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Physicochemical Properties of *Plukenetia volubilis* L. Seeds and Oxidative Stability of Cold-pressed Oil (Green Nut Oil)

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Cold-pressed oil from the seeds of *Plukenetia volubilis* L. (green nut oil, GNO) has been shown to be rich in n-3 fatty acid content, similar to flaxseed and perilla oils. These fatty acids are readily oxidized at high temperatures and under UV irradiation, limiting their potential applications. We therefore set out to clarify the physicochemical properties of green nuts. In addition, to explore means of the degradation process, we investigated the oxidation characteristics of GNO and the effects of oxidation on fatty acids. Results showed that GNO was stable up to 140°C and exhibited greater UV resistance than the other oils. This may be related to the 10% lower α-linolenic acid content of GNO, compared to the other oils, as well as the presence of γ- and δ-tocopherols. GNO was capable of tolerating a certain degree of heat processing.

Keywords: green nut oil, flaxseed oil, perilla oil, heating, UV irradiation, fatty acid, tocopherol

Introduction

Plukenetia volubilis L. is a perennial climbing plant in the Euphorbiaceae family that is native to the Peruvian Amazon River basin (Antonio, 1999). The seeds, which are borne in a green star-shaped fruit, are known locally as Inca Inchi, Sacha Inchi or green nuts. There are thought to be 8,000 cultivated and wild varieties. In Peru, the seeds have long been called Inca peanuts, and are prepared for consumption by roasting, boiling or after milling into powder and are also used medicinally, although medicinal use is limited (Gotoh, 2006; The Japan Food Chemical Research Foundation, 2005).

The cold-pressed oil from seeds of certain varieties of *P. volubilis* exhibits a unique, refreshing flavor and light texture, and is rich in n-3 fatty acids. Not only does the oil have superb flavor, having won a gold medal at the International Edible Oil Expo in the non-olive oil category in 2004 (La Revue, 2004), experiments have shown that the oil has a high anti-oxidative capacity, suggesting its potential to reduce oxidative DNA damage (Fukushima *et al.*, 2010). In addition, compared to flaxseed and perilla oils, *P. volubilis* seed oil have high α-linolenic acid content, which is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in living organisms (Kayama, 1995; Wada, 2008). The oil is thought to have anti-inflammatory properties and offers protection against blood clots, coronary heart disease and high blood pressure (Sawai, 2009; Williams, 2011; Okuyama, 2001; Ogawa *et al.*, 2009; Albelt *et al.*, 2005; Hu *et al.*, 1999). Individuals who either cannot or do not eat fish on a daily basis, or who only eat fish infrequently, can supplement their n-3 fatty acid intake with flaxseed oil or perilla oil (Editorial Department, 2005, i). However, as these fatty acids are readily oxidized at high temperatures, their potential applications are limited. As such, identification of new n-3 fatty acid sources, in addition to flaxseed and perilla oils, that are heat-resistant would be extremely beneficial from the standpoint of human health maintenance.

We therefore set out to clarify the physicochemical properties of green nuts. In addition, in order to explore means of the degradation process, we investigated the oxidation characteristics of cold-pressed oil from green nut seeds (GNO) and the effects of oxidation on fatty acids. Moreover, to further characterize GNO, a comparison was made with flaxseed and perilla oils, which are excellent sources of n-3 fatty acids.

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Materials and Methods

Sample preparation *P. volubilis* L. seeds (green nuts) were produced in the Peruvian lowlands and harvested (and de-hulled) in 2009 by the NPO Arco Iris. Using a blender, seeds were ground into a powder, and then subjected to moisture, ash, and lipid content analyses. Analyses of protein and dietary fiber contents were conducted on powdered samples from which fat was removed.

Proximate composition Analysis of the proximate composition of green nuts was conducted in accordance with the Fifth Edition of the Standard Tables of Food Composition in Japan (2005). The methods used were "Analytical manual for standard tables of food composition in Japan (fifth revised and enlarged edition)" (Council for Science and Technology Ministry of Education, Culture, Sports, Science, Subcommittee Resource Survey Committee on Food Ingredients, 2005) for protein, moisture, lipid and ash contents. Dietary fiber was analyzed using a dietary fiber assay kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Results were expressed as a percentage on a wet weight basis.

