Research Article

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Effects of rosemary (Rosmarinus officinalis L.) extracts and dry ice on the physicochemical stability of omega-3 fatty-acid-fortified surimi-like meat products

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Abstract

BACKGROUND: Lipid peroxidation entails major quality degradation in omega-3 (ω-3) fatty-acid-fortified surimi-like meat products upon storage. Currently, the use of label-friendly alternatives to synthetic antioxidants is encouraged in the industry. Hence, we aimed to examine the applicability of the hurdle-technology concept, using an 80% (v/v) ethanol solution to obtain rosemary extracts (REs) containing substantial amounts of polyphenol, and dry ice (DI) which can create a cryogenic environment, on the physicochemical stabilities of ω-3 fatty-acid (FA)-fortified meat products after manufacturing and storage periods. The polyphenolic profiles of the REs were also investigated.

RESULTS: Carnosol and rosmarinic acid are major phenolic components in REs. Furthermore, DI addition during the chopping procedure increased ($P < 0.05$) whiteness values and hardness of products, while total ω-3 and ω-6 FAs were relatively well preserved ($P < 0.05$) in products with flaxseed oil premixed with RE. During 14-day storage at 4 °C, combined treatment with RE and DI decreased ($P < 0.05$) thiobarbituric acid reactive substance (TBARS) levels and the centrifugation loss of products. Single or combined treatment with RE and/or DI decreased ($P < 0.05$) TBARS levels in products after 60 days of storage at −20 °C.

CONCLUSION: Due to the antioxidant-polyphenol profile of REs and a possible oxygen exclusion of DI treatment under atmospheric pressure during food manufacturing, application of the hurdle-technology concept, using treatment with both RE and DI, can reduce lipid peroxidation and maintain a greater water-holding capacity of ω-3 FA-fortified meat products upon storage.

Keywords: dry ice; fatty acid profile; physicochemical properties; polyphenolic profile; omega-3 fatty-acid fortified surimi-like meat products; rosemary extract

INTRODUCTION

Approximately 19 million spent hens are slaughtered annually in Taiwan.[1] Due to toughness and lack of palatability, only few traditional culinary products made of spent hen meat (e.g., chicken floss, chicken essence, salt-flavored chicken) are available in local markets. In view of this situation, a surimi technology may be applied for protein recovery from spent hen breast meat. In order to increase the usage of spent hens, diversify product types, and satisfy consumers' demands for meat products, an ω-3 fatty-acid (FA)-fortified surimi-like meat product was successfully developed, as reported in our previous study.[2] However, lipid peroxidation is a major challenge when ω-3 FAs are incorporated into processed meat products.[3] Not only their susceptible structure with multiple double bonds, but also exposure to oxygen and fluctuating temperature during processing, may lead to easy degradation of FAs and increased production of oxidative substances, that is, peroxide, malondialdehyde, conjugated diene, etc., resulting in rancidity, off-odors, and other negative impacts on the products.[4] Accordingly, the quality stabilization of products is a critical issue that has been investigated over the years to comply with growing demands for ω-3 FA-fortified meat products.[3]

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Rosemary (*Rosmarinus officinalis* L.) extract has been reported to be an easy natural source of antioxidants for several meat products, preserving their appearance while retarding lipid peroxidation and extending their shelf life.[5,6] Its antioxidant properties are attributed to its phenolic components. Aside from their antioxidant potential in food systems, phenolic compounds in commercially available rosemary extracts also have antimicrobial, anti-inflammatory, and anticarcinogenic effects.[7,8] Consequently, rosemary extracts may be label-friendly alternatives to synthetic antioxidants (i.e., BHT, BHA, and TBHQ). Other procedures with antioxidant effects, such as addition of cold water or ice during processing, packaging under vacuum or modified atmosphere, and use of cold-chain systems during transportation and storage, are also employed to eliminate degradative external factors (i.e., heat, oxygen, and light), to retard lipid peroxidation.[9] Solid carbon dioxide (DI)) (dry ice) , generally recognized as safe,[10] has been used to chill or pre-freeze meat products in the food supply chain.[11] In addition to creating a cryogenic environment, its sublimation to gas under atmospheric pressure contributes to the exclusion of oxygen during food manufacturing. In this study, the polyphenol components of rosemary extracts (REs), obtained with an 80% (v/v) ethanol solution, were investigated. The possible impact of the hurdle-technology concept, using REs and DI, on the physicochemical stabilities of ω-3 FA-fortified surimi-like meat products was examined. Chicken surimi fortified with flaxseed oil was the template tested in this study.

MATERIALS AND METHODS

Materials and chemicals

Dry rosemary leaves, spent hen breast meat, and flaxseed oil were purchased from Tomax Enterprise Co., Ltd. (Taichung, Taiwan), Jia Zhen Frozen Food Co., Ltd. (New Taipei City, Taiwan), and Gut & Gerne (Stubenberg, Germany), respectively. Other additives, including sodium chloride, sodium polyphosphate/sodium pyrophosphate, sorbitol, and trehalose were obtained from Taiyen Industrial Co., Ltd. (Tainan, Taiwan), Chien-Yuan Inc. (Taipei, Taiwan), Roquette (Lestrem, France), and Hayashibara ShojiInc (Okayama, Japan), respectively. DI, used to lower the temperature while manufacturing chicken surimi ,was purchased from Mei Erh Lien Ltd. (Taipei, Taiwan). Ethanol (absolute), as well as individual phenolic acid/flavonoid, and fatty acid compounds were purchased from Merck Ltd. (Taipei, Taiwan) and Sigma-Aldrich Co. (St. Louis, MO).

Extraction of rosemary leaves and HPLC analysis of REs

Extraction procedure of rosemary leaves

Dry rosemary leaves were ground to powder and extracted with 80% (v/v) ethanol solution (1:20, w/v) using an ultrasonic cleaner (40 KHz) (DC150H, Taiwan Delta New Instrument Co., Ltd., New Taipei, Taiwan) at 50 °C for 30 min.[12] The supernatant was concentrated and evaporated at 30 ± 2 °C after vacuum filtration, and the remnant REs were lyophilized using a freeze dryer system (Model#: CoolSafe 110-9 Pro Freeze Drying, LaboGene Aps, Lynge, Denmark).

