω-3 PUFAs, although plant-derived sources are found to be more stable as sources of ω-3 PUFAs, mainly short-chain PUFAs, i.e. α linolenic acid (ALA, 18:3ω3) than marine sources, they are still sensitive to oxidation during the storage. Among all the common culinary oil sources, flaxseed oil is made up of relatively large amount of ALA, up to 50–60% (USDA, 2015). ALA can be converted to eicosapentaenoic acid (EPA, C20:5) and further into docosahexaenoic acid (DHA, C20:6) by desaturation and elongation in human body; however, the conversion efficacy is only 5% to EPA and less than 1% to DHA (Swanson, Block, & Mousa, 2012). Despite the fact that health-promoting effects of ALA have been thought to be the precursor of EPA and DHA, a growing number of studies have revealed positive influences of ALA towards human body independent from its precursor role (Rajaram, 2014).

Though ω-3 PUFAs are physiologically vital, their susceptibility to autoxidation poses a challenge as incorporated in food products during processing and storage (Wang et al., 2016; de Conto, Oliveira, Martin, Chang, & Steel, 2012). Lipid autoxidation not only results in rancidity but also produces toxic substances, leading to overall quality deterioration and shortening shelf life of products (Taneja & Singh, 2012). Consequently, an effective process to retard lipid oxidation is an important subject regarding products rich in ω-3 fatty acids in the food manufacturing. As literature mentioned, it has been indicated that plant-derived phenolic compounds have been demonstrated to possess

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great antioxidant abilities, and several reports also showed that some plant extracts are able to delay the lipid oxidation in fish or meat products during refrigerated storage, thus prolonging the shelf life and improving the quality (Ding, Wang, Yang, Chang, & Chen, 2015; Fu et al., 2011; Shi, Cui, Yin, Luo, & Zhou, 2014). Rosemary extracts, containing phenolic compounds, exert antioxidant abilities via their free radical scavenging abilities to interrupt lipid oxidative chain reaction in food products (Shah, Bosco, & Mir, 2014). Therefore, the aims of this study were to (1) determine the extraction condition of rosemary leaves with an optimal phenolic recovery and antioxidant ability, and (2) investigate the antioxidant abilities of plant-derived omega-3 fatty-acid rich oil (flaxseed oil) premixed with the rosemary extracts (REs) via the accelerated oxidative stability test (Schaal oven test, 60 °C).

2. Materials and methods

2.1. Materials and chemicals

Dry rosemary leaves (*Rosmarinus officinalis* L.), flaxseed oil, and butylated hydroxytoluene (BHT), ethanol (95%) were purchased from Tomax Enterprise Co., Ltd. (Taichung, Taiwan), Gut & Gerne (Stubenberg, Germany), Sigma-Aldrich Co. LLC. (St. Louis, MO, USA), and Eco Chemical Co., Ltd. (Miaoli County, Taiwan), respectively. All other chemicals used in this study were purchased from Sigma-Aldrich Co. LLC. with a chemical grade of analysis. Alpha-tocopherol (α-TOCO) was kindly provided by Gemfont Ltd. (Taipei, Taiwan). BHT and α-TOCO were used to compare the oxidative stability of flaxseed oil with rosemary extracts (REs) in this study.

2.2. Extraction of rosemary leaves

Yields, phenolic contents, flavonoid contents, tannin contents, and *in vitro* antioxidant capacities of REs in different ethanol extraction concentrations (0, 30, 50, 80, and 95%) and extraction periods (15, 30, 60, 120, and 180 min) were evaluated. The extraction process of rosemary leaves was carried out as the following procedures. First, dry leaves were ground into powder. According to the suggested ratio of rosemary leaves and solvent for an efficient extraction (1:20, w/v, g dry leaves/mL solvent) of phenolcarboxylic acids, carnosic acid and rosmarinic acid (Zu et al., 2012), different concentrations of ethanol prepared with deionized distilled water (ddH_2O) was added to rosemary powder. The mixtures were underwent an ultrasonic bath (40 KHz) at 50 °C, followed by a vacuum filtration. The supernatant was evaporated at 30 ± 2 °C in an oven to remove ethanol, and remained contents were stored at −80 °C and lyophilized by freeze dryer system (Model#: CoolSafe 110-9 Pro Freeze Drying, LaboGene Aps, Lynge, Denmark) and the RE powder was stored at −20 °C for further analyses.

2.3. Parameters of extraction condition

2.3.1. Extraction yield

The extraction yield is an important economic index for the industry. The extraction yield of REs was calculated as the following equation:

Extraction yield (%) = {[weight (g) of lyophilized RE powder]/[weight (g) of dried rosemary leaves]} × 100%

2.3.2. Total phenolic contents

Total phenolic contents were measured according to Folin-Ciocalteu method under a microplate scale described by a previous report (Bobo-García et al., 2015). Twenty-μL sample solutions (4-mg RE powder in one-mL MeOH) were mixed with 100-μL Folin-Ciocalteu reagent (1:4 v/v) and then shaken for 5 min, followed by adding 75-μL sodium bicarbonate (100 g/L). The absorbance was measured at 750 nm after a reaction for 2 h. The standard curve was plotted by using gallic acid (0.1–2 mg/mL), and total phenolic contents were calculated as mg gallic acid equivalent (GAE)/g extract.