Green nut, flax and perilla oils GNO (extracted in March 2009, and unsealed in September 2009 and 2010, prior to use in experiments) was provided by the NPO Arco Iris. Flaxseed and perilla oils were manufactured in August 2010 by Benibana Foods Co., Ltd. (Tokyo, Japan) and were opened in August 2010, prior to use in experiments.

UV irradiation and heat treatment of oils UV-irradiated GNO, flax oil and perilla oil were prepared by pouring 12.5 g of each oil into separate 9-cm-diameter Petri dishes and exposing each to UV light (254 nm, model GL15; Toshiba Corp., Tokyo, Japan) on a clean bench for 5, 10, or 15 h. The intensity of UV radiation was 240 $\mu W/cm^2$. Heat-treated oil was prepared by placing 55 g of each oil into a 27-cm-diameter Teflon-coated frying pan on an electric hob, such that the sample depth was 2 cm, followed by heating at 80, 100, 120, 140, 160 or 180°C for 10 min. Oil temperature was measured using a digital thermometer (model TX10-03; Yokogawa Electric Corp., Tokyo, Japan).

Characterization of oils The iodine value of GNO was evaluated by the titration method using an iodine monochloride solution (Pharmaceutical Society of Japan, 1990), and the saponification value was determined using KOH ethanol (Pharmaceutical Society of Japan, 1990). Peroxide value (PV) in oxidized oils was determined in accordance with the method of the Japan Oil Chemists' Society (2003, a) and carbonyl value (CV) was determined by the butanol method using decenal as the standard (Japan Oil Chemists' Society, 2003, b).

Tocopherol content Tocopherol content was analyzed by high-performance liquid chromatography (HPLC) with a PEGASIL column (4.6 × 250 mm, 5 μm, 60Å; Senshu Corp., Tokyo, Japan) fitted with a pre-column (4.6 × 30 mm) of the same type. The mobile phase, consisting of a 5:6:1000 (v/v/v) mixture of acetic acid, 2-propanol and n-hexane, was passed through the column (maintained at 40°C) at a flow rate of 1.0 mL/min. The effluent was monitored with a fluorescence detector (excitation, 298 nm; emission, 325 nm). Each oil sample was dissolved in a fixed volume of n-hexane and injected into the HPLC column. Authentic standards of α-, β-, γ-, and δ-tocopherols were obtained from Eisai Co. (Tokyo, Japan).

Preparation of fatty acid methyl esters Methyl esterification of fatty acids was accomplished as follows. First, oils were poured into test tubes with screw caps. Sodium methoxide reagent (0.5 mol/L) was then added, and after mixing by agitation, the air in the test tubes was replaced with nitrogen. Test tubes were then heated for 10 min using a block heater maintained at 50°C. After allowing the samples to cool to room temperature, samples were neutralized by the addition of acetic acid. Equal amounts of ultrapure water and n-hexane were added to the samples and then mixed. After centrifuging for 5 min at 2400 rpm, the supernatant was transferred to a new test tube. Hexane was then added to the remaining aqueous layer and the centrifugation procedure and supernatant transfer was repeated three times. After removing water from the collected supernatant using anhydrous sodium sulfate, the solvent was evaporated from the samples using a rotary evaporator without causing the samples to dry and harden. Next, after adding a solution containing the internal standard to the samples, and completely removing the solvent using nitrogen gas, final test samples were prepared by dissolving and mixing them with acetone. Fatty acid reference samples consisted of methyl esters of palmitic acid and stearic acid (Sigma-Aldrich Co., USA), as well as oleic acid, linoleic acid, and α-linolenic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Fatty acid composition Fatty acid composition was analyzed by gas-liquid chromatography (GC). GC was performed with a model GC-4000 (GL Sciences Inc., Tokyo, Japan), using a 0.25 mm × 30 m InertCap Pure-Wax column (film thickness, 0.25 μm) with a split ratio of 50:1. Flow rate of the carrier gas (He) was 1.20 mL/min. Temperature was increased from 70°C to 190°C at 15°C/min, maintained at 190°C for 5 min, increased to 240°C at 4°C/min, and maintained at 240°C for 30 min. Methyl undecanoate (C11:0; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was used as an internal standard. The concentrations of individual fatty acids (mg/g oil) were calculated by means of the internal standard and the sample oil weight. Peaks were confirmed by their mass spectra using gas chromatography-mass spectrom-

etry (JMS-AX500; JEOL Ltd., Tokyo, Japan) operated with the same GC conditions. Electron-impact mass spectra were obtained at an ionization energy of 70 eV.

