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Oxidative stability of chia seed oil and flax seed oil and impact of rosemary (*Rosmarinus officinalis* L.) and garlic (*Allium cepa* L.) extracts on the prevention of lipid oxidation

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Abstract

Chia seed and flax seed oils are rich in polyunsaturated fatty acids, but are susceptible to oxidative deterioration. The aim of this study was to determine the oxidative stability of chia seed and flax seed oils and enhance the stability using rosemary or garlic extracts. During accelerated storage at 65 °C for 14 days, the antioxidant abilities of rosemary or garlic extracts were evaluated and compared with those of butylated hydroxy toluene, ascorbyl palmitate, and α-tocopherol using peroxide value, conjugated dienoic acids, free fatty acid, thiobarbituric acid value analysis. The profile of volatiles, fatty acid composition, and the tocopherol contents in the treated and/or untreated oils were also determined. Active ingredients of rosemary and garlic extracts were also determined. Rosemary extract was found to provide higher oxidative stability than garlic extract after 14 days in most assays (e.g., the CDA values of 4.8% for rosemary extract and 5.2% for garlic extract in chia seed oil). The contents of γ-tocopherol, linoleic acid, and α-linolenic acid were well retained in the functional oils treated with the two extracts. After accelerated storage, the content of the major odor-active volatiles varied based on the type of oil. Our findings show the potential of natural aromatic plant extracts with respect to improving the oxidative stability of functional oils.

Keywords: Rancimat, Accelerated storage, Rancidity, Volatile compound, Peroxide value, Linolenic acid, Tocopherol

Introduction

Flax (*Linum usitatissimum*) seeds and chia (*Salvia hispanica*) seeds are rich sources of α-linolenic acid. Omega-3 fatty acids (e.g., eicosapentaenoic acid (EPA), decosahexaenoic acid (DHA), α-linolenic acid)-rich diet is associated with the prevention of heart diseases. Flax seeds have been widely used as raw materials for production of functional oils. Flax is widely cultivated in more than 50 countries [1]. Meanwhile, chia is native to Mexico and Guatemala [2]. Chia seeds contain the highest proportion of α-linolenic acid (0.6 g/g of oil) among all known vegetable sources [3]. Further, chia seed oil contains essential fatty acids such as α-linolenic acid and linoleic acid, which comprise ~ 80% of the total fatty acid content [4]. Furthermore, chia seed intake has been reported to be associated with significantly reduced levels of serum triglycerides and low-density lipoproteins and increased levels of high-density lipoprotein [5].

However, edible oils with high polyunsaturated fatty acid content are susceptible to oxidative deterioration. Oxidation destroys the essential fatty acids (EFA) and may result in the production of toxic compounds and oxidized polymers. The lipid oxidation increases during thermal treatment. Destruction of EFA occurs at relatively high level during thermal treatment. For example, when heated as mixture at 90 °C for 60 min, only 60% of linoleic acid (LA), 50% linolenic acid (LNA), 30% arachidonic acid (AA), and 20% docosahexaenoic acid

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(DHA) were remained [6]. Free radicals formed from the oxidation of oils can cause reversible or irreversible damage to biological molecules such as DNA, proteins, and/or lipids. The latter can cause cancer, heart disease, and arthritis, and can accelerate the aging of organisms [7]. Additionally, oxidation of oils decreases their appeal because of rancid odor produced due to the generation of odor-active volatiles.

Addition of antioxidants may improve the oxidative stability of oils rich in polyunsaturated fatty acids such as flax seed and chia seed oils. Synthetic antioxidants, such as butylate hydroxyanisole (BHA), butylate hydroxytoluene (BHT), and tertbutyl hydroquinone (TBHQ), are commonly added to edible oils during processing to improve their oxidative stability; however, they could pose some health risks [8]. Therefore, antioxidants from natural sources have received great attention in the context of preventing the lipid oxidation of edible oils [9].

Rosemary (*Rosmarinus officinalis* L.) extracts efficiently prevent lipid oxidation in various oils [8, 10]. For instance, compared with common antioxidants such as α-tocopherol and ascorbyl palmitate, rosemary extract had better antioxidant activity with respect to sunflower oil (based on peroxide and anisidine values) [11]. The antioxidant activity of rosemary extract can be majorly attributed to phenol diterpen, carnosol, and carnosic acid [12]. Furthermore, the extract of garlic bulbs improved the stability of sunflower oil in response to thermal oxidation [8]. The antioxidative property of garlic (*Allium sativum* L.) bulb extract can be attributed to its high organosulfur content [13]. Therefore, the extracts of rosemary and garlic bulb have the potential to prevent the oxidation of flax seed and chia seed oils.

There is little information regarding the oxidative stability and volatile changes in flax seed and chia seed oils during storage. Malcolmson et al. studied the stability of flax seeds in the context of storage, but not that of flax seed oil [14]. Wiesenborn et al. determined the changes in volatile compounds in screw-pressed flax seed oil, but not in regular flax seed oil [15]. Additionally, to our knowledge, there is no study on the oxidative stability of chia seed oil treated with antioxidants during storage.

To address these issues, we investigated the changes in indicators of lipid oxidation, in chia seed and flax seed oils treated with extracts of rosemary or garlic bulb during accelerated storage at 65 °C for 14 days. Moreover, the residual tocopherol contents, fatty acid composition, and volatiles were evaluated. The oxidative stabilities of chia seed and flax seed oils treated with other antioxidants—commonly used in the edible oil industry, i.e., BHT, ascorbyl palmitate, and α-tocopherol—were also determined and compared.

Materials and methods

Chemicals, reagents, and oil samples

Acetic acid, chloroform, 1-butanol, 2-thiobarbituric acid, and other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). α-Tocopherol, γ-tocopherol, n-alkane standards and standards of volatiles such as hexanal, (2E)-2-octenal, (2E)-2-nonenal, benzaldehyde, 2-heptanone, 1-pentanol, 1-hexanol, 1-octen-3-ol, 2-pentylfuran, butanoic acid, hexanoic acid, octanoic acid, nonanoic acid, 3,4-dihydro-2H-pyran, α-pinene, and D-limonene were also purchased from Sigma-Aldrich. Stable isotope standards of n-hexyl-d_{13} alcohol and octanal-d_{16} were obtained from C/D/N Isotopes Inc. (Pointe-Claire, QC, Canada). Rosmarinic acid standard (98% purity) was obtained from Natural Product Institute of Science and Technology (www.nist.re.kr, Anseong, Korea). Nanopure water was prepared using a water purification system (Milli-Q Direct 8, Merck Millipore, Billerica, MA, USA). Cold-pressed chia seed oil was purchased from Organic Pure Foods (Florida, USA), whereas cold-pressed flax seed oil was purchased from Alliga (Richmond, Canada). The oil samples were delivered in air and immediately after purchase, the storage test was performed.

Preparation of rosemary and garlic bulb extracts

Extraction was performed according to the protocol proposed by Wang et al. and Alizadeh et al. with some modifications [16, 17]. First, the freeze-dried rosemary leaves and garlic bulb were ground to powder. The powder was sieved to ensure uniform size, and 80% ethanol was added at a ratio of 1:20 (w/v, g powder/mL solvent). The mixture was sonicated at 50 °C for 30 min in an ultrasonic bath (Branson Ultrasonics Corporation, Danbury, USA), and was then evaporated to remove ethanol using a rotary evaporator (n-1300, EYELA, Tokyo, Japan). The remaining samples were dehydrated by lyophilization and stored at − 80 °C for less than a month until treated to the oil samples. The extraction yield was calculated by dividing extracted amount (g) by used raw material (g). Extraction yield of garlic bulb and rosemary extracts were 38.4% and 39.8%, respectively.

Determination of rosmarinic acid, rosmanol, carnosol, and carnosic acid in the rosemary extract

Rosmarinic acid, rosmanol, carnosol, and carnosic acid in the rosemary were determined using an Agilent 1290 Infinity ultra-high performance liquid chromatography (UHPLC) system coupled to a 6470 triple quadrupole (QqQ) mass spectrometer (Agilent Technologies, Santa Clara, CA) according to Sánchez-Camargo's method with slight modification [18]. They were separated on a Zorbax eclipse plus C_{18} column (2.1 mm × 50 mm, 1.8 μm,

Agilent Technologies). The mobile phases were consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient condition was as follows: 0 min, 5% B; 0.35 min, 70% B; 6.2 min, 95% B; 9 min, 5% B. The flow rate and injection volume were 0.4 mL/min and 10 µL, respectively. Capillary voltage and nebulizer pressure were 4000 V and 30 psi, respectively. Drying gas flow rate and gas temperature were 10 L/min and 300 °C, respectively. Fragment voltage and collision energy were 125 V and 30 V, respectively. The multiple reaction monitoring (MRM) mode was utilized to analyze rosmarinic acid by measuring the area of m/z 360 (precursor ion) to m/z 161 (product ion). Three more transactions were monitored for rosmanol, carnosol, and carnosic acid, and the respective m/z values were as follows: m/z 345 (precursor ion) to m/z 283 (product ion), m/z 329 (precursor ion) to m/z 285 (production), and m/z 331 (precursor ion) to m/z 287 (production). Identification of rosmarinic acid was confirmed using an authentic standard and other compounds were tentatively identified by comparing their mass spectra with literature. The calibration curve of rosmarinic acid was used for relative quantification of rosmarinic acid, rosmanol, carnosol, and carnosic acid.

