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ORIGINAL ARTICLE

Importance of the higher retention of tocopherols and sterols for the oxidative stability of soybean and rapeseed oils

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Abstract Tocopherols and sterols were lost considerably during the refining process of vegetable oils, resulting in a dramatic decrease in the oxidation stability. However, the oxidation stability of vegetable oils was not directly proportional to tocopherol content, not mention to its synergistic interaction with sterol. Based on the peroxide values of oils with different content of tocopherols and sterols during storage at 65 °C, it was found that soybean oil (SO) had the best stability when the content of tocopherols and sterols was 0.14 and 1.09%, respectively, whereas the value in rapeseed oil (RO) was 0.06% and 1.14–2.90%. The optimal content of tocopherol in RO was lower than that in SO was due to the different tocopherol isoforms. Furthermore, it was found that the storage stability decreased significantly when adding a same content of α-tocopherol, compared with that by retaining more tocopherol isoforms existed in SO. It was suggested that retaining more tocopherol and sterol during vegetable oils refining improved the oxidation stability, however, it did not mean the more the better. The oxidation stability was dependent on both the content and the isoform. This study helps to define the effective contents of tocopherol and sterol under the moderate refining technology.

Keywords Storage stability · α-Tocopherol · γ-Tocopherol · δ-Tocopherol · Moderate refining

Introduction

Vegetable oils are good sources of tocopherols and sterols (Dunford 2004). Tocopherols are a group of fat-soluble antioxidants and can be divided into α-, β-, γ-, and δ- forms (Colombo 2010), whereas sterols constitute a major portion of the unsaponifiable fraction of oils (Moreau et al. 2002). The crucial effects of tocopherols (Kamal-Eldin and Budilarto 2015; Yi et al. 2011) and sterols (Hidalgo et al. 2009; Winkler-Moser et al. 2015) against oxidative reactions have been documented.

In the past, oil quality has mainly been defined by organoleptic parameters, such as taste, odor, and color. Therefore, the objective of oil refining has typically been to remove undesirable and pro-oxidative components, such as free fatty acids, phospholipids, pigments, and trace metals (Johnson 2008). Although refining extends the shelf-life of oils, there are also several disadvantages to this process, including the formation of harmful *trans* fatty acids, polymeric triacylglycerols, and oxidized triacylglycerols, as well as the loss of nutritional tocopherols and sterols (Dijkstra and van Duijn 2016; Farhoosh et al. 2009). Our laboratory had previously studied the effect of refining processes on minor components based on oil samples collected from seven representative oil plants in China. We found that the rates of tocopherol and sterol losses after refining varied from 10.22 to 75.88% and 21.60 to 2.62%

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for soybean oil (SO), respectively, and 6.57–70 and 18.02–85.26% for rapeseed oil (RO), respectively (Fang et al. 2014a). Importantly, dramatic losses in tocopherols in refined vegetable oils may result in substantial decreases in the protective power against autoxidation (Elisia et al. 2013; Fang et al. 2014b). Therefore, as attention has shifted to analysis and improvement of the nutritional aspects of vegetable oils, more emphasis has been placed on the contents of micronutrients, and moderate the refining process has been proposed.

However, refining processes yielding oils with higher tocopherol content are not necessarily more stable than those with lower tocopherol content. It was reported that there is a relative increase in hydroperoxide formation parallel to consumption of α-tocopherol in RO triglycerides when the α-tocopherol content is above 100 ppm (Huang et al. 1995). In another study, Anna-Maija et al. (1999) reported that when the tocopherol content is above 250 ppm, α-tocopherol has a pro-oxidative effect by promoting the formation of lipid hydroperoxides in corn oil triglycerides. Then the question was proposed that to what content the appropriate and effective tocopherol and sterol contents should be in vegetable oils.

To answer this question, we prepared oil samples with different contents of tocopherol and sterol using the purified rapeseed oil (RO) and soybean oil (SO) according to their original tocopherol and sterol compositions. This study provided a theoretical basis for defining the retention content of tocopherol and sterol in RO and SO under moderate refining conditions.