Statistical analyses Significant differences were determined by one-way ANOVA and Dunnett's test using SPSS. Differences with p-values of less than 0.01 or 0.05 were considered to be statistically significant.

Results and Discussion

Proximate composition of green nuts Table 1 shows the composition of general nutrients in green nuts. The composition was similar to that of dried peanuts, as recorded in the 2010 Standard Tables of Food Composition in Japan (Council for Science and Technology Ministry of Education, Culture, Sports, Science, Subcommittee Resource Survey Committee on Food Ingredients, 2010). However, the dietary fiber content of green nuts is higher than that of peanuts, accounting for 81% of carbohydrates in the former compared to 39% in the latter.

Chemical characterization of GNO The saponification and iodine values of GNO were determined to be 193 ± 1 and 185 ± 1, respectively. The saponification value was similar to that of soybean oil, while the iodine value represented by α-linolenic acid and linoleic acid contents was high, together accounting for more than 80% of the total fatty acid content.

Table 1. Components of green nuts and peanuts.

(per 100 g edible portion, %)

Components		Green nuts[1)]	Peanuts[2)]
Moisture		7.5	6.0
Lipid		52.2	47.5
Protein		24.2	25.4
Carbohydrate[3)]		13.4	18.8
Ash		2.7	2.3
Detail of carbohydrate (%)			
Glucide[4)]		18.7	60.6
Dietary Fiber	IDF[5)]	72.4	37.2
	SDF[6)]	9.0	2.1

1) Mean (n = 5).
2) As recorded in "The 2010 standard tables of food composition in Japan".
3) Carbohydrate = 100 – (Moisture + Lipid + Protein + Ash).
4) Glucide = Carbohydrate – (IDF + SDF).
5) IDF: water insoluble dietary fiber.
6) SDF: soluble dietary fiber.

Effect of heat treatment and UV irradiation on the oxidative stability of GNO, flaxseed and perilla oils Figure 1 shows the effect of heat treatment on the oxidative stability of GNO, flaxseed and perilla oils. Oxidative stability was evaluated using the PV and CV of each oil. While it was not possible to accurately compare the oils, given that the initial