Determination of organosulfur compounds in garlic bulb extract

Organosulfur compounds in the garlic bulb extract was determined by headspace solid-phase microextraction (HS-SPME) coupled to a gas chromatography apparatus (7890B GC, Agilent Technologies, Santa Clara, CA) employing an Agilent 5975C mass selective detector. One gram of garlic bulb extract was mixed with an internal standard (octanal-d_{16}) in a 20 mL glass headspace vial to analyze the organosulfur compounds in the garlic bulb extract. The final concentration of internal standard was 10 ng/g. The vial was sealed with a magnetic crime cap (Gerstel, Baltimore, MD, USA). Organosulfur compounds of garlic bulb extract were extracted by a 2 cm solid-phase microextraction (SPME) fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, Supelco, Bellefonte, PA) at 40 °C for 30 min after 10 min of equilibration time. Organosulfur compounds in the garlic bulb extract were separated on a DB-WAX capillary column (30 m × 0.25 mm, i.d., 0.25 µm film thickness; Agilent Technology). The initial oven temperature maintained at 40 °C for 2 min. The oven temperature increased to 120 °C at a rate of 4 °C/min and maintained at 120 °C for 5 min. The temperature was increased to 250 °C at a rate of 20 °C/min and held for 5 min. The injection temperature was 250 °C with splitless mode. The carrier gas was helium (99.999% purity) at a rate 1 mL/min. The electron energy was 70 eV. Transfer line, quadrupole, and ion source temperature were 240 °C, 150 °C, and 230 °C, respectively. Mass spectra was obtained by scanning over a range of m/z 30−350 at a rate of 3.06 scans/s. Organosulfur compounds were identified by searching the Wiley 9 and NIST 8 mass spectral libraries with < 80% match score as a cut-off and by comparing their Kovats' retention index (RI). Concentrations were calculated as described previously [19]. Briefly, the extracted ion peak area of each compound was divided by the peak area of the extracted ion peak area of the internal standard. The area ratio was converted to relative concentration of the appropriate internal standard by multiplying by 10 ng IS/g sample. The results were used to report the composition of organosulfur compounds in the garlic extract. Relative concentration was calculated using following equation [20]:

$$\text{Relative concentration}\left(\frac{\text{ng}}{\text{g}}\right) = \frac{\text{extracted ion peak area}}{\text{extracted ion peak area of I.S.}} \times \text{I.S.}\left(10\frac{\text{ng}}{\text{g}}\right) \quad (1)$$

Preparation of chia seed and flax seed oil samples treated with rosemary or garlic bulb extracts

Extracts of rosemary or garlic bulb (final concentration, 1000 mg/kg) were added to the chia seed and flax seed oil samples. AP (ascorbyl palmitate), BHT, and α-tocopherol (final concentrations, 2 mM, 1 mM, and 0.4 mM, respectively) were added to plain chia seed oil or flax seed oil and mixed using a homogenizer (T18 digital ultra turrax, IKA works, Staufen, Germany), according to the guidelines proposed by EFSA (European Food Safety Authority) [21]. Rosemary extract, garlic bulb extract, and AP have been accorded a GRAS (Generally Recognized as Safe) status by the FDA (U.S. Food and Drug Administration) [22]. Plain chia seed oil and flax seed oil were used as control.

Determination of thermal oxidative stability using the rancimat test

The thermal oxidative stability of the oil samples was analyzed using Rancimat 743 (Metrohm, Herisau, Switzerland). The Rancimat assay was performed according to the protocol proposed by Yang [23]. Three grams each of untreated and treated (with antioxidants) chia seed oil and flax seed oil were accurately weighed into each reaction vessel. The target temperature and airflow rate were set at 120 °C and 20 L/h, respectively. The results were expressed in induction period (IP), which was automatically determined based on the inflection

point of the curve using the 743 Rancimat software 1.1 supplied by the company. Each experiment was done in triplicate.

Determination of storage stability using the schaal oven test

Schaal oven test was used as an accelerated stability test of chia seed and flax seed oils. In Schaal oven test, the fatty food sample is usually stored in an oven maintained at ~ 65 °C. During storage, lipid oxidation indicator values (e.g., peroxide value) and volatiles by GC have been often analyzed for oxidative stability test of oils.

Experimental conditions were established based on those recommended by Aladedunye and Matthäus [24], and treated and untreated samples (5.0 g each) were placed in a 20-mL amber borosilicate glass sample vial (Restek Corporation, Bellefonte, PA, USA). Nitrogen gas was flushed into the headspace, and the vials were capped. The samples were stored at 65 °C for 14 days, and oxidative stability was measured after 0, 1, 3, 5, 7, and 14 days. For each sample, different vials were used, and no vial was repeatedly sampled during storage. The Scchaal oven test was performed three times.

Determination of changes in lipid oxidation indicator values

The peroxide value (POV) of the oil samples stored under accelerated storage was determined based on the AOCS method Cd 8–53 [25]. Conjugated dienoic acids (CDA) were determined based on the AOCS method Ti 1a-64 [25], whereas the free fatty acids (FFA) were analyzed by the AOCS method Aa 6–38 [25]. Thiobarbituric acid (TBA) value was determined by the AOCS method Cd 19–90 [25].

Analysis of the fatty acid composition using GC–MS

Fatty acid composition of the chia seed and flax seed oil samples was determined in accordance with the AOCS Official Method Ce 1 k-09 [25] after minor modifications. Briefly, 1.5 mL of 0.5 N methanolic NaOH was added to 25 mg oil and heated at 100 °C for 10 min. After cooling, the fatty acids were derivatized to fatty acid methyl esters (FAME) using 14% boron trifluoride methanol solution and heated again. Before methylation, triundecanoic acid was added as the internal standard (100 mg/L). Then, isooctane (2 mL) and saturated NaCl (1 mL) were added. After centrifugation for 15 min at 9000*g*, the supernatant in the isooctane layer was collected. Anhydrous sodium sulfate was used to remove residual water.

Fatty acid composition was analyzed using Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA) coupled to Agilent 5977B mass selective detector (Agilent Technologies). The FAME were separated on a DB-wax capillary column (30 m × 0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Santa Clara, USA). Injection volume of 1 µL, split ratio of 1:5, and injection temperature of 250 °C were employed. The GC oven was operated as follows: 50 °C for 1 min, increased to 200 °C at a rate of 25 °C/min and maintained for 3 min, increased to 230 °C at a rate of 3 °C/min (final temperature) and maintained for 18 min. The equilibration time was 5 min. The flow rate of He (99.999%) was 1.0 mL/min. The scan rate and electron energy were 2.9 scans/s and 70 eV, respectively. The transfer line temperature, quadrupole temperature, and electron impact ionization source were 250 °C, 150 °C, and 230 °C, respectively. The scan range was m/z 45–350. The identification of fatty acids was confirmed using the retention times and mass spectra of the standards of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 cis 9), linoleic acid (C18:2 cis 9,12), and α-linolenic acid (C18:3 cis 9, 12, 15) methyl esters. Fatty acid composition was expressed as % fatty acids of the oils [26].

Analysis of the tocopherol contents using HPLC

Tocopherol contents of the treated and untreated oil samples was determined in according to the method of Zou et al. [27]. Briefly, 1.0 g of the sample was mixed with 10 mL n-hexane and filtered through a 0.22 µm filter. Tocopherol content was determined using a high performance liquid chromatography system (Agilent 1260 Infinity II LC system, Agilent, Santa Clara, CA, USA) equipped with Agilent 1200 series VWD detector. Tocopherol was separated on a Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 µm, Agilent Technologies). Mobile phase was 98% aqueous methanol (v/v). The flow rate was 0.8 mL/min and injection volume was 10 µL. Tocopherol was monitored at 292 nm of wavelength. Quantification of α-and γ-tocopherol was achieved using external calibration curves of the authentic standards. Limit of detection (LOD) and limit of quantification (LOQ) of α-tocopherol were 3.3 µg/g and 9.8 µg/g, respectively. LOD and LOQ of γ-tocopherol were 3.3 µg/g and 9.9 µg/g, respectively.

Analysis of changes in volatile compounds in the oil samples by HS-SPME/GC–MS

The volatiles present in the oil samples were analyzed using headspace solid-phase microextraction (SPME) coupled to a gas chromatography apparatus (7890B GC, Agilent Technologies, Santa Clara, CA) employing an Agilent 5977B mass selective detector. After equilibration for 5 min at 60 °C, SPME fiber (2-cm-long DVB/Car/PDMS fiber; Supelco, St. Louis, MO, USA) was exposed for 30 min into the headspace vial. DVB/Car/PDMS SPME fiber is generally used for analysis of flavor

compounds including volatiles and semi-volatiles for C3-C20 (molecular weight of 40–275). Then, the fiber was desorbed for 3 min in a gas chromatograph injection port. Samples were prepared in duplicate (n = 2). Because the Scchaal oven test sampling was performed three times, the 6 different samples were injected per storage point.

