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Research article

Reducing free acidity and acrolein formation of omega-3-rich oils by blending with extra virgin olive oil during microwave heating

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Abstract: In recent years, microwave food processing has been increasingly utilized worldwide. Omega-3-rich oils, which have various health benefits, must be protected from heat treatment, including microwave heating, due to their thermosensitivity. In this study, we investigated oxidative stability of blends of omega-3-rich oils, such as flaxseed, sesame, and perilla oils, with extra virgin olive oil (EVOO) in order to reduce concentration of acrolein during microwave heating. Microwaving flaxseed oil increased the free acidity and the formation of undesirable and irritating odors (e.g., acrolein). In contrast, microwave treatment of EVOO resulted in a much lower level of free acidity and acrolein formation. Blending 10% EVOO in flaxseed oil enhanced the antioxidant capacity and effectively reduced free acidity and acrolein formation during microwave heating. The enhancing effect was also partially achieved in flaxseed oil blended with both 10% refined olive oil and α-tocopherol, which are bioactive components in EVOO. Similarly, blending 10% EVOO in other omega-3-rich oils, including sesame oil and perilla oil, also decreased free acidity and acrolein formation during microwave heating. These results suggest that blending with EVOO facilitates the use of omega-3-rich oils in microwave food processing while retaining their health benefits.

Keywords: microwave heating; omega-3; flaxseed oil; extra virgin olive oil; oxidation; acrolein

1. Introduction

Microwave heating has gained worldwide popularity as an alternative to conventional food processing methods in modern households. It has high energy efficiency and reduces cooking time compared to conventional heating. Therefore, microwave technology has been used in the food industry as a promising green technology for processes such as warming, drying, thawing, baking, pasteurizing, and sterilizing foods [1−4]. Despite considerable advantages over conventional food processing methods [5], objectionable compounds can be produced in microwaved foods. Particularly, microwave heating of different vegetable oils and fats leads to the formation of free radicals that rapidly react with atmospheric oxygen to produce hydroperoxides and secondary oxidation products [6−9].

Polyunsaturated omega-3 fatty acids, especially long-chain eicosapentaenoic and docosahexaenoic fatty acids, which are mainly found in fish oil, are well-known, health-promoting nutrients that contribute to the amelioration of age-related diseases, including cardiovascular diseases [10,11]. These two long-chain omega-3 fatty acids are highly susceptible to lipid oxidation, which can lead to serious problems, such as the loss of shelf-life, functionality, nutritional value, consumer acceptability, and fish oil safety [12]. Linolenic acid, a long-chain omega-3 fatty acid, is also found in a variety of plants and seed oils, such as flaxseed (*Linum usitatissimum* L.[*Linaceae*]), sesame (*Sesamum indicum* L. [*Pedaliaceae*]), and perilla (*Perilla frutescens* L. [*Lamiaceae*]) oils. It has been recommended that omega-3-rich oils should be prevented from extensive heat treatment during cooking since they are sensitive to heat, oxygen, and light [13]. In addition, linolenic acid is the main source of acrolein, which is an irritating and off-flavored compound formed during the heating of vegetable oils at high temperatures [14]. Thus, omega-3-rich oils are unsuitable for microwaving and conventional heat processing [15].

Olive oil is among the core components of the Mediterranean diet and extra virgin olive oil (EVOO) has numerous health benefits reported due to its high monounsaturated oleic acid content. It is also rich in antioxidants, such as tocopherols, phenols, and carotenes; hence it possesses the highest tolerance to heat among the edible oils [16]. Domestic microwaves are mostly used at a power of 700 W or less depending on the required food processing technique [17,18]. Intense microwave heating at 500 W increases free acidity and acrolein formation, even in EVOO, whereas this effect is hardly observed at 150 W [19]. This suggests that EVOO as a source of a natural antioxidant may be utilized to improve the oxidative stability of omega-3-rich oils during microwave heating at a lower power level. The aim of this study was to develop a method for preventing the thermal increase in free acidity and acrolein formation of omega-3-rich oils such as flaxseed, sesame, and perilla oils during low-power microwave heating using EVOO.