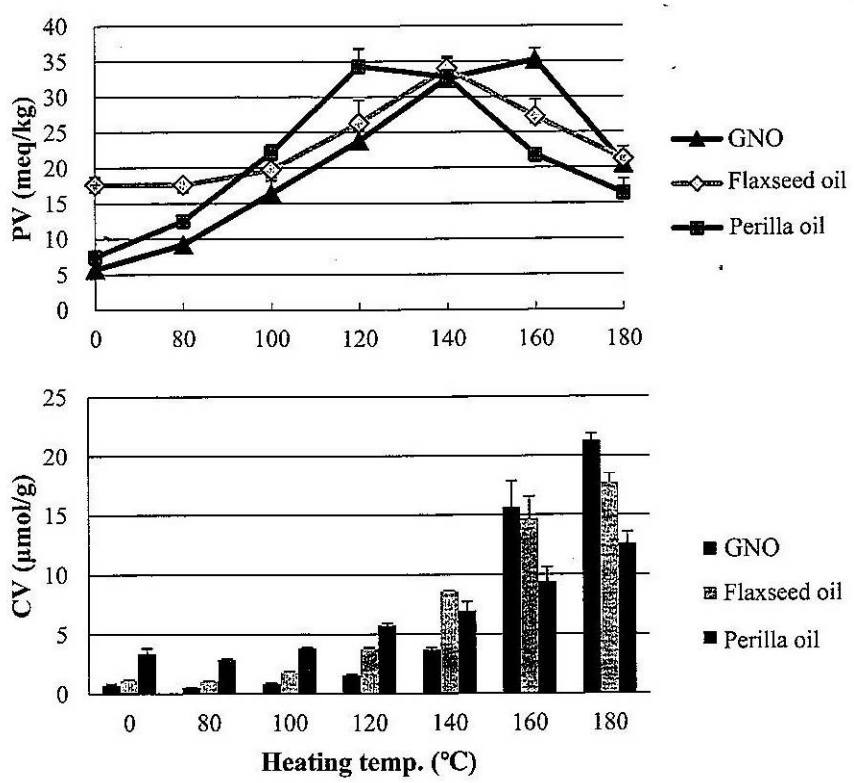

Fig. 1. Changes in peroxide value (PV) and carbonyl value (CV) with heat treatment.
Sample oils (55 g) were heated in a Teflon-coated frying pan on an electric hob for 10 min. Data represent mean ± SD (n = 6).

PV of the three oils differed, the data are presented here in order to characterize GNO. GNO exhibited the lowest PV up to 140°C, but the highest values at 160 and 180°C. The PV of GNO, flaxseed and perilla oils peaked at 160°C, 140°C, and 120°C, respectively. No differences were observed in the CV of any of the unheated oils or oils after the 10-min heat treatment at 80°C. However, above 100°C, the CV increased with increasing temperature. While the CV was the highest for perilla oil up to 120°C, flaxseed oil exhibited the highest CV at 140°C. The CV of GNO increased sharply at 160°C and 180°C, exceeding that of the other two oils. The fact that the PV of individual oils increased with heating temperature and declined after peaking could be attributable to the decomposition of peroxide into carbonyl compounds. It is assumed that the decomposition of peroxide in perilla and flaxseed oils increased markedly at around 120°C and 140°C, respectively, resulting in accelerated conversion to carbonyl compounds (Luna *et al.*, 2006). Although the CV in GNO appeared to be stable up to 140°C, the PV and CV increased between 140°C and 160°C, suggesting that the inflection point in oxidation rate and the threshold for edibility of GNO lie in this temperature range.

Figure 2 shows the influence of UV irradiation on the PV and CV of the three types of oil. By subjecting the oils to both typical and harsh UV exposure conditions, we are able to observe their respective physicochemical properties. PV was higher in all oils exposed to UV light than in those not exposed. As was reported previously (Kato *et al.*, 2008; Seto *et al.*, 1990; Miraliakbari and Shahidi, 2008; Shibasaki, 2000) fats and oils are readily photooxidized when exposed to UV light at wavelengths less than 300 nm. Similarly, in our experiments, we observed that PV increased with UV exposure time. At 15 or fewer hours of UV exposure, PV was highest in flaxseed oil, followed by perilla oil and GNO. Although the PV of GNO after 5 h of UV exposure was only 23, likely putting it into the edible category, the PV increased markedly to 97 after 10 h of UV exposure. The PV of flaxseed oil was already high (174) after 5 h of UV exposure, suggesting that it would be inedible. Perilla oil PV was between those of flaxseed oil and GNO, increasing to 33 and 144 after 5 and 10 h of UV exposure, respectively. At all UV exposure times other than 10 h, CV were highest in perilla oil, followed by flaxseed oil and GNO. CV of GNO remained low (5.7) up to 10 h of UV exposure, but increased