2.3.3. Total flavonoid contents

Total flavonoid contents were determined by using aluminum chloride colorimetric method described by a previous report (Liu, Lin, Wang, Chen, & Yang, 2009). Briefly, 250-μL sample solution (4-mg RE powder in one-mL MeOH) was mixed with 75 μL of 5% sodium nitrite and the mixture was stood for 5 min. Then 125 μL of 2% aluminum chloride and 125 μL of one-M sodium hydroxide were added. The absorbance was measured at 510 nm after a reaction for 10 min. The standard curve was plotted by using +(−) catechin (0.125–1.25 mg/mL), and total flavonoid contents were calculated as mg catechin equivalent (CE)/g extract.

2.3.4. Condensed tannin content

Condensed tannin contents were determined by using vanillin assay described by a previous report (Su et al., 2014). Two-hundred μL sample solution (4-mg RE powder in one-mL MehOH) was reacted with 3 mL of the mixture of 4% vanillin and 30% sulfuric acid solution prepared in methanol (1:1, v/v). The absorbance was measured at 510 nm after a reaction for 20 min at room temperature. The standard curve was plotted by using +(−) catechin (0.125–2 mg/mL), and condensed tannin content was calculated as mg catechin equivalent (CE)/g extract.

2.3.5. DPPH scavenging ability

DPPH scavenging ability was determined by using a method described by a previous study (Ye, Dai, & Hu, 2013) with slight modifications. Two-hundred μL sample solution (4-mg RE powder in one-mL MeOH) was mixed with 50 μL of one mM DPPH solution for 30 min in the dark. The absorbance of the mixture against blank was measured at 517 nm. The scavenging activity was measured through the percentage of scavenging DPPH radicals. EC_{50} value is the effective concentration that 50% DPPH radicals are scavenged.

2.3.6. Inhibition of conjugated dienes

When fats or oils are oxidized, the formation of conjugated dienes gives a rise to an absorption peak at 230–235 nm. An inhibition of conjugated dienes was determined by using the method described by previous reports (Lingnert, Vallentin, & Eriksson, 1979; Vaisali, Belur, & Regupathi, 2016) with slight modifications. Briefly, 0.1-mL sample solution (4-mg RE powder in one-mL MeOH) was added to 2-mL linoleic acid emulsion. The mixture was placed in the dark at 37 °C for 15 h. Then 0.2-mL solution (with or without RE powder) was mixed with 7 mL of 80% ethanol solution and the absorbance was measured at 234 nm.

Inhibition of conjugated dienes (%) = [(O.D. $_{\text{blank}}$-O.D. $_{\text{sample solution}}$)/O.D. $_{\text{blank}}$] × 100%

O.D. $_{\text{blank}}$ stood for the absorbance of solvent only while O.D. $_{\text{sample solution}}$ stood for the absorbance of sample solution.

2.3.7. Trolox equivalent antioxidant capacity (TEAC) assay

TEAC assay was determined by using the method described by Liyana-Pathirana and Shahidi (2006) with slight modifications. The $ABTS^{\cdot+}$ solution was prepared by mixing 2,2′-azino-bis (3-ethyl benzthiazoline sulfonic acid) (ABTS), H_2O_2, and peroxidase with the final concentration of 100 μM, 50 μM and 4.4 unit/mL, respectively. REs were dissolved in MeOH (250 μg/mL), and then an aliquot of the extract was reacted with the $ABTS^{\cdot+}$ solution (270 μL) for 3 min, followed by the absorbance determined at 734 nm. The scavenging ability of $ABTS^{\cdot+}$ was calculated relative to Trolox, and the TEAC value was expressed as μmole Trolox equivalent (TE)/g extract.