2. Materials and methods

2.1. Materials

Oils labeled as flaxseed and sesame oils (NID, Tokyo, Japan), perilla oil (Benibana Foods Co., Ltd., Tokyo, Japan), EVOO (Shodoshima Healthyland Co., Ltd., Kagawa, Japan), and refined olive oil (ROO; Toyo Olive Co., Ltd., Kagawa, Japan) were purchased from the market. The percentage composition of fatty acids in flaxseed, sesame, and perilla oils, EVOO, and ROO used in this study are listed in Table 1. The fatty acid composition of the olive oils met the international official regulations edited by the International Olive Council (IOC) [20] and the European Community [21]. The amount of α-tocopherol (TP) in flaxseed oil and EVOO was 1.6 and 24.6 mg per 100 g of oil, respectively. Medium-chain triglyceride (MCT) oil was purchased from Nisshin OilliO Group, Ltd.

(Tokyo, Japan). Acrolein (purity >95%) was purchased from Tokyo Chemical Industry (Tokyo, Japan).

Table 1. Summary of the fatty acid composition of oils (% fatty acids).

Fatty acids	Flaxseed oil	Sesame oil	Perilla oil	EVOO	ROO
Myristic acid (C14:0)	ND	ND	ND	ND	ND
Palmitic acid (C16:0)	5.6 ± 0.2	5.9 ± 0.2	5.9 ± 0.4	13.8 ± 0.6	8.5 ± 0.5
Palmitoleic acid (C16:1)	ND	ND	ND	1.3 ± 0.1	0.4 ± 0.0
Heptadecanoic acid (C17:0)	ND	ND	ND	ND	ND
Heptadecenoic acid (C17:1)	ND	ND	ND	ND	ND
Stearic acid (C18:0)	3.9 ± 0.2	1.6 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	3.3 ± 0.2
Oleic acid (C18:1)	17.5 ± 0.7	16.2 ± 0.6	15.3 ± 0.8	70.2 ± 0.9	75.2 ± 3.5
Linoleic acid (C18:2)	18.3 ± 0.7	14.2 ± 0.6	12.8 ± 0.7	7.8 ± 0.3	6.6 ± 0.2
α-Linolenic acid (C18:3)	50.8 ± 2.0	58.4 ± 2.3	59.1 ± 3.4	0.8 ± 0.2	0.6 ± 0.0
Arachidic acid (C20:0)	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	ND
Eicosenic (C20:1)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	ND
Behenic acid (C22:0)	0.1 ± 0.0	ND	ND	0.1 ± 0.0	0.1 ± 0.0
Lignoceric acid (C24:0)	ND	ND	ND	ND	ND

ND = Not detected.

2.2. Microwave heating procedure

A household microwave oven (Panasonic NE-EH229, Osaka, Japan) was operated at 150 W. Three samples (20 g) for each treatment were weighed into Petri dishes (diameter: 10 cm, height: 2 cm) [15], and then heated in a microwave oven at the center of the rotating plate (diameter: 28 cm) for 3, 6, 9, 12, and 15 min, with three independent experiments performed under the same conditions. All samples were cooled rapidly and analyzed shortly thereafter.

2.3. Analytical procedures

The free acidity (FA) of the oil samples was measured using an OxiTester (CDR, Ginestra Fiorentina, Italy) [19,22,23]. Aliquots of the oil sample (2.5 μL) were added to a prefilled cuvette. The fatty acid composition of oils was analyzed on a 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with autosampler, flame ionization detector and a 30 m DB-23 capillary column (Agilent Technologies) with internal diameter 0.25 mm and film thickness of 0.25 μm. α-TP content of the oils was analyzed on an LC-20AT HPLC system (Shimadzu Co., Kyoto, Japan) equipped with fluorescence Detector (Shimadzu RF-10AXL) and a YMC-Pack SIL-06 column (YMC Co., Ltd., Kyoto, Japan; particle size, 5 μm; length, 250 mm; internal diameter, 4.6 mm).