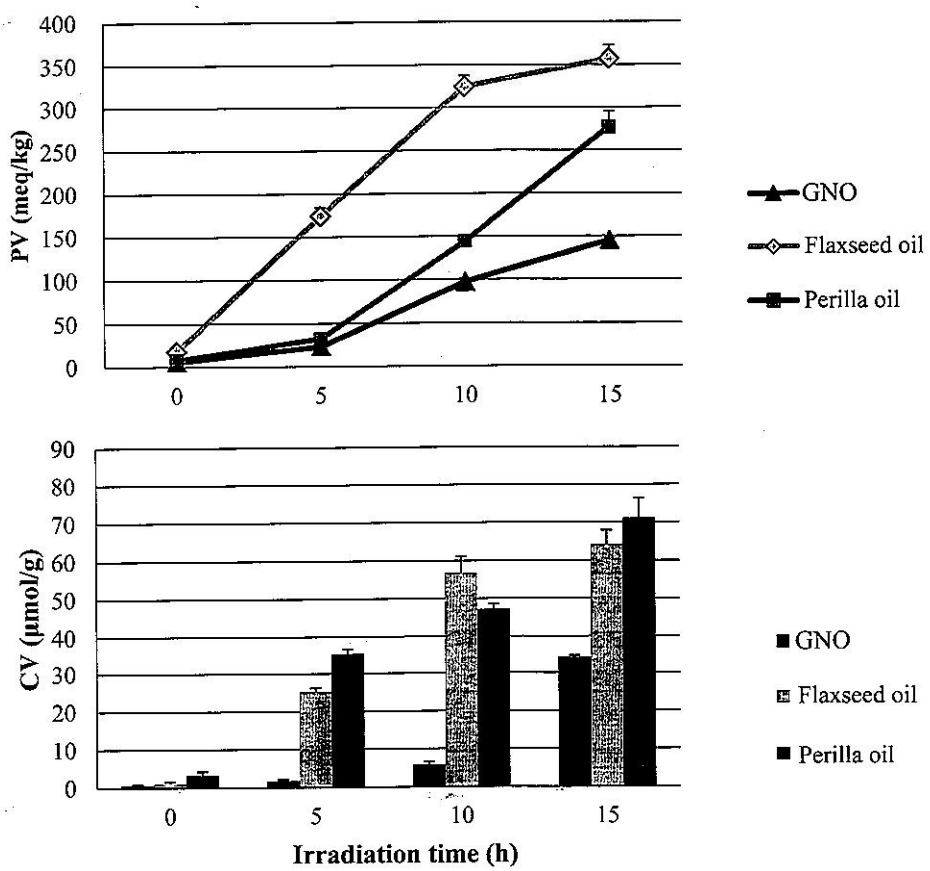

Fig. 2. Changes in peroxide value (PV) and carbonyl value (CV) with UV irradiation. Each oil (12.5 g) was irradiated with UV light (254 nm, intensity of 240 μW/cm^2) in a Petri dish (9 cm in diameter). Data represent mean ± SD (n = 6).

sharply to 34.4 after 15 h of UV exposure, and remained approximately half of the perilla oil value. The CV of GNO exceeded 50 after 20 h of UV exposure. While this value is not sufficiently low in terms of food safety (Kojima, 2007), it appears that GNO remains edible when heat-treated for 10 min at 140°C or less and when exposed to UV light for 5 to 10 h or less.

As chlorophyll promotes photosensitized oxidation, we measured the chlorophyll pigment content of each oil and found chlorophyll pigments were greatest in flaxseed oil. That said, the chlorophyll pigment content of flaxseed oil was low, approximately 1/40 that of olive oil (Endo *et al.*, 1998). Chlorophyll pigment content of GNO was even lower, approximately 1/6 that of flaxseed oil, and only trace amounts of chlorophyll pigments were detected in the perilla oil. Chlorophyll pigments in GNO did not change substantially upon heat-treatment and did not have a sizable impact on oxidation, even after UV exposure.

Changes in fatty acid composition of GNO, flaxseed and perilla oils Table 2 shows changes in fatty acid composition of the three oils upon heat treatment. The tested oils contained a large amount of polyunsaturated fatty acids (PUFAs) and linoleic and α-linolenic acids. Specifically, all oils were rich in α-linolenic acid.

Heat treatment of each oil resulted in decreased PUFAs. The treatment temperature affected the rate of fatty acid decline. In particular, a significant decrease in α-linolenic acid content was observed in flaxseed oil at 120°C, GNO at 140°C, and perilla oil at 160°C. Therefore, heating temperature is an important factor in retaining sufficient levels of α-linolenic acid for nutritional benefit.