A DB-Wax capillary column (30 m length × 0.25 mm inner diameter (i.d.), 0.25 μm film thickness; Agilent Technologies) was used to separate the volatiles. The temperature of the injection port was 260 °C, and the splitless mode was employed. A 0.75-mm i.d. inlet liner (Agilent Technologies) was used. The GC oven was operated as follows: 40 °C for 2 min, increased to 120 °C at a rate of 4 °C/min and kept for 5 min, and finally increased to 250 °C at a rate of 20 °C/min and maintained for 5 min. The flow rate of He (99.999%) was 1.0 mL/min. The electron impact ionization source temperature, quadrupole temperature, and transfer line temperature were set as 230 °C, 150 °C, and 280 °C, respectively. The electron energy was 70 eV. The volatiles were scanned at a range of m/z 29–400 at 3.06 scans/s.

Volatiles were identified by comparing their mass spectra and retention times with those of the standards, if available. Those for which standards were not available were tentatively identified by searching the Wiley 9 and NIST 8 mass spectral libraries with < 80% match score as a cut-off and/or by comparing their Kovats' retention index (RI). Volatile concentrations were calculated as described previously [19]. Briefly, the extracted ion peak area of each compound was divided by the peak area of the extracted ion peak area of an internal standard (n-hexyl-d_{13} alcohol for alcohol, acid, furan and other compounds; octanal-d_{16} for aldehyde and ketone compounds). The area ratio was converted to relative concentration of the appropriate internal standard by multiplying by 10 ng IS/g sample. The results were used to compare the composition of volatiles in the oil samples. Relative concentration was calculated using equation (1).

Statistical analysis

All experiments were performed in triplicate. The results are depicted in the form of average and standard deviation. Statistical analysis was conducted using IBM SPSS statistics software 23 (v. 23.0, SPSS, Inc., Chicago, IL). Multivariate analysis of variance was performed to evaluate the effect of antioxidant type, storage time, and antioxidant type × storage interaction on the oxidative stabilities of antioxidant-treated chia seed oil and flax seed oils. Significant differences among the oil samples were determined using Student's t-test or one-way ANOVA followed by a post-hoc test, the Duncan's multiple range test, at $p < 0.05$.

Results and discussion

Determination of rosmarinic acid, rosmanol, carnosol, and carnosic acid in the rosemary extract and organosulfur compounds in the garlic bulb extract

Rosmarinic acid, rosmanol, carnosol, and carnosic acid in the rosemary extract were determined using an UHPLC-QqQ and (Table 1). Sum of the four compound amounts were 19.4 mg/g DW in the rosemary extract. Carnosic acid (8.1 mg/g DW) and carnosol (8.6 mg/g DW) showed higher levels than rosmarinic acid and rosmanol in the rosemary extract. This agrees with previous studies [18, 28]. Bioavailability of carnosol has not investigated in humans; however, bioavailability of carnosic acid intragastrically at 90 mg/kg was reported as 65.09% [29].

Organosulfur compounds in the garlic bulb extract were identified/quantified using a HS-SPME/GC–MS (Table 2). Five organosulfur (i.e., allyl sulfide, 3,4-dimethylthiophene, methyl 1-propenyl disulfide, dimethyl trisulfide, and diallyl trisulfide) were identified in the garlic bulb extract. Sum of the organosulfur compound contents were 205.0 ng/g DW in the garlic bulb extract. The major organosulfur compound in the garlic bulb extract was diallyl trisulfide (186.9 ng/g). This result was corresponded with a previous study [30].

Table 1 Contents of rosmarinic acid, rosmanol, carnosol, and carnosic acid in rosemary extract

Compound	RT[a] (min)	Molecular formula	MRM[b] transition (m/z)	Concentration (mg/g DW)
Rosmarinic acid	2.3	$C_{18}H_{16}O_8$	360 → 161	2.3 ± 0.4
Rosmanol	3.1	$C_{20}H_{26}O_5$	345 → 283	0.5 ± 0.0
Carnosol	3.9	$C_{20}H_{26}O_4$	329 → 285	8.1 ± 0.5
Carnosic acid	4.3	$C_{20}H_{28}O_4$	331 → 287	8.6 ± 0.2

[a] RT means retention time

[b] MRM refers to multiple reaction monitoring

Table 2 Organosulfur compound composition in garlic bulb extract

Compound	Extract ion[a]	Unknown RI[b]	Literature RI	Concentration (ng/g DW)
Allyl sulfide	*45*, 41, 73	1153	1148	14.4 ± 0.4
3,4-Dimethylthiophene	*111*, 112, 97	1256	1250	9.6 ± 0.5
Methyl 1-propenyl disulfide	*73*, 120, 79	1284	1292	4.4 ± 0.2
Dimethyl trisulfide	*126*, 45, 79	1356	1362	19.7 ± 0.6
Diallyl trisulfide	*41*, 73, 113	1773	1773	186.9 ± 40.2

[a] Italic letters mean quantifier ion

[b] RI refers to retention index

Table 3 Induction period times (h) of untreated and antioxidant-treated chia seed oil and flax seed oil as determined by Rancimat tests

Antioxidants	Chia seed oil	Flax seed oil
Control	0.43 ± 0.01 d	0.37 ± 0.02 D
BHT[a]	1.41 ± 0.07 a	1.62 ± 0.25 A
α-Tocopherol	0.54 ± 0.06 d	0.53 ± 0.06 CD
Ascorbyl palmitate	1.21 ± 0.13 b	1.16 ± 0.20 B
Garlic extract	1.16 ± 0.22 c	1.07 ± 0.18 C
Rosemary extract	1.30 ± 0.06 ab	1.17 ± 0.20 B

[a] BHT refer to butylate hydroxytoluene. Mean values followed by different lowercase letters indicate significant differences in the induction period times (h) within chia seed oil at $p < 0.05$. Mean values followed by different capital letters indicate significant differences in the induction period time within flax seed oil at $p < 0.05$

Thermal oxidative stability using the rancimat test

Initially, seven single antioxidants, namely ascorbyl palmitate (AP), catechin, chitosan, collagen, quercetin, resveratrol, and eight plant extracts, namely extracts of black tea (*Camellia sinensis*), garlic bulbs, green tea (*Camellia sinensis*), lemon balm (*Melissa officinalis*), oregano (*Origanum vulgare*), onion (*Allium cepa* L.) skin, rosemary, and thyme (*Thymus vulgaris*) were added to the chia seed oil, and Rancimat tests were performed. The extracts of rosemary and garlic bulbs showed the highest antioxidant abilities among the plant extracts, whereas AP showed the highest antioxidant power among the single antioxidants. Thus, these were chosen for further investigations.

Rancimat tests were performed to compare the abilities of the antioxidants with respect to improving the thermal oxidative stability of chia seed and flax seed oils (Table 3). Higher oxygen induction time (IT) means better thermal oxidative stability. Oil samples without antioxidants (i.e., control) showed lower IT compared with antioxidant-added oils, and the chia seed and flax seed oils showed IT in the range of 0.37–0.43 h. The IT varied significantly depending on the type of antioxidant used ($p < 0.05$). For chia seed oil samples, BHT and rosemary extract showed the highest antioxidant power ($p < 0.05$). BHT also showed the highest antioxidant power for flax seed oil followed by rosemary extract and AP ($p < 0.05$). In both oils, α-tocopherol showed the lowest antioxidant activity among the tested antioxidants ($p < 0.05$).

Oxidative stability during accelerated storage

Lipid oxidation indicator values

Lipid oxidation indicator values during the accelerated storage of chia seed and flax seed oils after antioxidant treatments are shown in Fig. 1. The multivariate analysis of confirmed the effects of antioxidant type and storage time as well as two-way interactions between antioxidant type and storage time variance for all of the lipid oxidation indicator values (i.e., CDA, POV, FFA, and TBA) ($p < 0.05$). The addition of antioxidants to chia seed and flax seed oils resulted in lowered lipid oxidation indicator values than untreated oils, with the various rates depending on the antioxidant type at different time points.

Figure 1a, b present the changes in the CDA values of fresh chia seed oil (3.1%) and flax seed oil (3.4%), respectively at day 0. These CDA values increased significantly as the storage period was increased ($p < 0.05$). Particularly, between day 5 and 7 of storage days, the CDA values increased rapidly for both oils. At day 14, highest CDA values (range of 5.6 − 6.0%) were achieved for flax seed oil and chia seed oil. CDA value is an indicator of early lipid oxidation. The addition of antioxidants to chia seed and flax seed oils resulted in lowered CDA than untreated oils, with the various rates depending on the antioxidant type. At day 14, chia seed oil and flax seed oil showed the lowest CDA values of 4.8% and 5.0%, respectively, after the addition of rosemary extract and followed by garlic extract. The values were significantly lower than those observed upon using BHT and α-tocopherol, which are the commonly used antioxidants in the edible oil industry ($p < 0.05$). Thus, the extracts of rosemary and garlic displayed more effective potential with respect to preventing initial oxidation than BHT and α-tocopherol.