2.4. Quantification of acrolein

The headspace of the oil samples was analyzed using the method proposed by a HERACLES II electronic nose (Alpha MOS, Toulouse, France) [24] equipped with two identical gas chromatography columns working in parallel mode, namely a non-polar column (MXT-5; length, 10 m; internal diameter, 0.18 mm; film thickness, 0.4 μm) and a polar column (MXT-WAX; length, 10 m; internal diameter, 0.18 mm; film thickness, 0.4 μm), which produced two chromatograms simultaneously. The electronic nose was also equipped with an HS100 autosampler (CTC Analytics AG, Zwingen, Switzerland) to automate sample incubation and injection. The analytical conditions were as follows: an aliquot of the oil sample (2.0 g) was placed in a 20-mL vial, sealed with a magnetic cap, and placed in the autosampler, which was then placed in a HERACLES shaker oven and incubated for 15 min at 60 ℃ with shaking at 500 rpm. The headspace was sampled (5 mL) using a syringe and injected into the gas chromatograph. The thermal program started at 40 ℃ (held for 10 s) and then increased to 250 ℃ at a rate of 1.5 ℃ /s. The final temperature was maintained for 60 s, and the total separation time was 120 s. The data were acquired and processed using the AlphaSoft v2020 software (Alpha MOS). To determine the amount of acrolein in the oil samples, a standard curve was established [24]. Different concentrations of acrolein were prepared in MCT oil and subjected to flash gas chromatography electronic nose analysis. The amount of acrolein in the oil samples after microwave heating was determined from the standard curve.

2.5. Total antioxidant capacity assay

The antioxidant power of the oil samples was evaluated using the PAO-SO test kit (Japan Institute for the Control of Aging, Nikken Seil Co., Ltd., Shizuoka, Japan), according to the manufacturer's instructions [25,26]. This test is based on evaluating the Cu^{+} levels derived from the reduction of Cu^{++} by the action of antioxidant substances in the sample. Cu^{+} forms a stable complex with bathocuproine, whose typical optical absorption at 490 nm was determined using a microplate reader (SH-9000 Lab; Corona Electric Co., Ltd., Ibaraki, Japan). The values of antioxidant power detected in the samples were compared using a curve generated from the standard substance provided in the kit at known concentrations and expressed in μM.

2.6. Statistical analysis

Data are presented as the mean ± standard deviation (SD) from three replicates. The statistical significance of differences between the two groups was analyzed using the Student's *t*-test in Microsoft Excel. The Tukey-Kramer test in Microsoft Excel was used to identify significant differences among the means of multiple groups. The data were analyzed by one-way analysis of variance, followed by the Tukey-Kramer test in Microsoft Excel. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Microwave heating causes increases in free acidity and acrolein formation of flaxseed oil

The temperature increases during the microwave heating of flaxseed oil and EVOO, as shown in Figure 1A. The temperature increased with increasing heating time and reached 124–129 ℃. There were no significant differences of the temperature changes between the two oils.

The results from fatty acid hydrolysis in Figure 1B show the change in FA values during microwave heating of flaxseed oil and EVOO. The FA value of the flaxseed oil increased significantly during heating. It was higher for flaxseed oil heated in a microwave oven than for the oil heated in a pan at 150 °C [27]. Such an increase was also reported by a previous study in which flaxseed oil was subjected to microwave heat at 1000 W [15]. In contrast, the FA value of EVOO was maintained below the limit established by the IOC and the European Regulation (0.80%) for extra virgin oils [20,21], which is consistent with previous results [19]. These observations suggest that flaxseed oil is sensitive to microwave heating whereas EVOO is more tolerant.