Choo *et al.* (2007) reported that in flaxseed oil heat-treated at 150°C for 3 or 6 min, linolenic acid content declined, while that of palmitic acid, stearic acid, oleic acid, and linoleic acid increased. However, in our experiments, all fatty acids decreased in heat-treated oils relative to oils that were not heat-treated. This discrepancy suggests that both heat treatment temperature and heating time influence the changes in fatty acid content. Based on these results, we conclude that, among the three oils, GNO experiences the smallest decline in fatty acid content upon heating, and is therefore the most thermostable.

Table 2. Effect of heat treatment on the fatty acid content of green nut, flaxseed and perilla oils.

Oil	Temperature (°C)	Fatty acid (mg/g oil)				
		16:0	18:0	18:1	18:2	18:3
Green nut	non	30.5 ± 1.2	24.7 ± 2.0	56.6 ± 1.8	211.2 ± 3.4	384.5 ± 9.5
	80	29.7 ± 1.3	25.5 ± 1.7	55.8 ± 1.9	202.7 ± 8.0	368.2 ±13.6
	100	31.5 ± 0.2	26.0 ± 0.2	54.9 ± 0.2	200.0 ± 3.6	353.6 ± 9.4
	120	28.8 ± 1.5	23.7 ± 1.6	53.3 ± 4.0	188.2 ± 5.3	342.5 ± 3.8
	140	28.5 ± 2.7	24.9 ± 0.3	53.0 ± 1.3	187.3 ± 5.3	339.2 ± 5.4*
	160	31.1 ± 3.9	24.2 ± 2.1	54.9 ± 7.7	186.6 ± 22.2	328.3 ±34.6*
	180	26.1 ± 0.8	23.5 ± 2.0	48.7 ± 3.1	175.6 ± 7.9*	320.2 ± 7.0**
Flaxseed	non	28.6 ± 2.1	20.1 ± 2.8	62.1 ± 5.4	101.1 ± 2.4	395.8 ± 18.0
	80	18.7 ± 1.2**	15.6 ± 0.4*	45.4 ± 1.8**	67.0 ± 2.7**	375.0 ± 25.7
	100	20.9 ± 0.2**	15.4 ± 1.4*	50.1 ± 3.3*	80.8 ± 3.7*	326.7 ± 9.8*
	120	17.9 ± 1.2**	14.0 ± 1.0**	45.9 ± 2.8**	74.6 ± 7.9*	301.3 ± 12.8**
	140	17.4 ± 2.0**	12.7 ± 1.8**	42.5 ± 6.6**	72.4 ± 19.0**	294.8 ± 37.4**
	160	20.9 ± 2.5**	13.4 ± 1.7**	46.0 ± 6.8**	70.8 ± 9.8**	295.0 ± 17.2**
	180	23.8 ± 0.6*	15.1 ± 1.1*	47.0 ± 4.6**	67.8 ± 6.2**	255.0 ± 21.1**
Perilla	non	54.8 ± 1.4	20.1 ± 2.8	146.2 ± 3.2	100.1 ± 1.0	573.3 ± 22.0
	80	45.0 ± 0.9**	15.5 ± 1.3	108.5 ± 1.9**	80.2 ± 1.8**	514.7 ± 33.3
	100	42.3 ± 1.0**	13.2 ± 0.7**	107.4 ± 3.6**	85.3 ± 5.6*	531.3 ± 45.4
	120	46.2 ± 2.8**	18.1 ± 2.3	119.7 ± 7.8**	79.0 ± 5.4**	500.5 ± 54.2
	140	45.4 ± 3.1**	17.2 ± 0.9	118.9 ± 10.7**	76.1 ± 8.4**	467.5 ± 45.4*
	160	36.0 ± 2.0**	14.0 ± 1.2**	115.2 ± 13.4**	66.8 ± 8.9**	410.8 ± 30.0**
	180	26.0 ± 0.8**	10.9 ± 3.1**	67.5 ± 1.6**	45.2 ± 2.0**	305.6 ± 32.4**

Sample oils (55 g) were heated in a Teflon-coated frying pan on an electric hob for 10 min.
16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, α-linolenic acid.
Data represent mean ± SD (n = 6). Significant differences were determined by one-way ANOVA and Dunnett's test.
**$p < 0.01$ or *$p < 0.05$ indicate a significant difference from non-heating.