POV changes in the oil samples during the 14 days of storage are shown in Fig. 1c, d. This is especially important as POV is widely used as an indicator of early

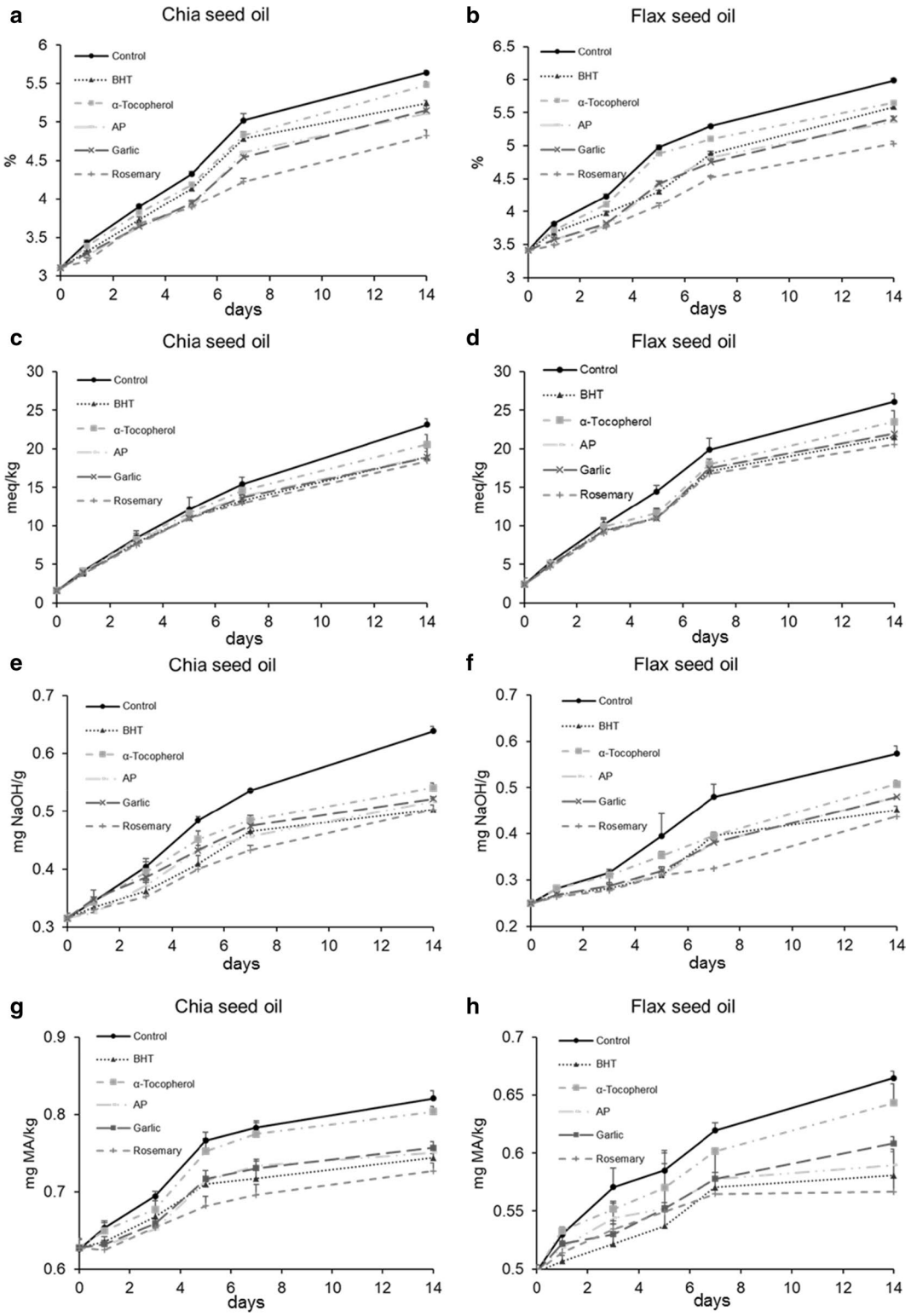

Fig. 1 Oxidative stabilities of antioxidant-treated chia seed oil and flax seed oil during accelerated storage and based on the conjugated dieneoic acid values of (**a**) chia seed oil and (**b**) flax seed oil; peroxide values of (**c**) chia seed oil and (**d**) flax seed oil; free fatty acid values of (**e**) chia seed oil and (**f**) flax seed oil; thiobarbituric acid values of (**g**) chia seed oil, and (**h**) flax seed oil

rancidity of oils. The POVs of chia seed oil and flax seed oil increased significantly with an increase in the storage period ($p < 0.05$). The POV of chia seed oil (control) showed 14.4-fold increase from 1.6 to 23.1 meq/kg after 14 days of accelerated storage, and that of the flax seed oil (control) increased 8.3 times. The addition of antioxidants significantly lowered the POVs of both chia seed and flax seed oils ($p < 0.05$). However, there was no significant difference among the antioxidant-treated chia seed and flax seed oils. After 3 days of accelerated storage, the POVs were under limit peroxide value of edible oils (10 meq/kg according to Codex Alimentarius recommended value). There were no significant differences among antioxidant-treated oils after 3 days of storage ($p > 0.05$). Therefore, adding natural antioxidants, such as rosemary and garlic extracts, will help prevent the development of rancidity in chia seed oil and flax seed oil to a similar degree of BHT based on POV data.

FFA values are commonly used as a secondary indicator of lipid oxidation during the storage of fats and oils. Triglycerides often get hydrolyzed during storage, and FFAs get dissociated from the glycerol backbone. Figure 1e, f present the changes in FFA values of the oil samples during accelerated storage, and show significant increases during accelerated storage ($p < 0.05$). After 14 days, chia seed oil and flax seed oil had the highest FFA values: 0.64 and 0.57 mg NaOH/g, respectively. Antioxidant-treated oil samples showed significantly lower FFA values than the controls, regardless of the antioxidant type ($p < 0.05$). There was no significant difference between the FFA values of rosemary extract and BHT-treated chia seed oil samples. Meanwhile, the rosemary extract-treated flax seed oil had the lowest FFA value ($p < 0.05$), even lower than that of BHT. Thus, rosemary extract seems to prevent the oxidation of chia seed and flax seed oils more effectively than BHT, or at least, to a similar degree based on FFA data.

Changes in TBA of the oil samples are shown in Fig. 1g, h. TBA assays measure the various aldehyde products produced during lipid peroxidation, particularly malondialdehyde. Hydroperoxide, the initial oxidation product of polyunsaturated fatty acids, reacts with oxygen to form malondialdehyde, a secondary oxidation product. The TBA value of chia seed oil was significantly increased from 0.63 to 0.78 mg MA/kg after 14 days of accelerated storage, whereas that of the flax seed oil was significantly increased from 0.50 to 0.62 mg MA/kg ($p < 0.05$). The TBA values of the antioxidant-treated oil were lower than those of the control, depending on the antioxidant type. The addition of rosemary extract resulted in the lowest TBA value in chia seed oil samples, followed by BHT ($p < 0.05$). In the case of flax seed oil, rosemary extract, AP, and BHT showed lower TBA values than α-tocopherol ($p < 0.05$).

In summary, based on the CDA, POV, FFA, and TBA data, the oxidative stability of chia seed and flax seed oils was enhanced upon using rosemary and garlic extracts with respect to that of untreated oils. The antioxidant activity of rosemary extract can be majorly attributed to carnosol, carnosic acid, and rosmarinic acid [31] and that of garlic extract can be attributed to its high organosulfur (e.g., diallyl sulphide) content [13]. In most assays, antioxidants from plant sources, i.e., rosemary and garlic extract, showed similar or better performance than the antioxidants commonly used in the edible oil industry, i.e., BHT and α-tocopherol. In bulk oil, polar antioxidants are more protective in lipid oxidation by being oriented in the air-oil interface compared to nonpolar antioxidants because nonpolar antioxidants are remained in the oil phase as mentioned in polar paradox [32]; thus, carnosic acid and rosmarinic acid present in the rosemary extract may play an important role to protect against lipid oxidation of the oils. In oils rich in long chain-fatty acids, high γ-tocopherol content and low α-tocopherol show synergetic antioxidant effect with carnosic acid rich in rosemary extract [33]. Thus, polar antioxidants in rosemary extract and garlic extract may have synergetic effect with endogenous γ-tocopherol in the chia seed and flax seed oils.

Fatty acid composition

The results of the fatty acid composition analysis using GC–MS are shown in Fig. 2. Palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), and α-linolenic acid (C18:3) were the fatty acids found in chia seed and flax seed oils. α-Linolenic acid was the major polyunsaturated fatty acid and its contents in chia seed oil and flax seed oil were 54.1% and 44.1%, respectively. In accordance with our result, the α-linolenic acid content of chia seed oil and flax seed oil was reported to be as high as 52.0% to 63.4% and 39.9% to 60.4%, respectively [34, 35]. Chia seed and flax seed oils showed omega-6 (α-linolenic acid)/omega-3 (linoleic acid) ratio of 2.5. Essential omega-6/omega-3 ratio of 5 is recommended by EFSA [36]; however, the ratio of omega-6/omega-3 in western diet is 15–17 [37]. Chia seed and flax seed oils showed lower ratio of omega-6/omega-3 fatty acids compared to soybean oil (7.4:1) and palm oil (13.1:1).