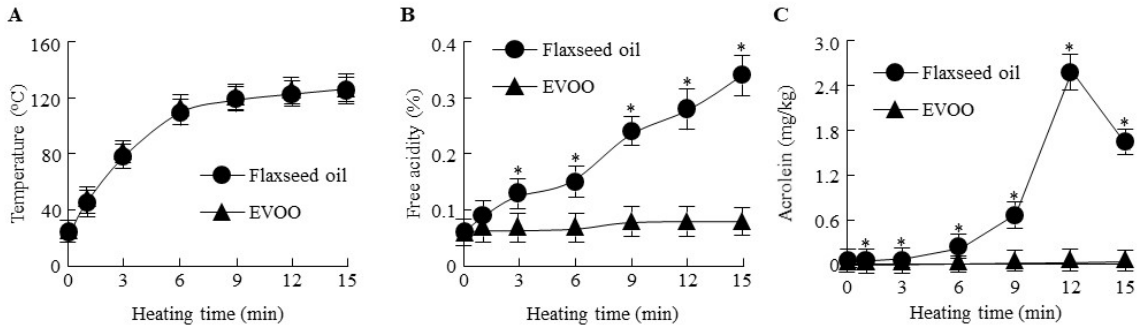

Figure 1. Changes in flaxseed oil and EVOO during microwave heating based on temperature (A), free acidity (B), and acrolein (C). Asterisks indicate mean values at the same time point ($p < 0.05$; Student's *t*-test) for triplicates.

Omega-3 fatty acid rich-oils, such as fish oil, have an unpleasant smell even at low oxidation levels because during the early stages of oil oxidation, they produce acrolein as the major volatile compound, which may affect consumer acceptability of the oil [28]. When vegetable oils rich in linolenic acid are heated, acrolein, which is an irritating and off-flavor compound, is also formed in the oil [14]. Owing to the strong impact of acrolein on flavor deterioration, the formation of this key volatile compound during microwave heating should be monitored to evaluate the sensory quality of lipids that are susceptible to oxidative degradation [19]. Figure 1C shows the changes in the formation of acrolein in flaxseed oil and EVOO during microwave heating. The amount of acrolein in flaxseed oil rapidly increased after heating for 6 min, reaching a maximum (2.58 mg/kg) at 12 min, after which the acrolein level decreased rapidly. The maximum amount of acrolein observed at the 12 min time point was still lower than the amount formed during frying at 180 °C [29]. Acrolein is a reactive monomer with a high tendency to polymerize [30]. It is possible that the excessive formation of acrolein increases its polymerization rate, which results in a rapid decrease in the levels of acrolein. Conversely, microwave heating produced only a very small increase in acrolein formation in the EVOO during heating. These results were consistent with previous results, which suggested that high amount of acrolein is formed during heating of cooking oils with high polyunsaturated fatty acid content, such as safflower oil, even at a low temperature (180 °C), except for EVOO owing to its low content of polyunsaturated fatty acids [31].

These observations suggest that microwave heating at 150 W causes an increase in free acidity and acrolein formation of flaxseed oil, but not EVOO.

3.2. Reducing the free acidity and acrolein formation of flaxseed oil by blending with EVOO

Changes in free acidity and acrolein formation after microwave heating of flaxseed oil blended with different concentrations of EVOO were investigated. Figure 2 shows the FA values and acrolein levels after microwave heating for 15 min and 12 min, respectively. These blends effectively maintained both the FA value and acrolein content at a low level. The reduction rates depended on the concentration of EVOO in the blends with flaxseed oil. This suggests that the increase in free acidity and acrolein formation of flaxseed oil after microwave heating can be reduced by blending with EVOO, since they might be caused by antioxidant factors in EVOO other than α-TP.