Materials and methods

Materials

Refined RO and SO were obtained from the Wilmar Oil Refinery (Wilmar, China) and Huifu Oil Refinery (Huifu, China), respectively. Both of the oil samples were refined by chemical refining technology and meet the requirements of China national standards. α-Tocopherol (purity >96%), β-tocopherol (50 mg/mL, dissolved in hexane, purity >96%), γ-tocopherol (purity >96%), δ-tocopherol (purity >90%), betulin (purity >98%), 5α-cholestane (purity >98%), N-methyl-N-trimethyl silylheptafluorobutyramide, and 1-methyl imidazole were obtained from Sigma-Aldrich (St. Louis, MO, USA). Two kinds of sterols (purified from SO and RO, respectively with a purity >95%) were purchased from China Oil & Foodstuffs Corporation. n-Heptane (HPLC-grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Thin-layer chromatography (TLC) plates (MK6F silica gel, 60 Å) were purchased from Whatman (Clifton, NJ, USA). All the other common chemicals and solvents (analytical-grade) used in below measurements were purchased from China.

Purification of RO and SO

The purification of RO and SO was performed according to the method of Afinisha Deepam et al. (2011) with modifications. Briefly, a glass column (40 cm in length, 2.5 cm in inner diameter) was packed with 50 g silica gel (60–120 mesh). Oil samples (20 g) were adsorbed on silica gel and packed in the column. To remove tocopherols and sterols from oils, the loading column was washed with 400 mL hexane then the fractions were evaporated and then repeated.

Sample preparation

Based on the main tocopherol isoforms and ratios in the collected RO and SO, tocopherol standards were added into the oils to obtain samples with different concentrations of tocopherols. Samples with different concentrations of sterols were obtained by adding a mixture of sterol esters isolated from the deodorizer distillates of SO and RO. The main types of sterol forms and proportions of each form were analyzed and were in accordance with the SO and RO sample (for details see Table 2).

Tocopherol analysis

Analysis of tocopherol was performed according to the AOCS Official method (AOCS Ce 8-89) using a HPLC (e2695; Waters Corporation, USA) equipped with a scanning fluorescence detector (mode 2475) on a silica gel Lichrosorb Si-60 column (particle size: 5 μm; 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain). Identification and quantification of chromatographic peaks were performed by comparison with the peaks observed in α-, β-, γ-, and δ-tocopherol mixed standard solutions (1, 2, 3, 4, 5, or 10 μg/mL). An external calibration curve was prepared for the standard to calculate the amounts of these four tocopherol forms in oil samples. All analyses were carried out in triplicate, and the results are reported as means.

Sterol analysis

Analysis of sterol was performed according to the ISO method (ISO 12228) using a gas chromatography on an instrument equipped with a flame ionization detector (6890 N, Agilent Technologies, Palo Alto, CA, USA) with brief modification in the mass of oil samples when saponified. For SO samples, the mass of oil was 0.75 g while for RO samples, the mass was 0.25 g.

Accelerated storage test

Oil samples (15 g) were weighed in a 100-mL glass beakers (72 mm high with a diameter of 60 mm) and incubated for 12 days at 65 °C in a forced air oven. Four beakers were prepared for each oil sample. On days 0, 4, 8, 10, and 12, one beaker for each oil sample was removed, and its contents were subjected to routine analysis and tocopherol and sterol analysis. This experiment was carried out twice. Peroxide values (PVs) of samples were measured according to the AOCS methods Cd 8b-90.

Statistical analysis

All the measurements (tocopherol analysis, sterol analysis and PVs) were repeated at least three times. Data analysis was carried out using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA). One-way analysis of variance (ANOVA) was used, and least significant differences (LSDs) were calculated at p values of less than 0.05 ($LSD_{0.05}$ and $Ducan_{0.05}$).

Results

Analysis of tocopherol and sterol contents in SO and RO samples

The contents of tocopherols and sterols in the collected and purified SO and RO samples are shown in Table 1. Based on the two primary tocopherol forms and ratios in the collected oils (RO-T1S1 and SO-T1S1), tocopherol standards were added into RO and SO at mass ratios of 1:2 for α- and γ-tocopherol and 7:3 for γ- and δ-tocopherol, respectively. The oil samples with different contents of tocopherols and sterols were also measured to verify the contents (Table 2).