Linolenic acid content in the control decreased from 54.1% to 53.0% for chia seed oil and from 44.1% to 42.2% for flax seed oil at day 14 (compared with day 0). Chia seed and flax seed oil samples treated with antioxidants showed decreased loss of linolenic acid content, compared to that in the control. Particularly, the addition of

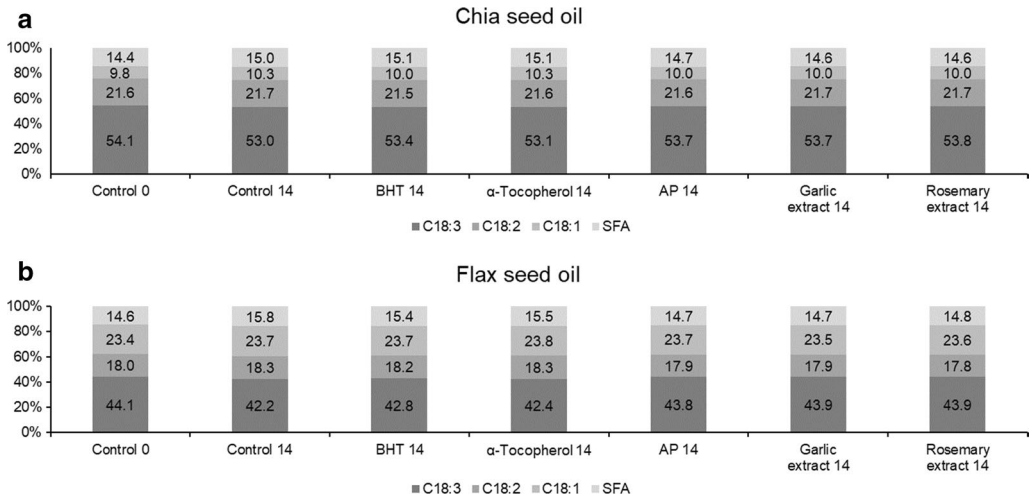

Fig. 2 Changes in fatty acid composition (%) of (**a**) chia seed oil and (**b**) flax seed oil treated with antioxidants at day 0 and day 14 of accelerated storage. The numbers of 0 and 14 refer to day 0 and day 14 of accelerated storage, respectively

rosemary or garlic bulb extracts resulted in decreased loss of linolenic acid content compared to that observed on using AP, BHT, or α-tocopherol.

Tocopherol contents

The results of tocopherol analysis using HPLC are shown in Fig. 3a, b. γ-Tocopherol was the major tocopherol in both chia seed and flax seed oils, which is consistent with the reports (422 and 520 μg/g, respectively) by Schwartz et al. [38] and Kulczyński et al.[39]. After 14 day storage, the γ-tocopherol content in the controls was significantly decreased by 14–17% (from 358 to 298 μg/g in chia seed oil; from 468 to 402 μg/g in flax seed oil) ($p < 0.05$), possibly because endogenous γ-tocopherol was utilized as an antioxidant.

After 14 days of storage, the γ-tocopherol content was significantly higher in the antioxidant-treated oils compared with that in the control ($p < 0.05$), indicating that

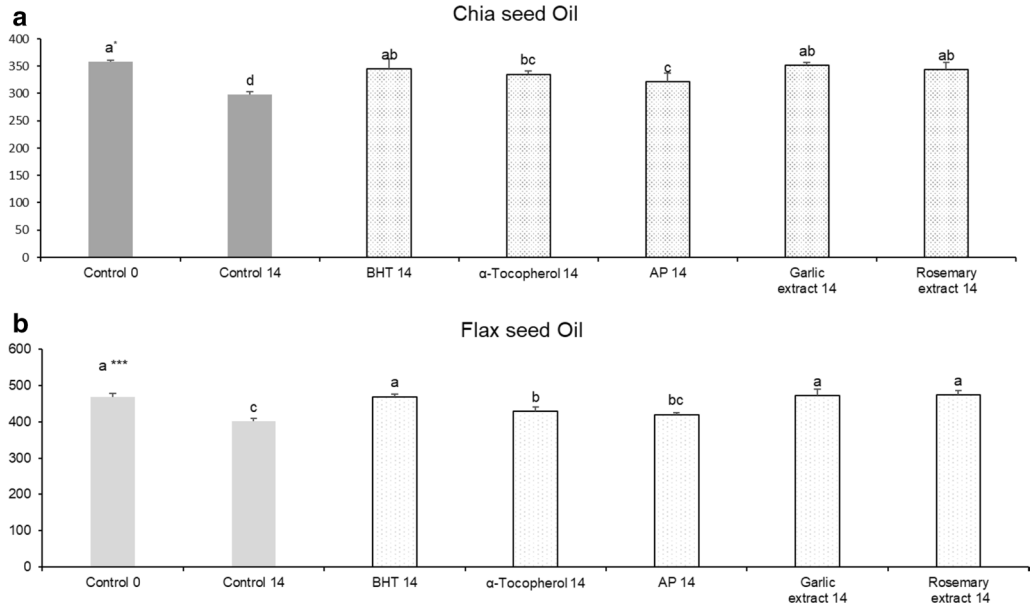

Fig. 3 γ-Tocopherol content (μg/g) in untreated (control) and antioxidant-treated (**a**) chia seed oil and (**b**) flax seed oil at day 0 and day 14 of accelerated storage. The letters indicate significant differences at $p < 0.05$ between antioxidants used in chia seed oil or flax seed oil

γ-tocopherol was not utilized as an antioxidant in the presence of other antioxidants. Moreover, the observed preservation of γ-tocopherol content was not significantly altered upon the addition of natural antioxidants, such as rosemary (344 and 474 μg/g in rosemary extract-treated chia seed and flax seed oil, respectively) and garlic extracts (351 and 472 μg/g in garlic extract-treated chia seed and flax seed oil, respectively), compared with that of BHT (345 and 469 μg/g in BHT-treated chia seed and flax seed oil, respectively) in both oils ($p > 0.05$). The preserved γ-tocopherol contents were not significantly different from fresh control samples at day 0 ($p > 0.05$). Extracts of rosemary and garlic retained the γ-tocopherol content, which can further prevent lipid oxidation. Thus, the oils treated with rosemary or garlic extracts may prolong shelf-lives. After 14 days of storage, α-tocopherol was not detected in all oils including α-tocopherol treated oils. It is likely that α-tocopherol was utilized as an antioxidant during accelerated storage; thus, it was not remained after accelerated storage.

The profiles of volatiles in fresh and stored oil samples using HS-SPME/GC–MS

There is little information regarding the changes in volatile compounds in flax seed oil, and to our knowledge there is no study regarding the volatiles of chia seed oil. Thus, herein, the profiles of plain chia seed and flax seed oil samples (untreated with antioxidants) were analyzed during accelerated storage (Tables 4–5, and Figs. 4,5).

Volatile compound identification

Representative GC chromatograms are shown in Fig. 4. A total of 34 volatile compounds, including 10 aldehydes, 3 ketones, 5 alcohols, 5 acids, 3 furans, and 8 additional compounds, were identified in fresh and stored chia seed oil and flax seed oil, and have been listed in Table 4. Aldehydes, ketones, alcohols, dienes, and acids are commonly formed volatiles in stored edible oils; these are responsible for unpleasant rancid odors and reduced shelf-life [40]. The presence of hexanal, nonanal, 2-octanal, 1-hexanol, hexanoic acid, octanoic acid, and α-pinene were confirmed by comparing with the standards.

Twenty volatiles, including hexanal, nonanal, 2,4-heptadienal, (2Z)-heptenal, 1-hexanol, hexanoic acid, 3-octen-2-one, and 2-pentylfuran were commonly observed in fresh and/or stored chia seed oil and flax seed oil samples. However, 2-pentanal, 2-octanal, (2E,4E)-decadienal, butanoic acid, nonanoic acid, 3-ethylfuran, and β-sabinene were only found in fresh and/or stored chia seed oil. Decanal, (2E)-nonenal, (2E)-decenal, (3E,5E)-octadien-2-one, 1-(4-ethylphenyl) ethanone, (2Z)-2-penten-1-ol, butanoic acid, nonanoic acid, and 5-pentyldihydro-2(3H)-furanone were only detected in fresh and/or stored flax seed oil. Additionally, various terpenes such as limonene were identified in both oils, which are the characteristic odor-active volatiles in chia seeds and flax seeds [41, 42]. Limonene is a consumer's favorite aromatic substance that is often added to detergents and foods.