HPLC analysis of REs

The phenolic acid, flavonoid, and phenolic diterpene components in REs were analyzed according to the method described by Lin *et al.*[13] The operation conditions of an HPLC system consisting of a Shimadzu LC-10AT HPLC pump system (Kyoto, Japan), a Shimadzu SCL-10A system controller module (Kyoto, Japan) and an S-3210 photodiode-array (PDA) detector (Schambeck SFD GmbH, Bad Honnef, Germany) were the following: stationary phase, Inspire C18 column (250 × 4.6 mm, 5 μm; Dikma Technologies Inc., Lake Forest, CA); mobile phase, acetonitrile (solvent A) and H_2O with 2% CH_3COOH (solvent B) (conditions: 2–4% A from 0 to 25 min and kept at 4% A from 25 to 40 min; 4–10% A from 40 to 50 min; 10–15% A from 50 to 60 min; 15–18% A from 60 to 110 min; 18–20% A from 110 to 115 min; 20–22% A from 115 to 135 min; 22–25% A from 135 to 150 min; 25–80% A 150–180 min; flow rate, 0.8 mL min^{-1}; detection at 200–700 nm; injection volume, 20 μL). Phenolic acid and flavonoid compounds in REs were identified and quantified by their retention times based on UV–Vis spectral data and using standard curves of authentic compounds.

Investigation of RE and DI impacts on physicochemical properties of chicken surimi fortified with flaxseed oil during chilled and frozen storage

The potential effect of premixing REs with flaxseed oil and DI addition during processing on the physicochemical stability of chicken surimi fortified with flaxseed oil was investigated in this study. The chicken surimi in each treatment were stuffed into 2 mL Eppendorf tubes and then stored in dark at 4°C for 0, 7, and 14 days, respectively, as well as at −20°C for 60 days. Color parameters, texture profiles, and FA profiles of the products were determined after manufacturing. Thiobarbituric acid-reactive substances (TBARS), centrifugation loss (CL), total sulfhydryl group (TSH) content, conjugated dienes, and peroxide value (POV) of products were measured at each storage period at both 4 and −20 °C.

Preparation of chicken surimi fortified with flaxseed oil

Chicken surimi fortified with flaxseed oil was manufactured as described in a previous study.[2] Spent hen breast meat was purchased locally (Jia Jen Ltd., Taipei, Taiwan), packaged in polyethylene bags and transported at −20 °C to the laboratory. Breast meat was minced in a laboratory blender (Model CSB-77TW, Cuisinart, East Windsor, NJ, USA). Distilled water was used for the first two washing steps, followed by a third washing step with 0.1% (w/v) NaCl (Taiyen Co., Tainan, Taiwan) solution. In each washing step, the minced meat was blended with distilled water or 0.1% NaCl solution at a ratio of 1:4 (w/w) for 5 min on ice. Each step of the washing process was followed by centrifugation (8000*g*, 15 min, 4 °C; Centrifuge 3700, Kubota Co., Osaka, Japan) for dehydration. The supernatant was poured out, and the recovered protein was stored at −20 °C in a sealed vacuum package. Once preparing the chicken surimi paste mixture, 170-g thaw proteins (about 0°C) (79.2%, w/w) recovered from spent hen breast was first chopped with 5.4-g NaCl (2.5%,w/w) and 0.6 g polyphosphate (0.3%, w/w). The mixture was then emulsified with 21.5-g flaxseed oil (10.0%, w/w) which is premixed with or without 4.3-mg REs (200 mg/kg oil). Then, a cryoprotectant mixture containing 8.6-g trehalose (4.0%, w/w) and 8.6-g sorbitol (4.0%, w/w) was added in the mixture. In addition, either no DI or 17 g of DI (DI/protein w/w ratio = 1:10) was added during each chopping stage (Fig. 1).

Color parameters

The same amount of raw chicken surimi in each treatment was heated at 95 °C in a water bath for 15 min, adjusted to room temperature for 1 h and sliced into 2 cm^3 cubes for color measurement. A color difference meter (Model NR-11, Nippon Denshoku Co., Tokyo, Japan) was calibrated on the CIE color space system

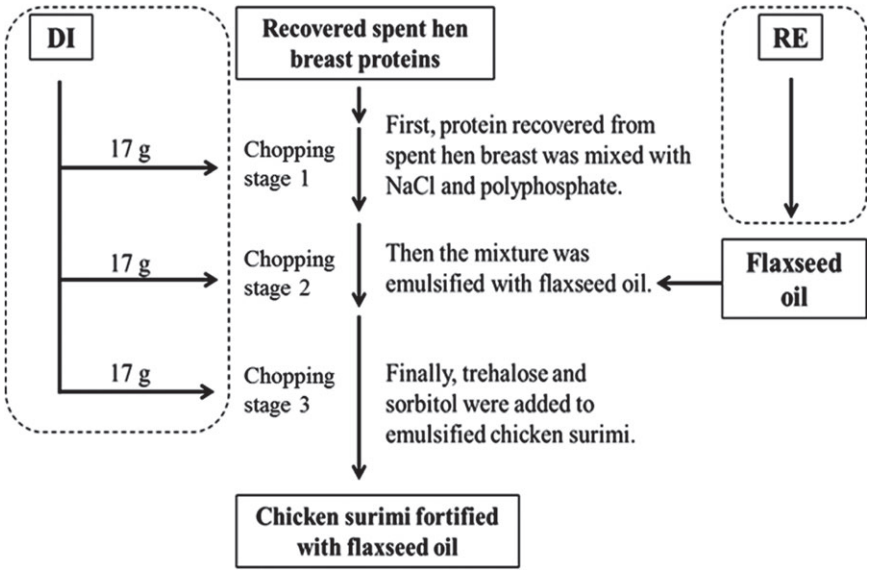


Figure 1. Preparation of chicken surimi fortified with flaxseed oil with or without treatment with RE and/or DI.

using a standard white tile and a black column. The L^* value indicates lightness ($L^* = 0$, darkness; $L^* = 100$, lightness); the a^* value indicates redness (+60 = red, −60 = green); and the b^* value indicates yellowness (+60 = yellow, −60 = blue). Whiteness was calculated as: $100-\sqrt{(100 - L*)^2 + a*^2 + b*^2}$.[2]