Edible oils with high levels of oleic acid and low levels of linoleic acid, such as olive oil, have higher oxidative stability [32,33]. Therefore, ROO, which contains no minor compounds with antioxidant activity [34], provided a lower level of FA and acrolein content compared to that from flaxseed oil after microwave heating (Figure 2A and 2B). This trend was consistent with that of EVOO, as it might be due to the low content of polyunsaturated fatty acids present in ROO (Table 1) [30]. The increases in free acidity and acrolein formation were also reduced in flaxseed oil blended with 10% ROO. EVOO contains substantial amounts of antioxidants, such as α-TP [35]. Tocopherol content is in relation with many factors and among them, the cultivar of origin and the fruit harvest date (the ripening index) [36]. The content of α-TP in EVOO used in the present study was 24.6 mg/100 g. The FA value and acrolein content were also reduced in flaxseed oil supplemented with α-TP at the same rate as that with 10% EVOO. Moreover, flaxseed oil blended with both 10% ROO and α-TP had additive effects on the reduction of the FA value and the acrolein content compared to when each component was added alone. However, these reduction values were still lower than those obtained for flaxseed oil blended with 10% EVOO.

To further evaluate the difference in free acidity and acrolein formation among the oil samples used in the blend, the initial antioxidant power of the oil samples was measured (Figure 3). The values of the antioxidant power were inversely proportional to the FA values and acrolein content of the oil samples (Figure 2A and 2B). The antioxidant capacity of flaxseed oil was enhanced by blending it with different concentrations of EVOO. An increase in the antioxidant capacity was achieved by adding 10% ROO or α-TP. Flaxseed oil blended with both 10% ROO and α-TP possessed higher antioxidant capacity than that with ROO or α-TP alone. In addition, flaxseed oil blended with 10% EVOO still exhibited a significantly higher antioxidant capacity than that with both 10% ROO and α-TP. These results suggest that EVOO contains an inhibitory agent (e.g., phenolic compounds) other than α-TP that reduces the free acidity and acrolein formation of flaxseed oil during microwave heating.

3.3. Reducing the free acidity and acrolein formation of other linolenate-rich oils by blending with EVOO

Finally, the free acidity and acrolein formation of other linoleate-rich oils, such as sesame and perilla oils, after microwave heating, were investigated (Table 2). The FA values of sesame and perilla oils increased from 0.03% to 0.19% and 0.05% to 0.13%, respectively, after microwave heating for 15 min. The acrolein contents of these oils dramatically increased from 0.002 to 2.16 mg/kg and 0.004 to 1.56 mg/kg, respectively, after microwave heating for 12 min. Sesame and perilla oils blended with 10% EVOO possessed significantly higher initial antioxidant capacity and showed lower levels of FA and acrolein content than those without EVOO, after microwave heating. These results suggest that EVOO can be used to reduce free acidity and acrolein formation when microwaving foods with thermosensitive omega-3-rich oils.