Stored chia seed and flax seed oils (day 14) contained more volatiles than fresh oils (day 0). For instance, (2E, 4E)-decadienal and 1-(4-ethylphenyl) ethanone were newly formed after the storage of chia seed oil, whereas (2E)-decenal was newly formed after storage of flax seed oil. At day 14, strong rancid odor was detected in both samples, and therefore, the storage test was stopped. The rancid odor may be caused by the formation of lipid oxidation-related volatiles.

Volatile compound quantification

The total volatile content in the chia seed oil and flax seed oil significantly increased during accelerated storage ($p < 0.05$) and is shown in Table 5. In particular, aldehydes, ketones, alcohols, furans, and acids either were newly formed or increased in concentration. However, the contents of some compounds such as terpenes decreased significantly after storage ($p < 0.05$).

The major volatile compounds vary in different oxidized unsaturated fatty acids. For example, with autoxidation of oleic acid, nonanal is produced from the 9- or 10-hydroperoxide; octanal and heptane from the 11-hydroperoxide; decanal and 2-undecenal from the 8-hydroperoxide; octane from the 10-hydrioperoxide [43]. Autoxidized linoleic acid derived volatiles include 2,4-decadienal, methyl octanoate, 3-nonenal, and 9-oxononanoate produced by cleavage on the 9-hydroperoxide; hexanal, pentane, 1-pentanol, and pentanal from the 13-hydroperoxide; 2-heptenal from the 12-hydroperoxide [43]. However, autoxidized linolenic acid produce decatrienal, methyl octanoate from the 9-hydroperoxide; 2,4-heptadienal from the 12-hydroperoxide; 3-hexenal and 2-pentenal from the 13-hydroperoxide; propanol and ethane from the 16-hydroperoxide [43]. The major volatile compounds reported in oxidized linoleic acid-rich oil are hexanal, 2-butane, heptanal, 2-heptenal, (2E)-octenal, nonanal, 2-decenal, 1-hexanol, and hexanoic acid [44–46]. Additionally, nonanal and 2,4-heptadienal are representative lipid oxidation products of oleic and linolenic acids [47]. 2-pentylfuran is an oxidation product of linoleic and linolenic acids [48]. Changes in selected lipid oxidation-related volatiles found in chia seed and flax seed oils during accelerated storage are shown in Fig. 5. Hexanal, nonanal, 2,4-heptadienal, (Z)-2-heptenal, 1-hexanol, hexanoic acid, 3-octen-2-one, and 2-pentylfuran were selected because they are reported as representative lipid oxidation volatiles and exhibited

Table 4 Identified volatile compounds in chia seed and flax seed oils during accelerated storage

No	Volatile compound	Measured RI	Literature RI[a]	Extracted ion[b]	Internal standard	Chia seed oil	Flax seed oil
	Aldehydes						
3[d]	Hexanal	1080	1080	44	octanal-d16	O[c]	O
5	2-Pentenal	1119	1073	55	octanal-d16	O	
14	(2Z)-Heptanal	1316	1318	83	octanal-d16	O	O
17[d]	Nonanal	1387	1387	98	octanal-d16	O	O
19[d]	2-Octanal	1420	1421	70	octanal-d16	O	
21	(2E,4E)-Heptadienal	1457	1457	81	octanal-d16	O	O
22	Decanal	1490	1490	73	octanal-d16		O
24	(2E)-Nonenal	1529	1529	55	octanal-d16		O
27	(2E)-Decenal	1695	1682	55	octanal-d16		O
28	(2E,4E)-Decadienal	1800	1800	81	octanal-d16	O	
	Ketones						
18	3-Octen-2-one	1394	1394	126	octanal-d16	O	O
23	(3E,5E)-Octadien-2-one	1510	1521	95	octanal-d16	O	O
29	1-(4-Ethylphenyl)ethanone	1821	1867	79	octanal-d16		O
	Alcohols						
9	1-Penten-3-ol	1163	1162	56	hexyl-d13 alcohol	O	O
10	2-Methyl-3-pentanol	1165	1167	59	hexyl-d13 alcohol	O	
15	(2Z)-Penten-1-ol	1321	1321	57	hexyl-d13 alcohol		O
16[d]	1-Hexanol	1355	1355	56	hexyl-d13 alcohol	O	O
20	1-Octen-3-ol	1451	1451	57	hexyl-d13 alcohol	O	O
	Acids						
25	Propanoic acid	1533	1534	74	hexyl-d13 alcohol	O	O
26	Butanoic acid	1665	1663	60	hexyl-d13 alcohol	O	
30[d]	Hexanoic acid	1849	1849	73	hexyl-d13 alcohol	O	O
33[d]	Octanoic acid	2067	2070	87	hexyl-d13 alcohol	O	O
34	Nonanoic acid	2100	2116	60	hexyl-d13 alcohol	O	
	Furans						
1	2-Ethylfuran	957	960	71	hexyl-d13 alcohol	O	O
12	2-Pentylfuran	1228	1228	138	hexyl-d13 alcohol	O	O
32	5-Pentyldihydro-2(3H)-furanone	2034	2035	57	hexyl-d13 alcohol		O
	Additional compounds						
2[d]	α-Pinene	1019	1019	93	hexyl-d13 alcohol	O	O
4	β-Sabinene	1113	1113	93	hexyl-d13 alcohol	O	
6	β -Pinene	1123	1125	93	hexyl-d13 alcohol	O	O
7	o-Xylene	1127	1142	91	hexyl-d13 alcohol	O	O
8	β-Myrcene	1160	1160	93	hexyl-d13 alcohol	O	O
11	D-Limonene	1189	1189	68	hexyl-d13 alcohol	O	O
13	p-Cymene	1264	1264	119	hexyl-d13 alcohol	O	O
31	Phenol	1989	2002	94	hexyl-d13 alcohol	O	O

[a] RI indicates retention index. RI values were obtained from https://www.nist.gov.or.flavornet.org

[b] Extracted ion from total ion scan was used for quantitation

[c] The circle represents presence of the volatile compound

[d] Verified using a standard. Other volatiles were tentatively identified by searching the Wiley 9 and NIST 8 mass spectral libraries with < 80% match score as a cut-off and/or by comparing their Kovats' retention index (RI)

Table 5 Changes in the relative concentrations of odor-active volatiles in chia seed and flax seed oils during accelerated storage