Texture profile analysis

Texture profile analysis (TPA) of cooked chicken surimi was performed at room temperature with a texture analyzer (Model TA.XTplus Texture Analyzer; Stable Micro Systems, Godalming, UK). The cylinder samples (1.0 cm diameter, 1.0 cm height) were subjected to a double compression test by using a cylindrical aluminum probe P/50 (aluminium cylinder, 50 mm diameter ; Stable Micro Systems). The samples were compressed to 75% of strain and the test speed was 5 mm/s. Texture profile parameters were presented as described by Wang *et al.* (2016).[2]

Fatty acid composition in chicken surimi

Lipids in raw chicken surimi, after each treatment, were extracted with chloroform and methanol (2:1, v/v),[14] and the fatty acid profile in the extracted lipid was further examined. FAs were transmethylated by the addition of 4 mL of 4% (w/v) methanolic sulfuric acid and heated at 90 °C in a water bath for 60 min.[2] The mixture was saponified by transfer through a sodium sulfate-filled Pasteur pipette and subsequently dried under N_2 at 60 °C in a water bath for 60 min. FA methyl esters (FAMEs) were resuspended in filtered isooctane. The FAMEs were analyzed using gas chromatography (Model 6890 N, Agilent, Santa Clara, CA) and a flame ionization detector, fitted with a highly polar stationary-phase SP-2560 column (100 m length, 0.25 mm inner diameter, 0.20 μm film thickness) (Supelco Inc., Bellefonte, PA). The injector and detector temperatures were maintained at 250 °C and 300 °C, respectively, while the initial and final column temperatures were 170 °C and 290 °C, respectively, with a 3 °C min^{-1} increase for 40 min. Helium was the carrier gas (0.75 mL min^{-1}) and a split ratio of 40 to 1 was used. FAs were identified by comparing their retention times with known standards (Sigma-Aldrich Co.). The amount of each identified FA was determined using standard curves of authentic compounds. The amount of each FA identified in raw chicken surimi was calculated by multiplying the lipid content in chicken surimi and was then given as mg/100 g chicken surimi.

Assay of conjugated dienes

Conjugated dienes in raw chicken surimi in each treated sample were assayed according to a previously described method.[15] Crude fat was extracted from 1 g of chicken surimi[14] and was then dissolved in 10-mL isooctane as the sample solution. Absorbance of the sample solution was measured at 232 nm against the blank of isooctane. Conjugated dienes were calculated using the equation:

$$\text{Conjugated dienes}\,(\%) = 0.84 \times \left[\left(A_s/bc\right) - K_0\right].$$

where A_s was absorbance of the sample solution, b the cuvette length (cm), c the concentration of sample solution (g L^{-1}), and K_0 the absorptivity by acid groups (0.03).

Peroxide values

Peroxide values (POV) of samples were determined using the International Dairy Federation (IDF) standard method (established following the Fe(II)-oxidation-based spectrophotometric method).[16] Crude fat was extracted from cooked chicken surimi[14] and was then dissolved in 1% (v/v) isooctane as the sample solution. Briefly, 300 μL sample solution was mixed with 5 mL of chloroform-methanol (7:3, v/v). Then 50-μL of redox solution containing iron(II) chloride and methanol/1-decanol/n-hexane (3 : 2 : 1, v/v/v) mixture containing ammoniumthiocyanate (30%, w/w) was added to the mixture and was vortexed for 2–4 s. The absorbance was measured at 500 nm against a blank after incubation for 5 min at room temperature in subdued light. POV was calculated using the following equation:

$$\text{Peroxide value}\ (\text{mEq.peroxide/kg extracted lipid}) = \left[\left(A_s - A_b\right) \times 10.83\right] / \left(55.84 \times m_0 \times 2\right).$$

where A_s and A_b were the absorbance of the sample solution and the blank, respectively, m_0 was the sample weight (g), 55.84 the

atomic weight of Fe^{3+} and 10.83 the slope of the Fe(III) calibration curve. The division by 2 was the unit expression converted from milliequivalents of oxygen to milliequivalents of peroxide.

Thiobarbituric acid reactive substance

TBARS of raw chicken surimi was measured according to a previously described method.[2] Samples were homogenized in PBS (pH 7.0) at a ratio of 1:10 (w/v) and the supernatant was collected after centrifugation at 800*g* and 4 °C for 15 min. The 60-μL supernatant reacted with 90 μL of thiobarbituric acid solution and 510-μL of trichloroacetic acid-hydrochloride solution. After vortexing, the mixture was heated at 95 °C for 30 min in a water bath, followed by cooling and centrifugation at 9000*g* and 4 °C for 3 min. Absorbance of the mixture was measured at 535 nm. The TBARS content was calculated using a molar extinction coefficient of 156 000 M^{-1} cm^{-1} and was expressed as mg Eq. MDA kg^{-1} surimi.

Total sulfhydryl (-SH) content

Total sulfhydryl content in raw chicken surimi was determined according to a previously described method[17] with slight modifications. Appoximately 1 mL of homogenate (10%, w/v) was mixed with 8 mL of buffer (0.086-M Tris, 0,09 M glycine, 4 mM EDTA, pH 8.0), followed by centrifugation at 10,000*g* and 4 °C for 15 min. Approximately 2.25 mL of supernatant was mixed with 0.25 mL of DTNB (10 mM), and absorbance at 412 nm was measured. The total sulfhydryl content of samples was calculated using a molar extinction coefficient of 13 600 M^{-1} cm^{-1} and was expressed as μmol g^{-1} surimi.

Centrifugation loss

Centrifugation loss of samples was measured according to a previously described method.[18] with a slight modification. Briefly, a *ca* 0.2-g sample was weighed and placed in the 1.5 mL tube with a filter paper, followed by centrifugation at 1000*g* and 4 °C for 1 h. Centrifugation loss of samples was calculated as the percentage of the difference between the original weight and that after centrifugation.