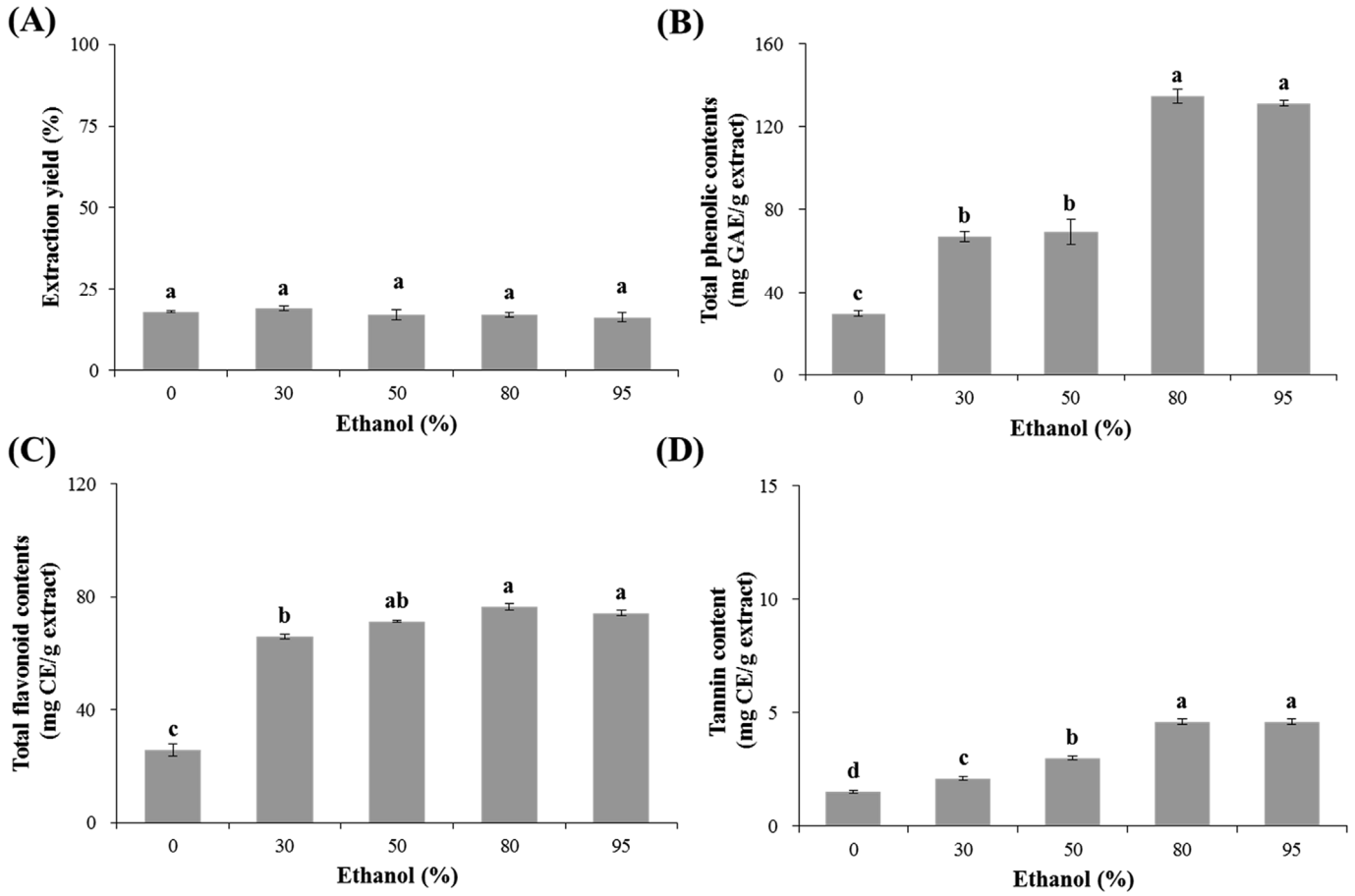

Fig. 1. Characteristics of rosemary extracts extracted with different ethanol concentrations (%). (A) Extraction yield (%). (B) Total phenolic contents (mg GAE/g extract). (C) Total flavonoid contents (mg CE/g extract). (D) Condensed tannin content (mg CE/g extract).
* Standard error bars are indicated (n = 3). Data bars with no common letters$^{(a-d)}$ denote significant differences among treatments by using Turkey's test ($p < 0.05$). 0% ethanol: distilled deionized water.

2.4. Determination of oxidative stability of flaxseed oil under a Schaal oven test condition (60 °C)

The Schaal oven test was carried out according to the methods from a previous report (Poyato et al., 2013) with slight modifications, as an incubation of oil samples premixed with or without antioxidants (RE powder, α-tocopherol, and BHT) stored in an oven with an air circulation at 60 °C. An oxidative stability of oil samples was evaluated at an interval of 24 h by measuring peroxide value, anisidine value, and Totox value of oil samples.

2.4.1. Peroxide value (POV)

Peroxide value was determined according to AOCS method Cd 8-53 (1998). Five grams of oil sample was first mixed with 30-mL acetic acid-chloroform solution. Then 0.5-mL saturated potassium iodine solution was added and the mixture was swirled for onemin, followed by adding 30-mL distilled deionized water and one mL of starch solution. The solution was titrated with 0.1-N sodium thiosulfate until purple color disappeared.

Peroxide value (meq O_2/kg oil) = [(S-B) × N × 1000]/ weight of sample (g)

S stands for titration of samples and B stood for titration of blank. N stands for the equivalent concentration of sodium thiosulfate.

Protection factor (Pf) of the antioxidant was determined as the following formulation:

Pf = IP (oil samples with antioxidants)/IP (oil sample without antioxidant)

IP stands for induction period, the time required to reach peroxide value of 20 meq O_2/kg oil.

2.4.2. Anisidine value (AV)

Anisidine value was determined according to AOCS method Cd 18-90 (1998). One-gram oil sample was dissolved in 25-mL isooctane, and measured the absorbance (A_b) at 350 nm against isooctane as a blank. Then 5-mL oil solution and isooctane were reacted with one-mL *p*-anisidine reagent (0.25 g/100 mL glacial acetic acid) relatively, followed by measuring the absorbance of sample solutions with *p*-anisidine reagent (A_s) against isooctane with *p*-anisidine reagent as a blank at 350 nm.

Anisidine value = [25 × (1.2A_s-A_b)]/weight of oil sample (g)

2.4.3. Totox value

Totox value was calculated by the values determined from peroxide value and anisidine value aforementioned (Sun-Waterhouse, Zhou, Miskelly, Wibisono, & Wadhwa, 2011). The formulation for Totox values was shown below:

Totox value = 2 × POV + AV

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine all

statistical analyses of data by SAS 9.2 (SAS Institute Inc., 2002). Differences were considered significant at 0.05 level, and differences between treatments were further compared by using Tukey's honest significant difference test.