Table 3 shows the changes in fatty acid composition by UV irradiation. In all tested oils, unsaturated fatty acid content tended to decline with increasing UV exposure time. A remarkable reduction was observed in linoleic and α-linolenic acids, similar to the case of heat treatment. While the α-linolenic acid content of GNO and flaxseed oil declined significantly after 10 h of UV exposure, the onset of this decline required longer exposure than with perilla oil. Similarly, a significant decrease in the contents of other fatty acids was observed in the perilla oil samples relative to GNO and flaxseed oil after 5 h of UV exposure. The magnitude of decrease in fatty acid content was smaller for GNO than for the other oils.

Although these observations are qualitative, with increasing UV exposure time, the three oils became darker in color and, although there were differences between these oils, the aroma of each eventually changed from refreshing to unpleasant. This change may be attributable to the progress of oxidation and the increase in acetaldehyde, 2-butanol and other breakdown products resulting from the decomposition of peroxide (Luna *et al.*, 2006). Flaxseed oil and perilla oil, in particular, were characterized by an unpleasant odor after only 5 h of UV exposure.

Although the data are not presented here, when the three oils were exposed to 2000 Lux of fluorescent light under the same conditions as in the UV exposure experiment, it was found that, in terms of changes in PV and CV, 1 wk of fluorescent light exposure corresponded to 5 to 10 h of UV exposure and 2 wk of fluorescent light exposure corresponded to 10 to 15 h of UV exposure.

Tocopherol contents in heated oils Table 4 shows the tocopherol contents of sample oils before and after heat treatment. GNO was rich in γ- and δ-tocopherols, with small amounts of α- and β-tocopherols. Flaxseed and perilla oils also contained tocopherols, but the total tocopherol content was very low compared to that in GNO. When each oil was heated at up to 140°C, degradation of tocopherols was observed. The combined γ- and δ-tocopherol contents of GNO declined by 15 and 40% when GNO was heated at 140°C and 180°C, respectively. Temperature-dependent changes in GNO tocopherol contents were opposite to those of PV, which increased up to 160°C, and CV, which increased with heat treatment temperature. Both δ- and γ-tocopherols have been shown to exhibit antioxidant activity towards fats and oils (Fukuzawa, 1978; Lea and Ward, 1959).

The fact that GNO in our experiment was stable up to 140°C and exhibited greater UV resistance than the other two oils may be related to the 10% lower α-linolenic acid content of GNO, compared to the other two oils, and the presence of γ- and δ-tocopherols. In addition, the observed decline in oxidative stability of GNO after heat treatment at 160°C or higher may be related to the marked decrease in γ-tocopherol upon heat treatment at 140°C or higher. In evaluating the presence of polyphenols, other compounds believed to exhibit antioxidant activity, it was found that the polyphenol contents of GNO and perilla oil were approximately half those of flaxseed oil. That said, the polyphenol content of all three oils was extremely low, and, as such, it is believed that the polyphenols did not contribute substantially to the antioxidant activity observed in this experiment. We will con-

Table 3. Effect of ultraviolet irradiation on the fatty acid content of green nut, flaxseed and perilla oils.