Statistical analysis

All analyses were conducted for three independently treated batches. The parameters for each tested batch per product were obtained with at least three analyses and arranged in a 2 × 2 factorial design to obtain the results concerning the physicochemical properties of chicken surimi fortified with flaxseed oil that had been either chilled or frozen during storage . The interaction effect of RE and DI, as well as two main effects (RE or DI), were analyzed. Differences were considered to be significance at a P value of 0.05. When a significant difference in the interaction effect was found, Tukey's honest significant difference (HSD) test at $P < 0.05$ was used to assess differences between combination treatments. All statistical analyses of data were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Identification of phenolic acid, flavonoid, and phenolic diterpene components in REs

The identification and quantification of phenolic acid, flavonoid, and phenolic diterpene components in our REs were determined using HPLC chromatography (Fig. 2a,b). *p*-Hydrobenzoic acid, sinapic acid, syrinic acid, rutin, *p*-anisic acid, rosmarinic acid, neohesperidin, diosmin, glycitein, luteolin, hesperetin, kamemferol, rosmanol, and carnosol were identified, and the phenolic diterpenes were the major components (rosmanol + carnosol = 88.76 mg g^{-1} extract), followed by phenolic acids and flavonoids. Among all phenolics, carnosol was the most abundant compound in our REs, followed by rosmarinic acid, diosmin, neohesperidin, and glycitein. According to the HPLC analysis of our REs, total amounts of phenolic acid, flavonoid, and phenolic diterpene compounds were 24.46, 38.36, and 88.76 mg g^{-1} extract, respectively. Previous studies reported that diterpenes (carnosic acid, carnosol, and rosmanol) and phenolic acids (rosmarinic acid) are major antioxidant compounds in rosemary.[19,20] The most abundant phenol was carnosol, accounting for approximately 56% of total phenolic compounds in our REs (Fig. 2b). This is in agreement with a previous report of a higher recovery of diterpenes by using a solvent with higher ethanol concentrations (70–100%).[21] Carnosol, a phenolic diterpene, is a derivative of carnosic acid that demonstrated an antioxidant activity comparable to that of BHA; additionally, it possessed anti-inflammatory, antioxidant, antimicrobial, and anticancer properties.[20] Rosmanol, another diterpene identified in our REs, was also proven to possess greater antioxidant ability than α-tocopherol or BHT.[19] In the present study we found a rosmarinic acid content of 23.63 mg g^{-1} REs. This acid is another strong antioxidant compound in rosemary leaves.[22] It is reasonable, then, that our REs should be a potential antioxidant.

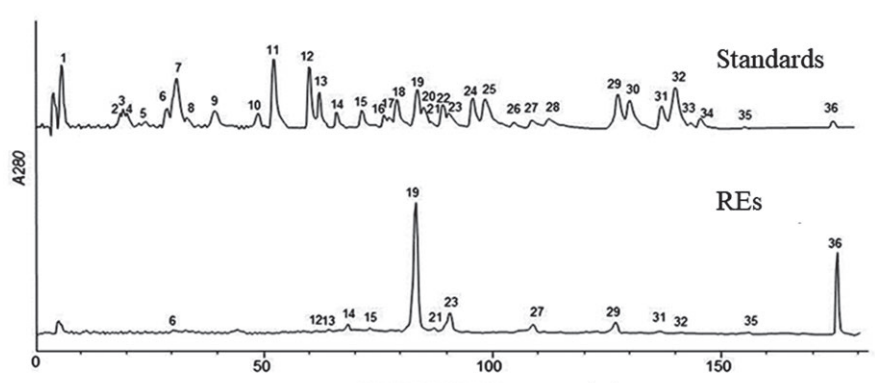
Effects of REs and DI on physicochemical properties of chicken surimi pastes fortified with flaxseed oil

Color parameters, texture profile, and fatty acid profile of chicken surimi fortified with flaxseed oil

With regard to the parameters of CIE color space system, although there were no ($p > 0.05$) interaction effects between RE and DI treatments as well as the main effect of RE on all parameters (L*, a*, b*, and calculated whiteness) of chicken surimi products, b* value (yellowness) was reduced ($p > 0.05$) by the DI treatment (Table 1). Whiteness is calculated based on the values of L^*, a^*, and b^* and, therefore, DI treatment increased ($P < 0.05$) the whiteness value of chicken surimi products. There were no ($P > 0.05$) effects on the interaction between RE and DI treatments, as well as no RE effect on indices of texture profile analysis of chicken surimi products (Table 1). Among all parameters, hardness, cohesiveness, resilience, and chewiness were increased ($P < 0.05$) with the addition of DI during processing, but adhesiveness and springiness were not ($P > 0.05$) influenced. As for the fatty acid composition of chicken surimi products, there was no interaction ($P > 0.05$) between RE and DI treatments. Total ω-3 and ω-6 polyunsaturated FAs (PUFAs) were effectively preserved ($P < 0.05$) via addition of RE. Especially, the contents of alpha-linoleic acid (ω-3), linoleic acid (ω-6) and arachidonic acid (ω-6) were higher ($P < 0.05$) in chicken surimi products with addition of RE, but the eicosatrienoic acid (ω-3) content was not ($P > 0.05$) influenced by addition of RE, while it was increased ($P < 0.05$) by addition of DI during processing.

The appearance of meat products is the key factor that influences the consumers' desire to purchase.[23] Based on our data, the chicken surimi products with the addition of DI during processing had a higher degree of whiteness, which may be due to (i) small ice crystal formation with the addition of DI, known to enhance the whiteness of products,[24] and (ii) lower yellow pigment generation from non-enzymatic browning reactions between lipid oxidative products and amines on proteins or lipids.[25] Moreover, textural

(a)

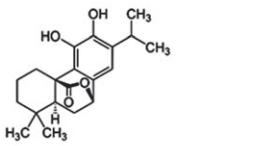


(b)

Compound (peak no.)	Content (mg/g)
p-hydroxybenzoic acid (6)	0.24 ± 0.01
sinapic acid (12)	0.13 ± 0.01
syringic acid (13)	0.18 ± 0.01
rutin (14)	4.21 ± 0.13
p-anisic acid (15)	0.28 ± 0.01
rosmarinic acid (19)	23.63 ± 1.36
neohesperidin (21)	10.27 ± 0.33
diosmin (23)	11.13 ± 0.50
glycitein (27)	9.36 ± 0.29
luteolin (29)	3.12 ± 0.09
hesperetin (31)	0.16 ± 0.01
kaempferol (32)	0.11 ± 0.01
rosmanol (35)	4.14 ± 0.20
carnosol (36)	84.62 ± 2.96
Total amount	151.58 ± 5.89

[1]Data is given as Mean±SEM (n=3).