Effects of tocopherol and sterol on the storage stability of RO

The storage stability of RO samples with different concentrations of tocopherols and sterols at 65 °C were evaluated according to changes in PVs (Table 3) during storage. Compared with the purified samples (RO-T0S0), retaining tocopherols and sterols did improve the stability significantly ($p < 0.05$). PV of the refined oils was limited to 5 mmol/kg oil (CODEX STAN 210-1999), based on this criterion, it was found that RO samples with a 0.06% content of tocopherols (the T1 group) have the best stability. At day 10, the PVs of the T1 group samples did not exceed 5 mmol/kg oil, while oils with higher contents of tocopherols have all exceed 5 mmol/kg oil. The pro-oxidant effect of tocopherols and sterols at high contents can be seen at the day 4 and 8. At day 4, PVs of oils with tocopherol and sterol did not differ significantly and were all lower than that of the T3S3 sample significantly ($p < 0.05$). At day 8, this effect was much more obvious that PVs of the T3S2 and T3S3 samples were significantly higher than those of the others ($p < 0.05$) and had already exceed the specified 5 mmol/kg oil. This study suggested that the content of tocopherol and sterol in RO should be lower than 0.18 and 1.76%, respectively. Furthermore, RO had the best storage stability when the retention content of tocopherols was 0.06% and the content of sterols can be retained to 2.90%.

Effects of tocopherol and sterol on the storage stability of SO

PVs of SO samples with different concentrations of tocopherol and sterol during storage at 65 °C were shown in Table 4. When removing tocopherol and sterol from SO, PV during storage increased significantly when compared with SO oils containing tocopherol and sterol ($p < 0.05$). As tocopherol increased from 0.004% (SO-T0S0) to 0.14% (SO-T3S1), PVs of SO decreased significantly ($p < 0.05$), however, when continued to increase the content of tocopherol or sterol resulted in only minor changes in PVs ($p > 0.05$) in 8 days. At day 10, PVs of all the oils have exceed the allowable value of 5 mmol/kg oil and exhibited significant differences. At a low sterol content of 0.35% (the S1 group), stability of SO was dependent on the content of tocopherol below 0.22% and further increased to 0.30%, the stability did not change significantly ($p > 0.05$).

Table 1 Content of tocopherols and sterols in the collected (T1S1) and purified (T0S0) RO and SO

Samples	Tocopherol (mg/100 g oil)					Total sterol (mg/100 g oil)
	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol	
RO-T1S1	20.97 ± 1.451	1.50 ± 0.032	40.31 ± 3.562	2.32 ± 0.021	64.57 ± 5.110	1141.49 ± 91.532
RO-T0S0	1.83 ± 0.362	0.21 ± 0.018	3.95 ± 0.234	0.40 ± 0.001	6.39 ± 0.614	76.95 ± 5.211
SO-T1S1	4.72 ± 0.491	2.42 ± 0.032	21.60 ± 1.681	9.75 ± 0.176	38.49 ± 2.381	349.10 ± 34.721
SO-T0S0	0.85 ± 0.015	0.80 ± 0.103	1.97 ± 0.361	1.24 ± 0.043	4.86 ± 0.436	30.55 ± 2.76

Values was shown as the means ± SDs of three replicates

Table 2 Content of tocopherols and sterols in the tested SO and RO samples

Content (%)	RO-T0S0	RO-T1S1	RO-T1S2	RO-T1S3	RO-T2S1	RO-T2S2	RO-T2S3	RO-T3S1	RO-T3S2	RO-T3S3
Total tocopherol	0.006	0.060	0.060	0.060	0.120	0.120	0.120	0.180	0.180	0.180
Total sterol	0.080	1.140	1.760	2.900	1.140	1.760	2.900	1.140	1.760	2.900
Content (%)	SO-T0S0	SO-T1S1	SO-T2S1	SO-T3S1	SO-T4S1	SO-T5S1	SO-T3S2	SO-T3S3	SO-T4S3	SO-T5S3
Total tocopherol	0.004	0.040	0.070	0.140	0.220	0.300	0.140	0.140	0.220	0.300
Total sterol	0.030	0.350	0.350	0.350	0.350	0.350	0.700	1.090	1.090	1.090

Table 3 PVs (mmol/kg) of RO samples with different concentrations of tocopherols and sterols during storage at 65 °C