No	Volatile compounds	Chia seed oil				Flax seed oil			
		Day 0	Day 3	Day 7	Day 14	Day 0	Day 3	Day 7	Day 14
	Aldehydes								
3*	Hexanal	4.74 ± 0.32 d	8.91 ± 0.63 c	15.02 ± 0.25 b	16.36 ± 0.58 a	1.78 ± 0.22 D	7.48 ± 0.32 C	10.31 ± 0.18 B	16.08 ± 0.32 A
5	2-Pentenal	0.40 ± 0.09 d	1.72 ± 0.12 c	2.44 ± 0.33 b	4.95 ± 0.35 a	n.d.[a]			
14	(2Z)-Heptanal	0.25 ± 0.02 d	0.63 ± 0.12 c	1.30 ± 0.12 b	1.51 ± 0.20 a	0.06 ± 0.01 D	0.12 ± 0.02 C	0.75 ± 0.09 B	1.32 ± 0.12 A
17*	Nonanal	0.04 ± 0.01 b	0.04 ± 0.01 b	0.05 ± 0.00 b	0.18 ± 0.03 a	0.15 ± 0.01 C	0.12 ± 0.06 C	0.31 ± 0.08 B	0.79 ± 0.03 A
19*	2-Octanal	0.25 ± 0.06 d	0.73 ± 0.11 c	1.26 ± 0.28 a	1.03 ± 0.08 b	n.d			
21	(2E,4E)-Heptadienal	20.21 ± 0.25 d	29.32 ± 0.15 c	55.15 ± 0.12 b	79.91 ± 0.32 a	1.59 ± 0.29 D	17.79 ± 0.29 C	27.71 ± 0.18 B	60.38 ± 0.33 A
22	Decanal	n.d				0.02 ± 0.01 D	0.46 ± 0.03 C	0.75 ± 0.08 B	1.24 ± 0.11 A
24	(2E)-Nonenal	n.d				0.02 ± 0.02 B	0.03 ± 0.01 B	0.05 ± 0.01 AB	0.07 ± 0.02 A
27	(2E)-Decenal	n.d				n.d	0.02 ± 0.00 C	0.04 ± 0.01 B	0.08 ± 0.02 A
28	(2E,4E)-Decadienal	n.d	0.08 ± 0.03 a	0.15 ± 0.02 a	0.13 ± 0.05 a	n.d			
	Ketones								
18	3-Octen-2-one	1.70 ± 0.34 d	2.03 ± 0.11 c	3.49 ± 0.10 b	4.78 ± 0.11 a	0.20 ± 0.01 D	0.49 ± 0.05 C	0.75 ± 0.03 B	0.90 ± 0.03 A
23	(3E,5E)-3,5-Octadien-2-one	4.26 ± 0.27 d	6.10 ± 0.36 c	9.61 ± 0.28 b	13.72 ± 0.22 a	4.70 ± 0.41 C	4.31 ± 0.66 C	13.33 ± 0.29 B	17.10 ± 0.22 A
29	1-(4-Ethylphenyl) ethanone	n.d	0.08 ± 0.01 a	0.10 ± 0.02 a	0.10 ± 0.05 a	n.d			0.10 ± 0.03
	Alcohols								
9	1-Penten-3-ol	0.02 ± 0.01 d	0.13 ± 0.02 c	0.16 ± 0.03 b	0.22 ± 0.03 a	0.94 ± 0.08 D	7.92 ± 0.09 C	12.34 ± 0.22 B	19.86 ± 0.18 A
10	2-Methyl-3-pentanol	0.17 ± 0.12 c	0.34 ± 0.11 c	0.77 ± 0.16 b	1.15 ± 0.32 a	n.d			
15	(2Z)-2-Penten-1-ol	n.d				n.d		0.01 ± 0.01	0.02 ± 0.01
16*	1-Hexanol	0.20 ± 0.02 b	0.20 ± 0.01 b	0.28 ± 0.03 a	0.29 ± 0.01 a	1.51 ± 0.03 B	1.69 ± 0.11 AB	1.76 ± 0.15 A	1.78 ± 0.23 A
20	1-Octen-3-ol	0.28 ± 0.03 d	1.07 ± 0.08 c	1.30 ± 0.1 b	1.52 ± 0.23 a	0.43 ± 0.08 D	1.45 ± 0.25 C	2.20 ± 0.31 B	2.75 ± 0.33 A
	Acids								
25	Propanoic acid	1.00 ± 0.29 d	1.69 ± 0.32 c	3.87 ± 0.09 b	5.06 ± 0.55 a	0.37 ± 0.06 D	1.88 ± 0.12 C	3.00 ± 0.18 B	4.82 ± 0.29 A
26	Butanoic acid	0.17 ± 0.03 b	0.29 ± 0.06 b	0.66 ± 0.18 a	0.75 ± 0.12 a	n.d			
30*	Hexanoic acid	1.23 ± 0.11 c	2.15 ± 0.31 b	3.15 ± 0.22 a	3.54 ± 0.43 a	1.31 ± 0.06 D	1.88 ± 0.15 C	3.62 ± 0.20 B	4.63 ± 0.39 A
33*	Octanoic acid	0.22 ± 0.08 d	0.38 ± 0.08 c	0.68 ± 0.13 b	1.99 ± 0.09 a	0.18 ± 0.10 D	0.43 ± 0.05 C	0.52 ± 0.22 B	1.19 ± 0.08 A
34	Nonanoic acid	1.21 ± 0.22 c	1.81 ± 0.21 b	2.12 ± 0.23 b	9.32 ± 0.55 a	n.d			
	Furans								
1	2-Ethylfuran	1.06 ± 0.09 b	1.85 ± 0.13 a	2.18 ± 0.2 a	1.96 ± 0.11 a	0.55 ± 0.23 D	0.78 ± 0.08 C	1.23 ± 0.21 B	1.52 ± 0.32 A
12	2-Pentylfuran	0.26 ± 0.02 d	0.63 ± 0.09 c	0.80 ± 0.06 b	0.92 ± 0.05 a	0.23 ± 0.01 D	0.61 ± 0.05 C	1.18 ± 0.07 B	1.35 ± 0.12 A
32	5-Pentyldihydro-2(3H)-furanone	n.d				0.01 ± 0.00 D	0.05 ± 0.01 C	0.07 ± 0.02 B	0.17 ± 0.01 A
	Additional compounds								
2	α-Pinene	4.42 ± 0.53 a	3.48 ± 0.32 b	3.25 ± 0.25 b	3.22 ± 0.22 b	3.47 ± 0.28 A	3.19 ± 0.31 A	3.18 ± 0.24 A	2.87 ± 0.12 B
4	β-Sabinene	0.50 ± 0.09 a	0.37 ± 0.10 ab	0.36 ± 0.03 b	0.32 ± 0.02 b	n.d			
6	β-Pinene	0.94 ± 0.12 a	0.77 ± 0.08 a	0.72 ± 0.13 a	0.71 ± 0.22 a	0.92 ± 0.06 A	0.80 ± 0.12 AB	0.73 ± 0.09 AB	0.66 ± 0.13 B
7	o-Xylene	0.23 ± 0.06 a	0.23 ± 0.01 a	0.23 ± 0.02 a	0.17 ± 0.06 a	0.48 ± 0.01 A	0.11 ± 0.03 B	0.03 ± 0.01 C	n.d
8	β-Myrcene	0.14 ± 0.03 a	0.13 ± 0.01 a	0.11 ± 0.03 ab	0.08 ± 0.01 b	0.17 ± 0.06 A	0.16 ± 0.02 A	0.04 ± 0.01 B	n.d
11	D-Limonene	0.14 ± 0.02 a	0.11 ± 0.05 ab	0.06 ± 0.01 bc	0.05 ± 0.00 c	0.05 ± 0.00 A	0.03 ± 0.01 B	0.01 ± 0.00 C	n.d
13	p-Cymene	1.55 ± 0.33 a	1.14 ± 0.13 b	1.02 ± 0.05 c	0.96 ± 0.21 d	1.43 ± 0.08 A	1.26 ± 0.11 AB	1.02 ± 0.12 BC	0.83 ± 0.22 C
31	Phenol	0.09 ± 0.01 a	0.09 ± 0.01 a	0.09 ± 0.02 a	0.10 ± 0.01 a	0.08 ± 0.01 A	0.09 ± 0.06 A	0.08 ± 0.02 A	0.09 ± 0.01 A
SUM		29.16 ± 3.57	46.62 ± 3.95	70.33 ± 3.79	100.63 ± 5.42	22.52 ± 2.53	66.33 ± 3.01	99.36 ± 3.79	166.67 ± 4.21

[a] n.d. indicates not detected. *Identification of the volatile compound was confirmed with authentic standards. The different lowercase letters indicate significant differences in the volatile concentrations at $p < 0.05$ within chia seed oil during storage. The different capital letters indicate significant differences in the volatile concentrations at $p < 0.05$ within flax seed oil during accelerated storage. Six samples were injected per storage point

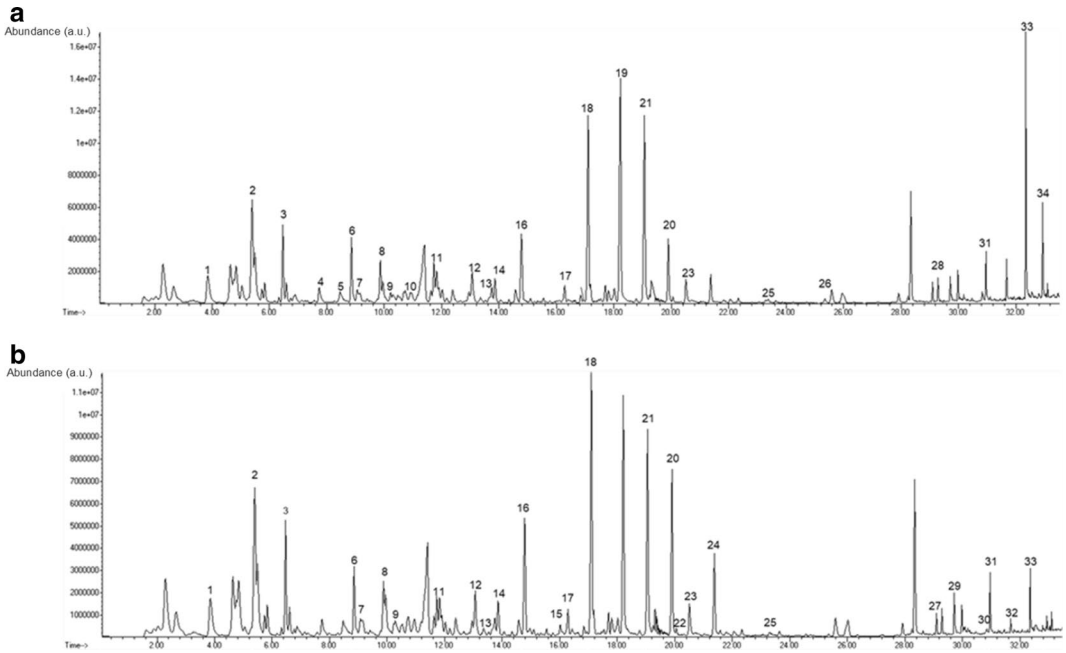

Fig. 4 Representative GC chromatogram of (**a**) chia seed oil and (**b**) flax seed oil samples stored at 65 °C for 14 days. Volatile code numbers are provided in Table 4

significant changes during accelerated storage in this study.

Storage markedly increased the concentrations of aldehydes in both chia seed and flax seed oils. Hexanal is commonly used as an oxidation marker, and its concentration in chia seed oil increased from 4.7 to 16.4 ng/g, whereas that of flax seed oil increased from 1.8 to 16.1 ng/g. The concentration of nonanal increased by two-fold in both oils. Nonanal has an aldehydic odor [49]. Furthermore, 2,4-heptadienal was the most abundant before and after storage in both oils among the identified volatiles. Its concentration in chia seed oil increased four times from 20.2 to 79.9 ng/g, whereas that in the flax seed oil showed the largest increase of 38-fold from 1.59 to 60.38 ng/g. Thus, 2,4-heptadienal may be used as an oxidation marker in chia seed and flax seed oils.