(c)



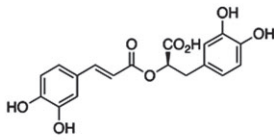


Figure 2. (a) HPLC chromatograms of phenolic compounds, (b) identification of phenolic acid, flavonoid, and phenolic diterpene components, and (c) chemical structures of two major phenolic compounds identified in rosemary extracts (REs) obtained using extraction with 80% (v/v) ethanol solution and 30 min ultrasound treatment. Peaks: gallic acid (1), catechin (2), carnosic acid (3), gentisic acid (4), chlorogenic acid (5), *p*-hydroxybenzoic acid (6), vanillic acid (7), caffeic acid (8), epicatechin (9), *p*-coumaric acid (10), ferulic acid (11), sinapic acid (12), syringic acid (13), rutin (14), *p*-anisic acid (15), naringin (16), myricetin (17), hesperidin (18), rosmarinic acid (19), quercitrin (20), neohesperidin (21), eriodictyol (22), diosmin (23), morin (24), daidzein (25), quercetin (26), glycitein (27), naringenin (28), luteolin (29), genistein (30), hesperetin (31), kaempferol (32), apigenin (33), isorhamnetin (34), rosmanol (35), and carnosol (36).

properties play an important role in the perception and acceptability of processed meat products, and processing conditions, as well as recipe, have a direct impact on the textural behavior of food products.[23] Hardness, cohesiveness, resilience, and chewiness of products were enhanced by the addition of DI during processing, leading to a more solid structure and a harder texture, which might be explained by the maintenance of low temperature resulting from addition of DI during the manufacturing process (chopping stage). The oil- and water-binding condition of the meat emulsion during processing has a great impact on textural properties, and the stability of the emulsion is greatly influenced by the temperature.[26] Since flaxseed oil is rich in PUFAs (~ 54% α-linolenic acid) and is fluid at room temperature, application of DI during the processing might assist in immobilizing oil, which would lead to a firmer texture of products via the creation of a cryogenic environment. Additionally, antioxidant techniques are also required in stabilizing flaxseed oil, to assure a certain amount of ω-3 PUFAs in food products. Alpha-linoleic acid (ALA), a major ω-3 PUFA in flaxseed oil, was substantialy retained in chicken surimi products (520.94 mg 100 g^{-1}) by the addition of RE, as compared to products without addition of RE (369.17 mg 100 g^{-1}), indicating that RE has great retention and antioxidant activity against ALA loss and deterioration, respectively, during processing. Further, ω-6 PUFA contents were approximately 50% higher in products with the addition of RE, leading to greater amounts of PUFAs but not to different ω-6/ω-3 PUFA ratios in surimi. With regard to the functional property, our products with the addition of RE contained 520.94 ± 53.64 mg of ω-3 PUFAs/100 g surimi, providing 33–45% of the recommended daily ALA intake of 1.1–1.6 g day^{-1}.[27] Meanwhile, ω-6/ω-3 PUFA ratios of all products remained within the range of 0.31–0.33, relatively lower than values obtained for most conventional meat products (ω-6/ω-3 ratio ≈ 15).[28] It is recommended that the ω-6/ω-3 PUFA ratio should not exceed 4 to support an optimal physiological status, in agreement with currently prevalent Western-diets.[29]

Lipid and protein qualities of chicken surimi fortified with flaxseed oil stored at 4 °C

Conjugated diene, peroxide, and TBARS values were employed as indices concerning the lipid oxidative status of our products during storage. Centrifugation loss and total sulfhydryl content were found to be indicators of protein deterioration in our products. Conjugated dienes are intermediate oxidative products of early-stage of lipid oxidation.[15] Figure 3a shows the results of conjugated dienes during 14 days of storage at 4 °C. The interaction of RE and DI was observed ($P < 0.05$), and single or combined

Table 1. Effects of rosemary extract and dry ice on color parameters, texture profile, and fatty acid composition of chicken surimi fortified with flaxseed oil after 1-day of storage at 4 °C