3. Results and discussion

3.1. Determination of the optimal extraction condition for rosemary leaves

No significant differences ($p > 0.05$) of extracted ethanol concentrations on extraction yields, ranging from 16 to 19% (Fig. 1A). However, rosemary extracts (REs) prepared from ultrasonic bath with different ethanol concentrations demonstrated different total phenolic, flavonoid, and condensed tannin contents, respectively (Fig. 1B–D). REs obtained by using 80 and 95% ethanol-solution extraction possessed higher ($p < 0.05$) total phenolic contents up to 131–135 mg GAE/g extract, followed by those using 50 and 30% ethanol solutions, containing 69 and 67 mg GAE/g extract. Meanwhile, water extracts of rosemary leaves (by using 0% ethanol solution) had the lowest ($p < 0.05$) total phenolic contents. REs obtained by using 80 and 95% ethanol-solution extraction contained higher quantities ($p < 0.05$), approximately 74 and 77 mg CE/g extract for flavonoids respectively, and both 5 mg CE/g extract for condensed tannin. The lowest ($p < 0.05$) total flavonoid and condensed tannin contents were observed in water extracts of rosemary leaves. DPPH scavenging ability and inhibition of conjugated dienes were also measured to evaluate *in vitro* antioxidant abilities of REs obtained by using different ethanol concentrations (Fig. 2). The REs extracted by using 80% ethanol-solution extraction displayed the higher ($p < 0.05$) DPPH scavenging abilities compared to those by using other ethanol concentrations, and its DPPH scavenging ability was even similar ($p > 0.05$) to that of BHT (positive control). Regarding an inhibition of conjugated dienes in a linoleic acid emulsion, REs obtained by using ethanol concentrations beyond 50% exhibited the greater ($p < 0.05$) abilities to prevent the formation of conjugated dines compared to those extracted from 30% ethanol solution and ddH_2O. The characteristics of REs obtained by using 80% ethanol-solution extraction for 15, 30, 60, 120, and 180 min were investigated (Table 1). The extraction yields showed no ($p > 0.05$) differences between various ethanol concentrations. Total phenolic contents (TPC) in REs increased ($p < 0.05$) as the extraction period reached 30 min and so did the DPPH scavenging ability. However, the advantages of longer extraction period on TPC and DPPH scavenging ability were not ($p > 0.05$) observed in 80% ethanolic extracts from treatments with over 30 min of extraction periods.

Total phenolic contents (TPC) has been an important index for evaluating the antioxidant abilities of herb extracts because phenols are major antioxidant compounds that are able to donate hydrogen atoms to free radicals (Fu et al., 2011). Although there were no significant differences on extraction yields, TPC varied among REs by using different ethanol concentrations. Based on our results, higher TPC in REs resulted from higher extraction concentrations of ethanol. A similar result was also reported in which an ethanol extraction with an ultrasonic equipment assists the extraction of TPC in fresh rosemary leaves (Rodríguez-Rojo, Visentin, Maestri, & Cocero, 2012). Total flavonoid contents (TFC) were also influenced by the ethanol concentrations, where the lower ethanol concentration resulted in smaller quantities of TFC, and the TFC level in water extracts was 24 mg CE/g extract. Condensed tannin contents in REs also increased as the extraction concentrations of ethanol elevated. Su et al. (2014) indicated that 80% ethanolic extract of litchi pulp possesses higher total flavonoid and tannin contents compared to aqueous counterparts which are similar to the results in our study (Fig. 1C and D). DPPH scavenging abilities of REs enhanced as the extraction concentrations of ethanol increased. This observation corresponded to the results from total phenolic contents, verifying that TPC levels in REs correlated with radical scavenging ability of REs, with the correlation coefficient (r) being 0.88. Similar results were demonstrated that both total phenolic compounds (determined by using HPLC) and antioxidant activity (determined by using $ABTS^{\cdot+}$) showed a good correlation ($r = 0.93$) in herbal extracts of the mint family Lamiaceae (Wojdyło, Oszmiánski, & Czemerys, 2007). An inhibition of conjugated-diene method was often used to simulate an antioxidant ability of rosemary extract emulsion. Our data showed similarly results where ethanolic extracts assist an inhibition of conjugated dienes and retard lipid oxidation in an emulsion system. Based on the results aforementioned, 80% ethanol solution with 30 min of ultrasonic extraction was consequently used for the optimal condition of REs with a higher antioxidant ability.

3.2. Total flavonoid, condensed tannin and antioxidant ability of rosemary extracts (REs) under 30 min of ultrasonic extraction period with 80% ethanol solution

Total flavonoid, condensed tannin contents, EC_{50} of DPPH scavenging activity, and TEAC levels of REs (80% ethanol extraction for 30 min) were investigated as well, and results are shown in Table 2. TFC and condensed tannin of REs was 46.63 mg CE/g, and 1.86 mg CE/

