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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 (dd$H_2O$) 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.

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