		Main effect						
		Rosemary extract (RE)		Dry ice (DI)		*P* value		
Parameter		−	+	−	+	RE	DI	RE × DI
Color parameters								
*L**		82.04 ± 0.43	81.58 ± 0.40	81.69 ± 0.40	81.93 ± 0.45	0.48	0.72	0.47
*a**		0.77 ± 0.21	0.82 ± 0.07	0.95 ± 0.19	0.65 ± 0.06	0.83	0.21	0.84
*b**		19.01 ± 0.47	18.36 ± 0.69	19.66 ± 0.36#	17.71 ± 0.48	0.30	0.01	0.40
Whiteness		73.81 ± 0.42	73.93 ± 0.40	73.10 ± 0.23	74.65 ± 0.19#	0.71	0.00	0.97
Texture profile analysis								
Hardness (g)		953.39 ± 86.72	891.51 ± 78.76	785.19 ± 14.31	1059.71 ± 79.78#	0.47	0.01	0.29
Adhesiveness (g × sec)		−1.63 ± 0.47	−3.75 ± 0.89	−3.00 ± 1.00	−2.38 ± 0.66	0.06	0.52	0.10
Springiness		0.56 ± 0.01	0.55 ± 0.01	0.54 ± 0.01	0.56 ± 0.01	0.58	0.28	0.28
Cohesiveness		0.36 ± 0.02	0.40 ± 0.02	0.35 ± 0.02	0.39 ± 0.02#	0.15	0.02	0.31
Resilience		0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.15 ± 0.01#	0.37	0.02	0.37
Chewiness (g)		187.55 ± 26.50	183.49 ± 25.95	155.57 ± 9.05	242.67 ± 16.68#	0.87	0.00	0.48
Fatty acid composition (mg/100 g chicken surimi paste)								
α-Linolenic acid	C18:3(ω-3)	369.17 ± 38.18	520.94 ± 53.64#	395.59 ± 56.04	494.51 ± 50.19	0.05	0.17	0.92
Eicosatrienoic acid	C20:3(ω-3)	0.31 ± 0.03	0.25 ± 0.09	0.19 ± 0.07	0.37 ± 0.03#	0.45	0.05	0.32
Linoleic acid	C18:2(ω-6)	112.41 ± 11.88	163.97 ± 17.48#	123.27 ± 19.02	153.10 ± 16.19	0.04	0.20	0.85
Dihomo-γ-linolenic acid	C20:3(ω-6)	0.15 ± 0.07	0.37 ± 0.14	0.15 ± 0.15	0.37 ± 0.06	0.19	0.17	0.62
Arachidonic acid	C20:4(ω-6)	1.77 ± 0.79	6.29 ± 1.56#	3.59 ± 2.20	4.47 ± 0.43	0.03	0.62	0.15
SFA		82.29 ± 9.44	120.86 ± 14.38	92.72 ± 17.29	110.43 ± 10.70	0.06	0.35	0.57
MUFA		165.20 ± 17.84	198.56 ± 25.91	155.06 ± 22.96	208.70 ± 16.91	0.29	0.11	0.92
PUFA		482.87 ± 50.60	692.62 ± 71.92#	523.52 ± 77.20	652.97 ± 66.74	0.04	0.18	0.87
Σω-3		369.48 ± 38.21	521.72 ± 53.75#	396.32 ± 56.36	494.51 ± 50.19	0.05	0.17	0.92
Σω-6		114.33 ± 12.46	170.62 ± 18.30#	127.01 ± 20.94	157.94 ± 16.52	0.03	0.20	0.76
ω-6/ω-3		0.31 ± 0.00	0.33 ± 0.02	0.31 ± 0.01	0.32 ± 0.00	0.05	0.56	0.11

1. Data are given as Mean ± SEM (n = 3). 2. # indicates a significant difference in the same main effect within the same testing parameter ($P < 0.05$). Main effect: RE (−, +) = RE (no addition of 80% (v/v) ethanolic rosemary extract, addition of 80% (v/v) ethanolic rosemary extract); DI (−, +) = DI (no dry ice addition, dry ice addition). 3. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

treatment with RE and DI indeed reduced ($P < 0.05$) the amount of conjugated dienes in the chicken surimi products compared with untreated products on Day 0. However, on Day 14, chicken surimi products that had undergone combined treatment with RE and DI had the lowest ($P < 0.05$) conjugated dienes, followed by those treated with either RE or DI, and those treated with neither. The POV was used to determine the quantity of peroxide compounds, primary oxidative products.[16] An interaction effect ($P < 0.05$) between RE and DI treatments existed upon storage (Fig. 3b). On Day 0, a single or combined treatment with RE and DI resulted in lower ($P < 0.05$) POVs in chicken surimi products than those in untreated products. On Day 7, chicken surimi products without any treatment had the highest ($P < 0.05$) POV, followed by products treated with only RE or DI, while the lowest ($P < 0.05$) POV was observed in products treated with a combination of RE and DI. On Day 14, DI treatment did not ($P > 0.05$) retard POV increases in products, while RE treatment apparently lowered ($P < 0.05$) the POV in products. Meanwhile, treatment with a combination of RE and DI resulted in the most effective ($P < 0.05$) retardation in the formation of peroxide compounds. Successively, TBARS was used to determine the quantity of the secondary oxidative product malondialdehyde (MDA). Immediately after manufacturing (Day 0), an interaction effect ($P < 0.05$) between RE and DI treatment on TBARS values of products was observed (Table 2). Impacts of two main effects (RE or DI treatment) on our chicken surimi products were not detected ($P < 0.05$) until 14 days of storage, when products with only the RE addition showed the lower ($P < 0.05$) TBARS value (26% lower than those seen without addition of RE). Incidentally, a correlation between TBARS values for fish fillets and sensory attributes indicated that TBARS values for fish below 0.58 mg kg^{-1} reflect absence of rancidity, values of 0.58–1.51 mg kg^{-1} reflect slight but acceptable rancidity, and values above 1.51 mg kg^{-1} reflect outright rancidity.[30] Based on the results shown in Table 2, our chicken surimi products stored at 4 °C for 14 days were slightly, but still acceptably, rancid.. With regard to protein quality, centrifugation loss and TSH contents were determined, representing the water-holding capacity and protein oxidation of chicken surimi products, respectively. On Day 0, RE-treated products had lower ($P < 0.05$) centrifugation loss. However, on Day 14 treatment with either RE or DI, as well as with a combination of RE and DI, reduced ($P < 0.05$) centrifugation loss (Table 3). Concerning protein oxidation, there were no ($P > 0.05$) differences among treatments in terms of TSH value upon storage (Table 3).

In addition to the external features of chicken surimi fortified with flaxseed oil,[2] the oxidative status has been a critical parameter in food preservation as oxidative products may cause, not only rancidity, but may also pose the risk of toxicity to consumers. Conjugated dienes, hydroperoxides, and MDA are sequentially formed when lipid peroxidation is initiated.[31] Primary oxidative products