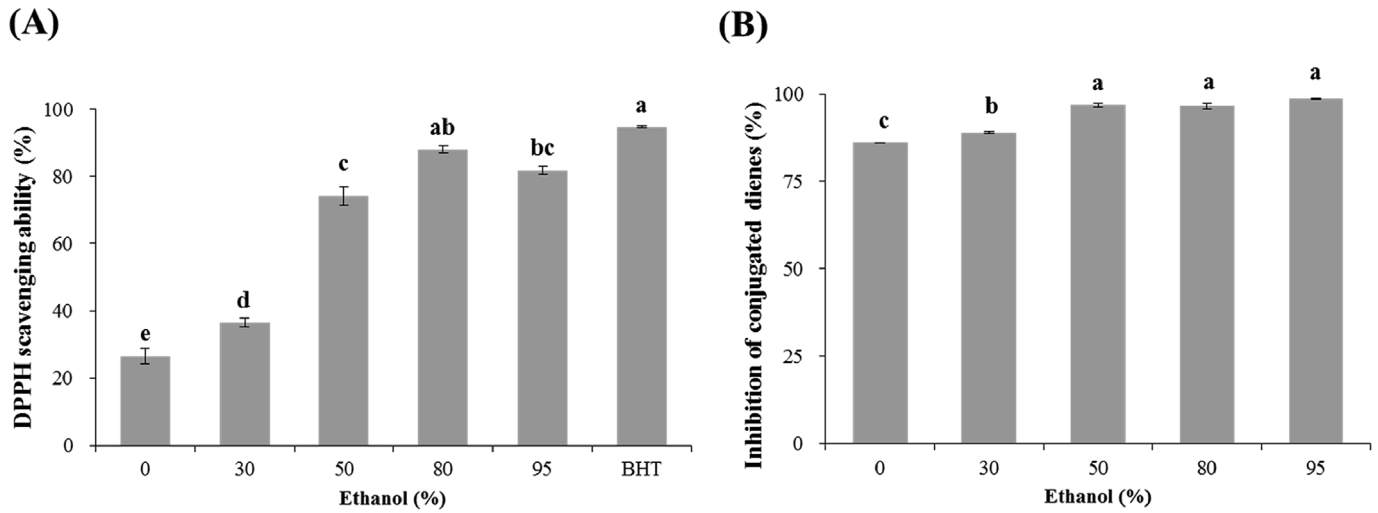

Fig. 2. *In vitro* antioxidant abilities of rosemary extracts extracted with different ethanol concentrations (%). (A) DPPH scavenging ability (%). (B) Inhibition of conjugated dienes (%). * Standard error bars are indicated (n = 3). Data bars with no common letters$^{(a-e)}$ denote significant differences among treatments by using Turkey's test ($p < 0.05$). DL: dry rosemary leaves; 0, 30, 50, 80, and 95: rosemary extracts extracted with different ethanol concentrations (%).

Table 1
Characteristics of 80% ethanolic extracts of rosemary from different extraction periods.

Extraction period (min)	15	30	60	120	180	BHT
Yield	15.00 ± 0.15[a]	14.69 ± 1.44[a]	13.36 ± 0.41[a]	16.90 ± 3.67[a]	17.36 ± 2.01[a]	
Total phenolic contents	108.30- ± 0.70[c]	161.07 ± 3.12[a]	160.70 ± 2.97[a]	158.43 ± 1.28[a]	144.93 ± 1.34[b]	
DPPH scavenging ability	94.07 ± 0.05[c]	94.84 ± 0.17[ab]	94.79 ± 0.20[ab]	94.59 ± 0.09[b]	94.68 ± 0.10[ab]	95.12 ± 0.25[a]

1. Data are given as Mean ± SEM (n = 3). Mean values with no common superscripts [(a-c)] within the same row indicate significant differences among treatments by using Turkey's test ($p < 0.05$).
2. Yield, total phenolic contents, and DPPH scavenging ability are expressed as %, mg GAE/g extract, and %, respectively.

Table 2
Total flavonoid, condensed tannin and antioxidant ability of 80% ethanolic extract of rosemary under 30-min extraction period (RE).

Total flavonoid (mg CE[a]/g)	Condensed Tannin (mg CE[a]/g)	EC_{50}[b] of DPPH radical scavenging activity (μg/mL)	TEAC (mmol TE[c]/g)
46.63 ± 0.74	1.86 ± 0.02	1.90 ± 2.51	0.87 ± 0.21

[a] CE means catechin equivalent.
[b] EC_{50} means the effective concentration of sample that can decrease DPPH radicals by 50%.
[c] TE means trolox equivalent.

g, respectively. Regarding its antioxidant abilities, EC_{50} of DPPH scavenging activity of RE was 1.9 μg/mL, and TEAC was 0.87 mmol TE/g.

Phenolic composition and free radical scavenging ability (i.e. DPPH scavenging ability, trolox equivalent antioxidant capacity) have been commonly applied to evaluate antioxidant properties of plant extracts. A previous report showed that there are 34.18 mg GAE/g of TPC and 9.87 mg quercetin/g of TFC in ethanolic rosemary extracts obtained by a constant shaking in flasks (Zhang, Wu, & Guo, 2016). EC_{50} indicated the concentration of antioxidants used to scavenge 50% of initial radicals, so the smaller of the values mean the better antioxidant ability of antioxidants. Furthermore, it was reported that an oil-soluble commercial rosemary extract product (V40, Vitiva d.d., Markovci, Slovenia) has an antioxidant activities of 28 μg/mL for EC_{50} based on the DPPH assay and 0.31 μmol TE/g (Bubonja-Sonje, Giacometti, & Abram, 2011). In addition, an EC_{50} value of 0.49 mg/mL from ethanolic extracts of rosemary has been reported to effectively retard lipid oxidation and extend the shelf-life of raw chicken meat during 15 days of storage at 4 °C (Zhang et al., 2016). Accordingly, our REs, which had an EC_{50} value of 1.9 μg/mL, might possess a great antioxidant potential.