Oil	Irradiation time (h)	Fatty acid (mg/g oil)				
		16:0	18:0	18:1	18:2	18:3
Green nut	0	30.5 ± 1.4	24.7 ± 2.4	56.6 ± 2.2	211.2 ± 4.2	384.5 ± 11.6
	5	32.0 ± 2.4	27.1 ± 4.6	54.8 ± 2.6	200.9 ± 7.9	363.2 ± 13.7
	10	31.6 ± 3.6	26.2 ± 2.1	57.0 ± 0.3	202.3 ± 1.0	358.2 ± 3.6*
	15	26.5 ± 0.5	22.4 ± 0.3	51.1 ± 0.8*	177.6 ± 8.1**	308.7 ± 7.6**
Flaxseed	0	28.6 ± 2.1	20.1 ± 2.8	62.1 ± 5.4	101.2 ± 2.4	395.7 ± 18.0
	5	24.5 ± 0.4	16.4 ± 2.0	55.5 ± 4.5	87.4 ± 4.1	349.3 ± 13.0
	10	21.8 ± 0.6*	15.3 ± 2.0	49.2 ± 3.2	72.2 ± 5.6*	334.6 ± 41.2*
	15	19.3 ± 0.9**	13.3 ± 1.2	43.8 ± 3.8	63.8 ± 3.9**	333.3 ± 6.9*
Perilla	0	54.8 ± 1.4	20.1 ± 2.8	146.2 ± 3.2	100.1 ± 1.0	573.3 ± 22.0
	5	37.5 ± 2.4**	18.3 ± 3.9	101.7 ± 6.7**	63.5 ± 1.7**	414.1 ± 25.8**
	10	31.2 ± 2.3**	16.4 ± 1.0	88.2 ± 3.0**	60.8 ± 1.9**	360.6 ± 27.9**
	15	30.0 ± 1.2**	14.6 ± 1.7*	86.6 ± 3.0**	53.7 ± 7.5**	354.4 ± 25.4**

Each oil (12.5 g) was irradiated by a UV light (254 nm, intensity of 240 μW/cm^2) at 20°C in a Petri dish (9 cm in diameter).
16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, α-linolenic acid.
Data represent mean ± SD (n = 6). **$p < 0.01$ or *$p < 0.05$ indicate a significant difference from non-irradiation.
Significant differences were determined by one-way ANOVA and Dunnett's test.

Table 4. Effect of heat treatment on the stability of endogenous tocopherols in green nut, flaxseed and perilla oils.

Oil	Temperature (°C)	Tocopherol (mg/100 g oil)				
		α	β	γ	δ	Total
Green nut	non	ND	ND	149.0 ± 6.5	60.0 ± 2.2	209.0 ± 5.2
	100	ND	ND	153.0 ± 3.4	62.4 ± 1.0	215.4 ± 2.4
	140	ND	ND	124.0 ± 4.2	63.9 ± 1.8	187.9 ± 5.0
	180	ND	ND	78.1 ± 2.2	52.4 ± 1.7	130.5 ± 4.0
Flaxseed	non	ND	7.4 ± 0.5	19.9 ± 1.0	ND	27.3 ± 1.2
	100	ND	7.2 ± 0.4	19.5 ± 1.0	ND	26.7 ± 1.1
	140	ND	7.2 ± 0.7	18.5 ± 0.6	ND	25.7 ± 0.3
	180	ND	4.6 ± 0.3	12.9 ± 0.6	ND	17.5 ± 0.7
Perilla	non	1.1 ± 0.3	ND	49.1 ± 0.7	ND	50.2 ± 0.7
	100	ND	ND	44.2 ± 3.9	ND	44.2 ± 3.9
	140	ND	ND	40.9 ± 1.4	ND	40.9 ± 1.4
	180	ND	ND	24.7 ± 1.6	ND	24.7 ± 1.6

Sample oils (55 g) were heated in a Teflon-coated frying pan on an electric hob for 10 min.
α, β, γ and δ-tocopherol content was analyzed by high-performance liquid chromatography.
ND, not detected. Data represent mean ± SD (n = 6).

tinue to evaluate the impact of other antioxidant compounds in the future.

Based on the above, it appears that, in contrast to traditional oils rich in n-3 fatty acids, which are not generally heat treated, GNO can tolerate a certain degree of heat processing. Finally, with regard to the long-term storage of the three oils tested, it is necessary to prevent light exposure in order to avoid photooxidation.

Conclusion

Similar to flaxseed and perilla oils, GNO has a relatively high content of the n-3 fatty acid, α-linolenic acid. However, the α-linolenic acid in GNO was found to be more stable than in the other oils, even after heat treatment up to 140°C and 10 h of UV exposure. This stability is believed to be due to GNO's lower α-linolenic acid content and high γ- and δ-tocopherol content, two compounds that exhibit high antioxidant activity with respect to fats and oils. Furthermore, polyphenols are only present in GNO in trace amounts and, thus, are not thought to contribute substantially to the oil's antioxidative properties.

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