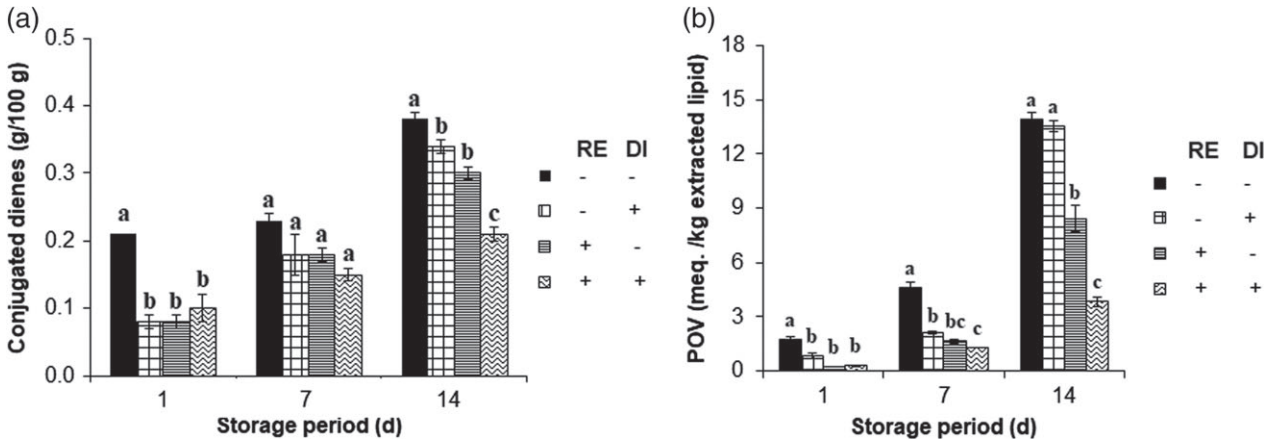


Figure 3. Effects of rosemary extract and dry ice on (a) conjugated dienes (%) and (b) peroxide value (POV) (mEq kg^{-1} extracted lipid) of chicken surimi fortified with flaxseed oil during 14 days of storage at 4 °C. 1. Standard error bars are shown (n = 3). 2. Data bars with no common letters denote significant differences among treatments ($P < 0.05$). Main effect: RE (−, +) = RE (no addition of 80% (v/v) ethanolic rosemary extract, addition of 80% (v/v) ethanolic rosemary extract); DI (−, +) = DI (no dry ice addition, dry ice addition).

Table 2. Effects of rosemary extract and dry ice on TBARS, centrifugation loss (CL), and total sulfhydryl group (TSH) content of chicken surimi fortified with flaxseed oil during 14 days of storage at 4°C

		The interaction effect of RE × DI				Main effect						
		(RE,DI)				RE		DI		*P* value		
Parameter	Storage period (d)	(−,−)	(−,+)	(+,−)	(+,+)	−	+	−	+	RE × DI	RE	DI
TBARS	0	1.09 ± 0.06[a]	0.79 ± 0.05[b]	0.78 ± 0.05[b]	0.77 ± 0.05[b]	0.94 ± 0.08	0.78 ± 0.03	0.93 ± 0.08	0.78 ± 0.03	0.03	0.01	0.02
	7	1.13 ± 0.03	1.00 ± 0.05	0.91 ± 0.10	0.97 ± 0.03	1.07 ± 0.04	0.94 ± 0.05	1.02 ± 0.07	0.98 ± 0.03	0.15	0.07	0.50
	14	1.42 ± 0.11	1.25 ± 0.02	1.00 ± 0.09	0.98 ± 0.06	1.34 ± 0.06[#]	0.99 ± 0.07	1.21 ± 0.11	1.12 ± 0.07	0.36	0.01	0.27
CL	0	23.40 ± 0.75	23.29 ± 0.53	19.47 ± 0.66	19.12 ± 1.01	23.35 ± 0.41[#]	19.29 ± 0.55	21.44 ± 0.99	21.20 ± 1.06	0.87	0.00	0.77
	7	20.94 ± 0.88	20.71 ± 0.24	20.57 ± 0.70	21.80 ± 1.02	20.83 ± 0.41	21.19 ± 0.62	20.76 ± 0.51	21.25 ± 0.53	0.37	0.65	0.54
	14	23.46 ± 0.19[a]	20.17 ± 0.31[bc]	20.72 ± 0.15[b]	19.57 ± 0.19[c]	21.82 ± 0.75	20.14 ± 0.28	22.09 ± 0.12	19.87 ± 0.21	0.00	0.00	0.00
TSH	0	2.43 ± 0.02	2.40 ± 0.03	2.43 ± 0.02	2.40 ± 0.03	2.41 ± 0.02	2.41 ± 0.02	2.43 ± 0.01	2.40 ± 0.02	0.86	0.95	0.40
	7	2.80 ± 0.04	2.81 ± 0.04	2.77 ± 0.02	2.78 ± 0.03	2.81 ± 0.03	2.77 ± 0.02	2.79 ± 0.02	2.79 ± 0.02	0.92	0.34	0.92
	14	2.43 ± 0.01	2.43 ± 0.0.01	2.43 ± 0.00	2.44 ± 0.00	2.43 ± 0.00	2.44 ± 0.00	2.43 ± 0.00	2.44 ± 0.00	0.17	0.39	0.39

1. Data are given as Mean ± SEM (n = 3). 2. Mean values without the common superscript letter[(a-c)] within the same row indicate significant differences among treatments ($P < 0.05$). # indicates a significant difference in the same main effect within the same testing parameter ($P < 0.05$). Main effect: RE (−, +) = RE (no addition of 80% (v/v) ethanolic rosemary extract, addition of 80% (v/v) ethanolic rosemary extract); DI (−, +) = DI (no dry ice addition, dry ice addition). 3. Units of TBARS, CL, and TSH are mg MDA e.q. kg^{-1} surimi, %, and μmol g^{-1} surimi, respectively.

were greatly reduced by combined treatment with RE and DI after 14 days of chilled storage. A previous study considered the acceptable limit of POV in meat products to be 5 mEq kg^{-1}, and, after storage at 14 °C for 14 days, chicken surimi fortified with flaxseed oil and treated with a combination of RE and DI displayed a POV of 3.86 mEq kg^{-1}, well below the acceptable limit.[32] Moreover, secondary oxidation products were lower following addition of RE in the later stage of chilled storage, indicating that RE exerted more persisting antioxidant activity compared with DI. The fact that application of plant extracts, that is, *Melissa officinalis*, can delay lipid oxidation in emulsified meat products with a high content of ω-3 FAs was demonstrated previously.[33] It was also reported that a natural antioxidant of an oregano and sage mixture can decrease hexanal and MDA levels in cooked chicken thigh and breast meat under refrigeration for 96 h.[15] Rosemary extract, in combination with nisin, can result in a significant extension of the shelf life of pompano fillets via improvement of physicochemical quality parameters (e.g. POV, thiobarbituric acid, total volatile basic nitrogen, trimethylamine, pH, *K* value, texture, and color) and of the sensory characteristics, as well as in reduction of microbial growth.[6] We also showed recently that our REs can more efficiently retard lipid oxidation when premixed with flaxseed oil, as compared with α-tocopherol and butylated hydroxytoluene.[12] Rosemary extracts are used commercially in foods and, among all compounds, lipophilic diterpenes carnosol and carnosic acid are primarily responsible for the antioxidant activity of rosemary extracts.[34,35] In our REs, phenolic diterpenes were the major components (rosmanol + carnosol = 88.76 mg g^{-1} extract) (Fig. 2b). Hence, we hypothesize that the retarded lipid oxidation in our chicken surimi products fortified with flaxseed oil is highly related to the polyphenolic profile of our REs. Water-holding capacity, defined as the ability of meat to maintain water under external pressure, is an important property that reflects protein quality and functionality in meat products. In the present case, a combined treatment with RE and DI improved the protein quality of products by decreasing the centrifugation loss by16% as compared to untreated products. Another index concerning protein quality is the TSH value. Oxidation-mediated formation of protein–protein