3.3. Effects of REs on the oxidative stability of flaxseed oil under Schaal oven test condition (60 °C)

In this study, Schaal oven test, an accelerated oxidative test, was carried out to determine the antioxidant ability of REs obtained by using 80% ethanol-solution extraction with 30 min of ultrasonic extraction in flaxseed oil incubated at 60 °C. Peroxide value, anisidine value, and Totox value were applied as indicators of oxidative status of flaxseed oil at each incubation period. Peroxide value was a test for measuring peroxide compounds in products, considered as primary oxidative substances (Pizarro, Esteban-Díez, Rodríguez-Tecedor, & González-Sáiz, 2013). During the incubation, flaxseed oil with an addition of REs had the lowest ($p < 0.05$) peroxide value, followed by those with the addition of BHT, CON (control, without antioxidant addition), and the addition of α-TOCO (Fig. 3A). Furthermore, protection factor (Pf) is calculated as the induction period reaching peroxide value of 20 meq O_2/kg of sample divided by that of control (without antioxidant addition). Protection factor (Pf) > 1 testifies to the antioxidative potential of additives while Pf < 1 demonstrates pro-oxidative potential of additives (Gramza-Michalowska, Sidor, & Hes, 2010). After a calculation, Pf values of flaxseed oils with our REs, α-TOCO, and BHT were 1.33, 0.83, and 1 respectively, demonstrating higher antioxidant abilities of RE than α-TOCO and BHT. Anisidine value is a measurement of α and β unsaturated aldehydes, second oxidative products of lipid peroxidation (Miraliakbari & Shahidi, 2008). Interestingly, α-TOCO addition did not reduce the anisidine value of flaxseed oil incubated at 60 °C while even a higher tendency was detected. No ($p > 0.05$) differences on anisidine values were observed between CON and BHT treatments, although there was a lower tendency in the BHT treatment. Only RE addition obviously reduced ($p < 0.05$) anisidine value of flaxseed oil which indicates a retardation of lipid peroxidation in the flaxseed oil storage. Totox value is calculated as a formulation of double times of peroxide value plus anisidine value, indicating overall oxidation of flaxseed oils in Schaal oven test (Wai, Saad, & Lim, 2009). Similar to peroxide and anisidine values, flaxseed oil with our RE addition showed the least ($p < 0.05$) quantities of oxidative products during incubation at 60 °C, followed by treatments of BHT, CON, and α-TOCO (Fig. 3C).

Regardless of primary and secondary oxidative products or overall oxidative status, our results showed that: (1) the addition of 100-ppm α-tocopherol did not effectively stabilize the antioxidant ability in flaxseed-oil samples, (2) the addition of 200-ppm BHT slightly but did not significantly retard lipid oxidation in flaxseed-oil samples, and (3) the addition of 200-ppm REs increased oxidative stability of flaxseed-oil samples. Interestingly, slight pro-oxidative effects found in α-tocopherol might be owing to the fact that it tends to be unstable and thus easily degraded in exposure to oxygen and free radicals or at higher temperature (Gregory, 1996). Similar results were demonstrated in a previous study (Hraš, Hadolin, Knez, & Bauman, 2000), where sunflower oil supplemented with 100 ppm α-tocopherol exhibits a pro-oxidative effect. Besides, significant losses of naturally occurring α-tocopherol in plant derived edible oils are found to be paralleled the formation of conjugated dienes after heating, confirming its potential pro-oxidative activity (Elisia, Young, Yuan, & Kitts, 2013). There is 300–400 ppm of naturally occurring tocopherol in flaxseed oil (USDA, 2015), which may act as a potential pro-oxidant factor during the storage. The ideal performance of our REs on the oxidative stability of flaxseed oil resulted from not only its phenolic components (carnosol and rosmarinic acid) (de Conto et al., 2012) but also a synergistic effect with self-containing tocopherols in flaxseed oil. It has been presumed that the phenolic components in our REs donate hydrogen atoms to α-tocopheroxyl radicals, reducing oxidized α-tocopherol in flaxseed oil, thus effectively delaying rancidity (Beddows, Jagait, & Kelly, 2000). Synergistic activities of rosemary extracts with α-tocopherol, ascorbyl palmitate, and citric acid in sunflower oil at a 60 °C incubation were observed by reducing formation of hydroperoxides (Hraš et al., 2000). In the sardine oil model system, the mixture of α-tocopherol (0.05%) and rosemary extract (0.02%) effectively delays the onset of oxidation for 5 days longer than either α-tocopherol or rosemary extract alone while its antioxidant activity is even comparable to that of BHA (Wada & Fang, 1992). Altogether, our REs exhibited the better stabilization of flaxseed oil during the storage.