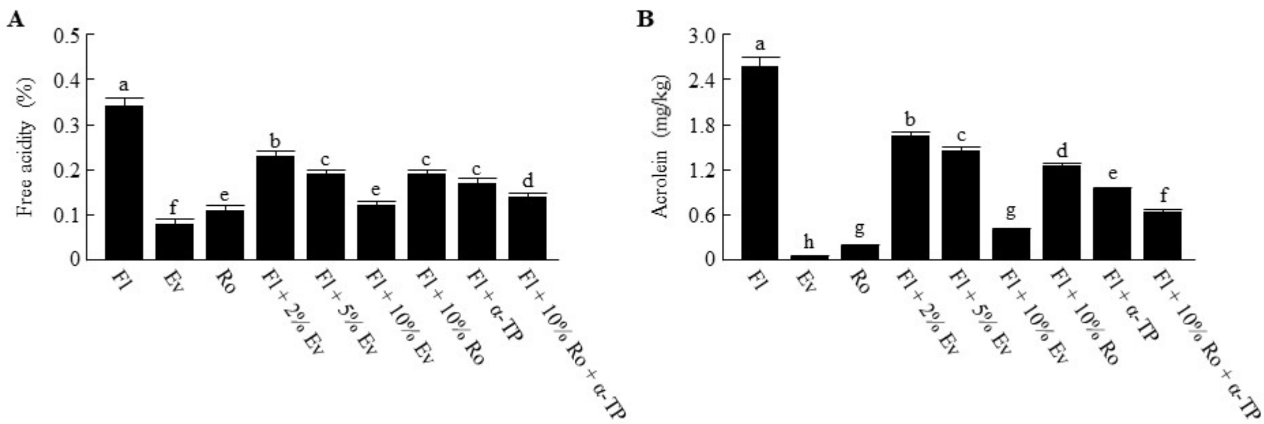

Figure 2. Free acidity (A) and acrolein formation (B) in flaxseed oil and EVOO after microwave heating for 15 min and 12 min, respectively. Mean values with different letters are statistically different ($p < 0.05$; Tukey-Kramer multiple comparison test). Fl, flaxseed oil; Ev, EVOO; Ro, ROO.

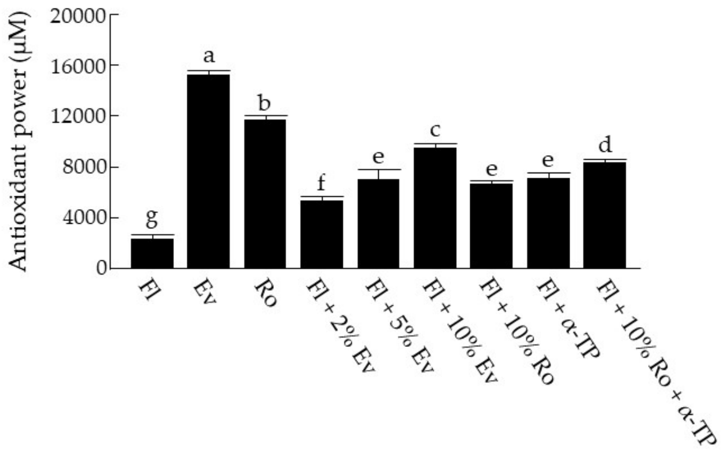

Figure 3. Initial antioxidant capacity among oil samples. Mean values with different letters are statistically different ($p < 0.05$; Tukey-Kramer multiple comparison test). Fl, flaxseed oil; Ev, EVOO; Ro, ROO.

Table 2. Free acidity, acrolein formation, and initial antioxidant power in sesame and perilla oils with/without 10% EVOO.

Oil samples	Free acidity (%)[1]	Acrolein (mg/kg)[2]	Initial antioxidant power (μM)
Sesame oil	0.19 ± 0.02	2.16 ± 0.078	3041 ± 214
Sesame oil + 10% EVOO	0.13 ± 0.02*	0.33 ± 0.010*	9452 ± 597*
Perilla oil	0.13 ± 0.05	1.56 ± 0.023	5272 ± 664
Perilla oil + 10% EVOO	0.05 ± 0.01*	0.33 ± 0.012*	12649 ± 839*

[1,2] Data are expressed as mean ± SD (n = 3) after microwave heating at 15 min[1] and 12 min[2]. Asterisks indicate significant differences between each oil blend with and without 10% EVOO ($p < 0.05$; Student's *t*-test).

4. Conclusions

Our results demonstrated that omega-3-rich oils, such as flaxseed, sesame, and perilla oils, are sensitive to microwave heating at 150 W, suggesting that these oils are unsuitable for cooking using a microwave. However, blending 10% EVOO in omega-3-rich oils could enhance the antioxidant capacity of the oils and maintain the FA values and low levels of acrolein formation in the oils during microwave heating. Therefore, EVOO can be a source of a natural antioxidant in prolonging food stability via its virtue in preventing increases in free acidity and acrolein formation. Since the consumption of EVOO also helps to reduce the risk of cardiovascular disease, blending EVOO in omega-3-rich oils not only allows the oils to be used safely in microwave food processing, but also promotes human health through the powerful cardioprotective properties.

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Conflict of interest

The author declares no conflicts of interest.

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