Table 3. Effects of rosemary extract and dry ice on TBARS, centrifugation loss (CL), and total sulfhydryl group (TSH) content of chicken surimi fortified with flaxseed oil after 60 days of storage at −20°C

		The interaction effect of RE × DI				Main effect						
		(RE,DI)				RE		DI		P value		
Parameter	Storage period (d)	(−,−)	(−,+)	(+,−)	(+,+)	−	+	−	+	RE × DI	RE	DI
TBARS	60	0.57 ± 0.04[a]	0.31 ± 0.06[b]	0.27 ± 0.02[b]	0.26 ± 0.05[b]	0.44 ± 0.07	0.27 ± 0.02	0.42 ± 0.07	0.29 ± 0.04	0.00	0.01	0.03
CL	60	33.30 ± 0.76	30.15 ± 1.84	30.95 ± 0.72	28.32 ± 0.53	31.72 ± 1.13	30.10 ± 1.06	32.12 ± 0.70	29.70 ± 1.27	0.64	0.31	0.14
TSH	60	3.27 ± 0.06	3.46 ± 0.08	3.37 ± 0.01	3.45 ± 0.09	3.36 ± 0.06	3.41 ± 0.05	3.32 ± 0.03	3.45 ± 0.06	0.44	0.53	0.09

1. Data are given as Mean ± SEM (n = 3). 2. Mean values without the common superscript letter[(a-b)] within the same row indicate significant differences among treatments ($P < 0.05$). # indicates a significant difference in the same main effect within the same testing parameter ($P < 0.05$). Main effect: RE (−, +) = RE (no addition of 80% (v/v) ethanolic rosemary extract, addition of 80% (v/v) ethanolic rosemary extract); DI (−, +) = DI (no dry ice addition, dry ice addition). 3. Units of TBARS, CL, and TSH are mg MDA e.q. kg^{-1} surimi, %, and μmol g^{-1} surimi, respectively.

cross-linkages caused denaturation and aggregation of muscle proteins. For instance, proteins with sulfhydryl groups would be oxidized and form disulfide bonds with one another, giving rise to lower values of TSH content.[17] Although treatment with RE and DI showed no effects, our previous report indicated that dried polyphenol-rich litchi flower significantly reduces TBARS values and centrifugation/purge losses, and also promotes higher TSH contents in emulsified meatballs, thus providing better quality under frozen storage.[36] As we know, the sublimation of dry ice to gaseous carbon dioxide can expel oxygen. Based on our results, the successful management of low oxidative substances (conjugated dienes, peroxides, and MDA) in chicken surimi fortified with flaxseed oïl via the addition of either RE or DI, or both, during chilled storage could be attributed to (i) the polyphenolic compounds in REs that provide either effective free radical scavenging ability by attacking unsaturated fatty acids or by inhibition of conjugated dienes (Fig. 3a) and (ii) the addition of DI, thus decreasing incremental heat and expelling oxygen during the chopping phase of the manufacturing process. Following the lipid oxidation in meat products, protein is a further target that would be attacked by free radicals or would undergo adduction of lipid oxidative products, leading to protein degradation. As the major component in meat products, protein provides not only nutritional value but also functionality. During 14 days of chilled storage (4 °C), treatment with either RE or DI, or with a combination of both, lowers centrifugation loss, indicating the better water-holding capacity of the products.

Effects of REs and DI on lipid and protein quality of chicken surimi fortified with flaxseed oil under storage at −20 °C

After 60 days of storage at −20 °C, single or combined treatment with RE and DI resulted in approximately 50% lower TBARS values of the products (Table 3). Although centrifugation loss and TSH contents in chicken surimi fortified with flaxseed oil were not ($P > 0.05$) influenced by treatment with REs, there was a tendency toward lower centrifugation loss in DI-treated chicken surimi products. In addition to storage at 4 °C, frozen storage (−20 °C) was used to simulate the storage conditions of semi-manufactured chicken surimi products. However, during frozen storage, the MDA content in all groups remained at low levels (0.26–0.57 mg MDA e.q. kg^{-1}) which are similar to values for fresh chicken breast meat (0.33–0.58 mg MDA e.q. kg^{-1}).[37] According to the results of this study, TBARS values of chicken surimi fortified with flaxseed oil can be reduced by individual or combined treatment with RE and/or DI, under frozen storage conditions for 60 days; however, the protein quality (CL and TSH) of our chicken surimi did not deteriorate after 60 days of storage at −20 °C.

CONCLUSION

Following the manufacturing process, the addition of DI during the chopping procedure of ω-3 FA-fortified surimi maintained a desirable appearance and textural characteristics, while premixing of flaxseed oil with our REs resulted in the retention of higher amounts of total ω-3 and ω-6 FAs in the products. Also, combined treatment with RE and DI effectively retarded lipid oxidation and alleviated protein degradation of the flaxseed-oil fortified chicken surimi after 14 days of chilled storage (4 °C) or after 60 days of frozen storage (−20 °C). Therefore, addition of our RE to the premixture of ω-3 FA lipids and DI, respectively, during the chopping procedure makes for a potentially prolonged shelf life of ω-3 FA-fortified surimi-like meat products in the industry.

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