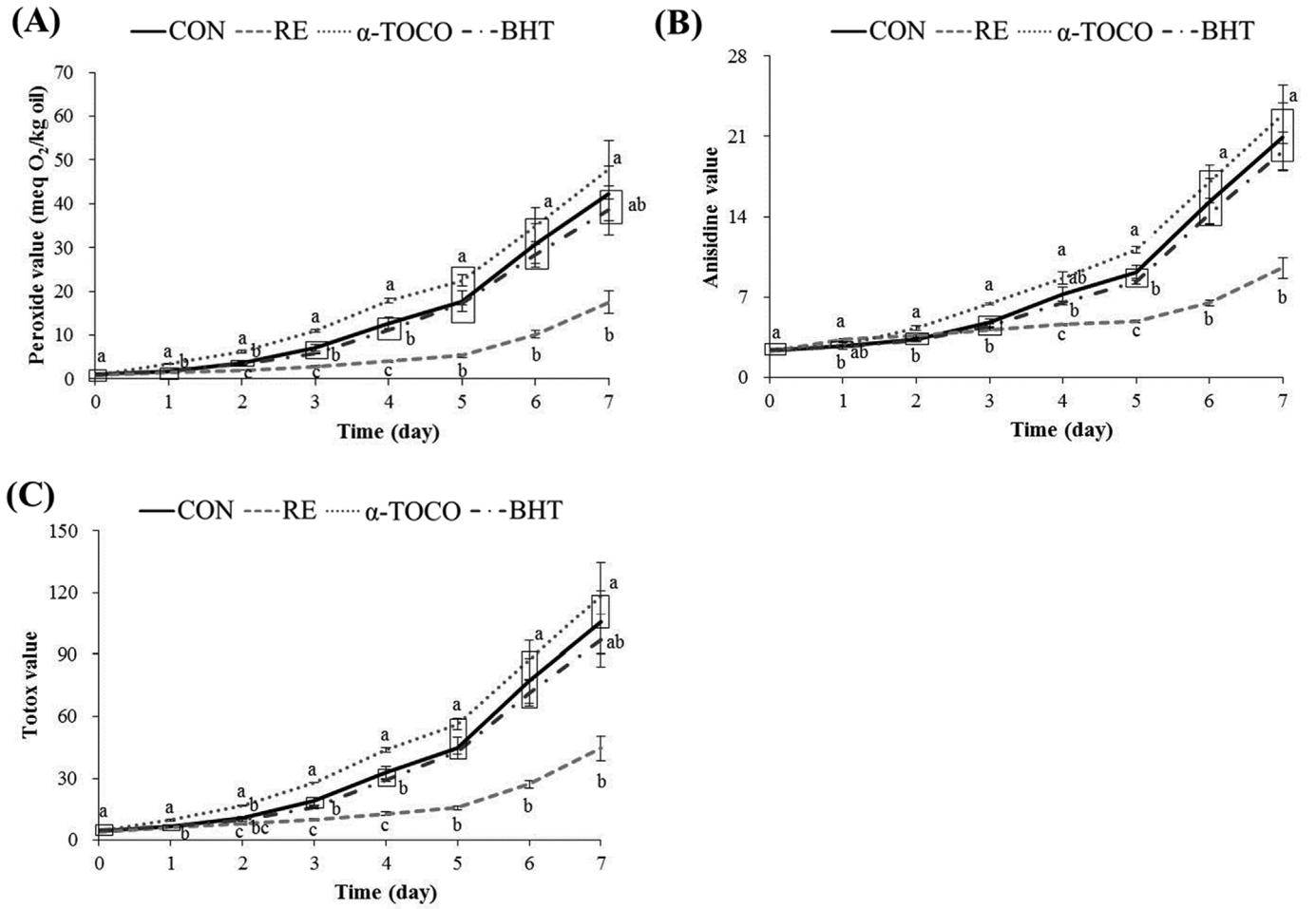

Fig. 3. Oxidative stability of flaxseed oil with the addition of different antioxidants under a Schaal oven test condition (60 °C). (A) Peroxide value (meq O_2/kg oil). (B) Anisidine value. (C) Totox value.
*Standard error bars are indicated (n = 3). Data bars with no common letters$^{(a-b)}$ denote significant differences among treatments by using Turkey's test ($p < 0.05$). CON: no antioxidant addition. RE: addition of 200 ppm rosemary extract by using 80% ethanol; α-TOCO: addition of 100 ppm α-tocopherol; BHT: addition of 200 ppm 2,6-bis(1,1-dimethylethyl)-4-methylphenol.

4. Conclusion

In conclusion, a 30-min ultrasonic extraction at 50 °C with 80% ethanol solution demonstrated the optimal extraction condition of dried rosemary leaves according to the data of DPPH scavenging ability, inhibition of conjugated dienes, yields, and phenolic compositions. Moreover, via a Schaal oven test of flaxseed oil incubated at 60 °C, our RE addition performed a favorable antioxidant ability compared to α-tocopherol and BHT addition in the flaxseed oil. Hence, due to a healthy scare of synthetic antioxidants, our REs, a natural source of antioxidant, should be a high potential for preservation of lipids, especially those containing a high ration of unsaturated fatty acids, i.e. ω-3 PUFAs.

References

AOCS (American Oil Chemists' Society) (1998a). *Official methods and recommended practices of the American Oil Chemists' Society, method Cd8-53. Peroxide value.* Champaign, IL, USA.
AOCS (American Oil Chemists' Society) (1998b). *Official methods and recommended practices of the American Oil Chemists' Society, method Cd18–90. p-Anisidine value.* Champaign, IL, USA.
Beddows, C. G., Jagait, C., & Kelly, M. J. (2000). Preservation of α-tocopherol in sunflower oil by herbs and spices. *International Journal of Food Science and Nutrition, 51*, 327–339.
Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *Journal of the Science of Food and Agriculture, 95*, 204–209.
Bubonja-Sonje, M., Giacometti, J., & Abram, M. (2011). Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. *Food Chemistry, 127*, 1821–1827.
de Conto, L. C., Oliveira, R. S. P., Martin, L. G. P., Chang, Y. K., & Steel, C. J. (2012). Effects of the addition of microencapsulated omega-3 and rosemary extract on the technological and sensory quality of white pan bread. *LWT-Food Science and Technology, 45*, 103–109.
Dellarosa, N., Laghi, L., Martinsdóttir, E., Jónsdóttir, R., & Sveinsdóttir, K. (2013). Enrichment of convenience seafood with omega-3 and seaweed extracts: Effect on lipid oxidation. *LWT - Food Science and Technology, 62*, 746–752.
Ding, Y., Wang, S. Y., Yang, D. J., Chang, M. H., & Chen, Y. C. (2015). Alleviative effects of litchi (*Litchi chinensis* Sonn.) flower on lipid peroxidation and protein degradation in emulsified pork meatballs. *Journal of Food and Drug Analysis, 23*, 501–508.
Elisia, I., Young, J. W., Yuan, Y. V., & Kitts, D. D. (2013). Association between tocopherol isoform composition and lipid oxidation in selected multiple edible oils. *Food Research International, 52*, 508–514.
Elkin, R. G., Ying, Y., & Harvatine, K. J. (2015). Feeding laying hens stearidonic acid-enriched soybean oil, as compared to flaxseed oil, more efficiently enriches eggs with very long-chain n-3 polyunsaturated fatty acids. *Journal of Agricultural and Food Chemistry, 63*, 2789–2797.
Fu, L., Xu, B. T., Xu, X. R., Gan, R. Y., Zhang, Y., Xia, E. Q., et al. (2011). Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry, 129*, 345–350.
Gramza-Michalowska, A., Sidor, A., & Hes, M. (2010). Herb extract influence on the oxidative stability of selected lipids. *Journal of Food Biochemistry, 35*, 1723–1736.
Gregory, J. F. (1996). Vitamins. In O. R. Fennema (Ed.). *Food chemistry* (pp. 531–616). New York: Marcel Dekker.
Hraš, A. R., Hadolin, M., Knez, Z., & Bauman, D. (2000). Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. *Food Chemistry, 71*, 229–233.
Lingnert, H., Vallentin, K., & Eriksson, C. E. (1979). Measurement of antioxidant effect in model system. *Journal of Food Processing and Preservation, 3*, 87–103.
Liu, S. C., Lin, J. T., Wang, C. K., Chen, H. Y., & Yang, D. J. (2009). Antioxidant properties