Lipid oxidation produces off-flavor and various volatiles are responsible for the off-flavor. For example, oxidized linoleic acid derived volatiles such as hexanal, (2Z)-octenal, (2E)-nonenal, 1-octene-3-one, 3-octene-2-one, and (2E)-octenal are known for their intense aroma impact by GC–olfactometry [50]. Methyl linolenic acid derived volatiles with intense aroma impact include (2E,6Z)-nonadienal, (Z)-1,5-octadien-3-one, (3E,5Z)-octadien-2-one, and (2Z)-3-hexenal [51]. Soybean oil with high flavor score (better ones) showed a negative correlation with pentane, pentanal, hexanal, (2E)-heptenal, (2E,4E)-heptadienal, (2E,4Z)-decadienal, and (2E,4E)-decadienal, analyzed by GC analysis [52]. The largely increased 2,4-heptadienal may be responsible for the intense off-flavor in chia seed and flax seed oils after 14 day accelerated storage.

Among the alcohols, the concentration of 1-hexanol in chia seed oil increased from 0.20 to 0.29 ng/g, whereas that of flax seed oil increased from 1.51 to 1.78 ng/g. 1-Hexanol is a secondary oxidation product of linoleic acid, and has a sweet and grassy odor [45]. The concentrations of hexanoic acid were increased significantly after accelerated storage in both chia seed oil and flax seed oil (from 1.23 to 3.54 ng/g and 1.31 to 4.63 ng/g, respectively). Hexanoic acid has a fatty, sweaty, and cheese-like odor [49]. Furthermore, increased concentrations of ketones were observed, specifically those of 3-octen-2-one. 3-Octen-2-one concentration increased in chia seed oil from 1.7 to 4.8 ng/g and in flax seed oil from 0.2 to 0.9 ng/g. Meanwhile, 2-pentylfuran concentration in chia seed oil and flax seed oil increased from 0.26 to 0.92 ng/g and 0.23 to 1.35 ng/g, respectively. 3-Octen-2-one has an oily and sweet odor [49], whereas 2-pentylfuran has an undesirable fruity, green, earthy, beany odor with a 6-ng/g threshold value [48].

Among the terpenes, the major odor-active compounds in chia seed and flax seed oils were α-pinene, o-xylene, β-myrcene, D-limonene, and p-cymene. Terpenes such as α-pinene, β-pinene, D-limonene,

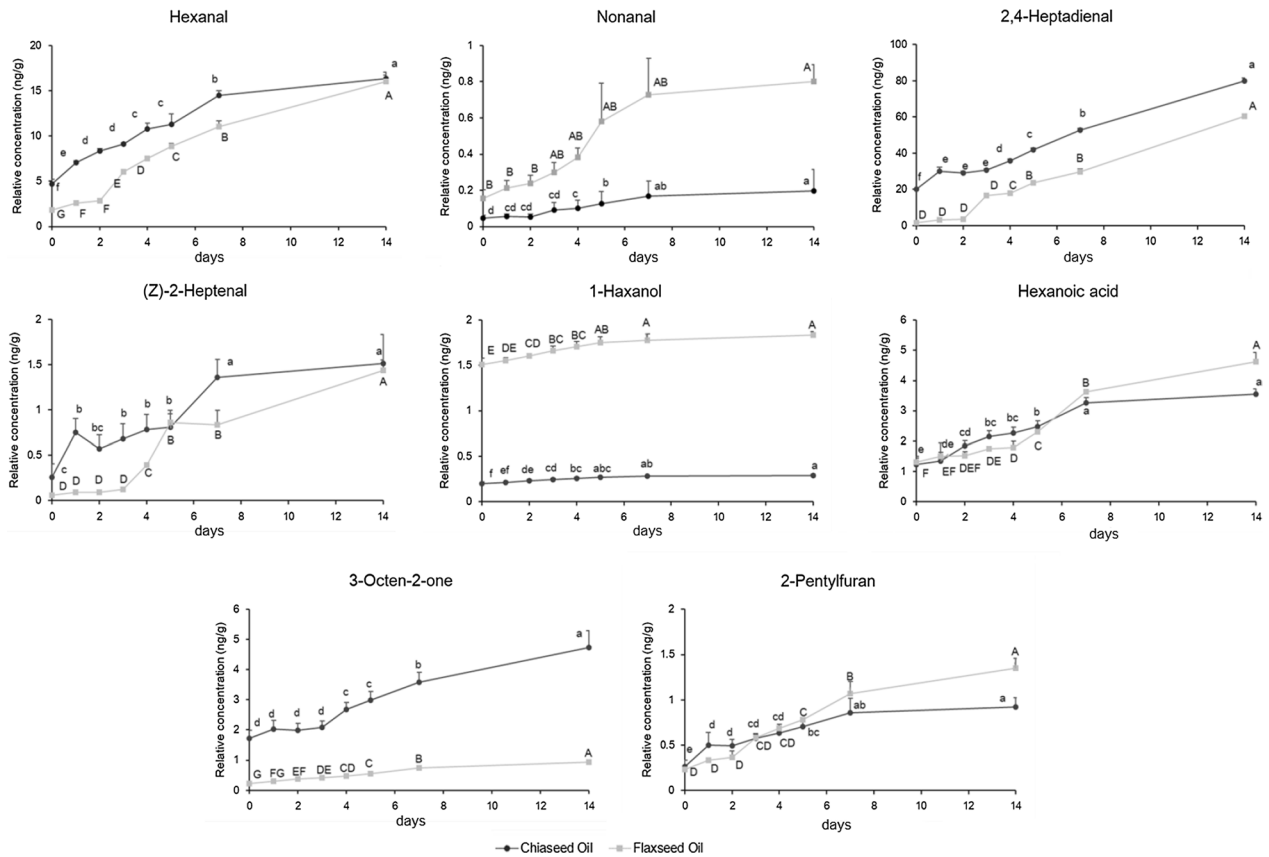

Fig. 5 Changes in the relative concentrations of lipid oxidation-related volatiles in chia seed oil and flax seed oil during accelerated storage. The different lowercase letters indicate significant differences in the volatile concentrations at $p < 0.05$ within chia seed oil during storage. The different uppercase letters indicate significant differences in the volatile concentrations at $p < 0.05$ within flax seed oil during accelerated storage. Six samples were injected per storage point

myrcene and cymene are responsible for the pleasant aromas of essential oils [53]. Terpene contents gradually decreased with some disappearing after accelerated storage. Therefore, the loss of terpenes may indicate quality loss with respect to chia seed and flax seed oils. In previous studies, stripped oils have often been used to compare the antioxidant power of the antioxidant-added oils [54]. However, we did not employ this stripping technique because we wanted to identify the retained concentration of naturally occurring antioxidants such as γ-tocopherol after storage.

Consumption of chia seed or flax seed oil could contribute to increase omega-3 fatty acid intake and provide the omega-6/omega-3 balance; however, they can be easily oxidized during storage and processing due to high α-linolenic acid content. However, little is known about their oxidation stabilities and how to protect oil from oxidation using natural antioxidants. To address this, the oxidation stabilities of untreated and treated (with extracts of rosemary or garlic) chia seed oil and flax seed oil were determined using Rancimat test and accelerated storage test. Additionally, the changes in fatty acid composition, tocopherol content, and volatiles related to lipid oxidation were investigated. The results were compared with those of oils treated with commonly used antioxidants such as AP, BHT, and α-tocopherol. The Rancimat test and the evaluation of the primary and secondary indicators of oxidation, i.e., CDA, POV, FFA, and TBA during accelerated storage revealed that rosemary extract prevents lipid oxidation more efficiently than AP, BHT, and α-tocopherol. Meanwhile, garlic extract prevented lipid oxidation better than α-tocopherol in most of the assays during accelerated storage. γ-Tocopherol, the main tocopherol present in the oils was better retained in the treated chia seed and flax seed oils after 14 day storage. In addition, 33 volatiles were identified, and some were

newly formed or have increased concentrations during accelerated storage. The volatile composition was dependent on the type of oil, and the amount of each volatile increased or decreased at a different rate. The concentration of lipid oxidation-related volatiles such as hexanal increased significantly, whereas that of terpenes such as limonene decreased significantly. The newly formed volatiles or the volatiles that increased at high rate such as 2,4-heptadienal can be used as markers for the lipid oxidation of chia seed and flax seed oils. Our study suggests that antioxidants from natural sources, i.e., rosemary extract and garlic extract could be successfully substituted in place of commonly used antioxidants such as BHT to protect oils against lipid oxidation.

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Authors' contributions
Conceptualization and funding acquisition: JL; Supervision: JL; Investigation and methodology: HJ, and IK; Formal analysis: HJ and SJ; Original draft preparation: HJ, IK and SJ; Review and editing: JL. All authors read and approved the final manuscript.

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Competing interests
The authors declare that there is no conflict of interest.

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