of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. *Food Chemistry, 114*, 577–581.
Liyana-Pathirana, C. M., & Shahidi, F. (2006). Antioxidant properties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. *Journal of the Science of Food and Agriculture, 86*, 477–485.
Miraliakbari, H., & Shahidi, F. (2008). Oxidative stability of tree nut oils. *Journal of Agricultural and Food Chemistry, 56*, 4751–4759.
Pizarro, C., Esteban-Díez, I., Rodríguez-Tecedor, S., & González-Sáiz, K. M. (2013). Determination of the peroxide value in extra virgin olive oils through the application of the stepwise orthogonalisation of predictors to mid-infrared spectra. *Food Control, 34*, 158–167.
Poyato, C., Navarro-Blasco, I., Calvo, M. I., Cavero, R. Y., Astiasarán, I., & Ansorena, D. (2013). Oxidative stability of O/W and W/O/W emulsions: Effect of lipid composition and antioxidant polarity. *Food Research International, 51*, 132–140.
Rajaram, S. (2014). Health benefits of plant-derived α-linolenic acid. *American Journal of Clinical Nutrition, 100*, 443S–448S.
Rodríguez-Rojo, S., Visentin, A., Maestri, D., & Cocero, M. J. (2012). Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering, 109*, 98–103.
Shah, M. A., Bosco, S. J. D., & Mir, S. A. (2014). Plant extracts as natural antioxidants in meat and meat products. *Meat Science, 98*, 21–33.
Shi, C., Cui, J., Yin, X., Luo, Y., & Zhou, Z. (2014). Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: Effect on lipid and protein oxidation. *Food Control, 40*, 134–140.
Sun-Waterhouse, D., Zhou, J., Miskelly, G. M., Wibisono, R., & Wadhwa, S. S. (2011). Stability of encapsulated olive oil in the presence of caffeic acid. *Food Chemistry, 126*, 1049–1056.
Su, D., Zhang, R., Hou, F., Zhang, M., Guo, J., Huang, F., et al. (2014). Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. *BMC Complementary and Alternative Medicine, 14*, 9–18.
Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Advances in Nutrition, 3*, 1–7.
Taneja, A., & Singh, H. (2012). Challenges for the delivery of long-chain n-3 fatty acids in functional foods. *Annual Review of Food Science and Technology, 3*, 105–123.
USDA (2015). *National nutrient database for standard reference release 28. Full report (all nutrients) 42231, oil, flaxseed, cold pressed. Food group: Fats and oils.* U.S. Department of Agriculture.
Vaisali, C., Belur, P. D., & Regupathi, I. (2016). Comparison of antioxidant properties of phenolic compounds and their effectiveness in imparting oxidative stability to sardine oil during storage. *LWT-Food Science and Technology, 69*, 153–160.
Wada, S., & Fang, X. (1992). The synergistic antioxidant effect of rosemary extract and α-tocopherol in sardine oil model system and frozen-crushed fish meat. *Journal of Food Processing and Preservation, 16*, 263–274.
Wai, W. T., Saad, B., & Lim, B. P. (2009). Determination of TOTOX value in palm oleins using a FI-potentiometric analyzer. *Food Chemistry, 113*, 285–290.
Wang, H. L., Chou, C. H., Yu, Y. S., Hsu, C. L., Wang, S. Y., Ko, Y. F., et al. (2016). Chicken surimi fortified by omega-3 fatty acid addition: Manufacturing and quality properties. *Journal of the Science of Food and Agriculture, 96*, 1609–1617.
Wojdyło, A., Oszmiánski, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry, 105*, 940–949.
Ye, C. L., Dai, D. H., & Hu, W. L. (2013). Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.). *Food Control, 30*, 48–53.
Zhang, H., Wu, J., & Guo, X. (2016). Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Food Science and Human Wellness, 5*, 39–48.
Zu, G., Zhang, R., Yang, L., Ma, C., Zu, Y., Wang, W., et al. (2012). Ultrasound-assisted extraction of carnosic acid and rosmarinic acid using ionic liquid solution from *Rosmarinus officinalis*. *International Journal of Molecular Sciences, 13*, 11027–11043.