Samples	0	4	8	10	12
RO-T0S0	3.33 ± 0.127^{b}	33.59 ± 0.100^{c}	66.03 ± 1.761^{d}	103.667 ± 1.753^{d}	146.83 ± 1.828^{e}
RO-T1S1	1.03 ± 0.042^{a}	3.35 ± 0.057^{a}	4.29 ± 0.099^{a}	4.72 ± 0.107^{a}	5.37 ± 0.071^{a}
RO-T1S2	1.12 ± 0.021^{a}	3.39 ± 0.071^{a}	4.25 ± 0.028^{a}	4.86 ± 0.270^{a}	5.58 ± 0.311^{a}
RO-T1S3	1.13 ± 0.028^{a}	3.36 ± 0.014^{a}	4.22 ± 0.050^{a}	4.92 ± 0.123^{a}	5.54 ± 0.255^{a}
RO-T2S1	1.10 ± 0.153^{a}	3.66 ± 0.158^{a}	4.26 ± 0.239^{a}	5.10 ± 0.196^{a}	7.62 ± 0.070^{b}
RO-T2S2	1.10 ± 0.078^{a}	3.65 ± 0.194^{a}	4.53 ± 0.260^{a}	5.80 ± 0.368^{a}	16.30 ± 0.196^{c}
RO-T2S3	1.08 ± 0.083^{a}	3.74 ± 0.143^{a}	4.61 ± 0.256^{a}	6.03 ± 0.506^{ab}	23.13 ± 0.848^{d}
RO-T3S1	1.05 ± 0.165^{a}	3.91 ± 0.196^{a}	4.76 ± 0.250^{a}	6.39 ± 0.475^{b}	7.62 ± 0.070^{b}
RO-T3S2	1.12 ± 0.078^{a}	4.16 ± 0.167^{a}	5.73 ± 0.515^{b}	7.45 ± 0.110^{b}	16.30 ± 0.196^{c}
RO-T3S3	1.13 ± 0.076^{a}	4.34 ± 0.151^{b}	6.83 ± 0.531^{c}	8.58 ± 0.271^{c}	23.13 ± 0.848^{d}

Values was shown as the means ± SDs of three replicates; the superscript letter corresponded to the significant analysis results and the different letter in the same column indicated a significant difference ($p < 0.05$)

Table 4 PVs (mmol/kg) of SO samples with different concentrations of tocopherols and sterols during storage at 65 °C

Samples	0	4	8	10	12
SO-T0S0	3.18 ± 0.103^{b}	32.15 ± 2.754^{d}	84.05 ± 4.154^{d}	118.83 ± 8.828^{h}	282.09 ± 6.116^{e}
SO-T1S1	0.79 ± 0.051^{a}	7.71 ± 0.151^{c}	33.79 ± 2.421^{c}	52.62 ± 3.020^{g}	84.05 ± 4.154^{d}
SO-T2S1	0.81 ± 0.013^{a}	5.18 ± 0.319^{b}	7.06 ± 0.457^{b}	24.21 ± 0.722^{f}	45.37 ± 0.362^{c}
SO-T3S1	0.83 ± 0.042^{a}	1.75 ± 0.064^{a}	3.10 ± 0.001^{a}	18.20 ± 0.141^{e}	44.19 ± 3.836^{c}
SO-T4S1	0.80 ± 0.007^{a}	1.82 ± 0.028^{a}	3.53 ± 0.014^{a}	14.68 ± 0.184^{de}	30.37 ± 2.524^{b}
SO-T5S1	0.81 ± 0.042^{a}	1.88 ± 0.035^{a}	3.95 ± 0.014^{a}	15.15 ± 0.276^{de}	41.79 ± 0.361^{c}
SO-T3S2	0.81 ± 0.007^{a}	1.74 ± 0.007^{a}	3.13 ± 0.057^{a}	12.19 ± 0.042^{c}	43.42 ± 0.863^{c}
SO-T3S3	0.75 ± 0.001^{a}	1.70 ± 0.050^{a}	3.13 ± 0.001^{a}	5.20 ± 0.113^{a}	14.21 ± 0.219^{a}
SO-T4S3	0.76 ± 0.021^{a}	1.77 ± 0.014^{a}	3.51 ± 0.191^{a}	8.32 ± 0.573^{b}	28.54 ± 2.029^{b}
SO-T5S3	0.76 ± 0.021^{a}	1.80 ± 0.071^{a}	3.82 ± 0.071^{a}	16.60 ± 0.403^{e}	29.32 ± 0.035^{b}

Values was shown as the means ± SDs of three replicates; the superscript letter corresponded to the significant analysis results and the different letter in the same column indicated a significant difference ($p < 0.05$)

In the contrary, at a high sterol content of 1.09% (S3 group), stability of SO decreased significantly as the increasing of tocopherol ($p < 0.05$). SO had the best storage stability when the retention content of tocopherol and sterol was 0.14 and 1.09%, respectively. Meanwhile, at this content of tocopherol, PV decreased significantly as the increasing of sterol ($p < 0.05$).

Effects of tocopherol forms on SO stability

Based on the result of the retention contents of tocopherols and sterols in RO and SO, it can be seen that besides the concentration, the forms of tocopherol also influenced the storage stability. To verify the effects of tocopherol forms on storage stability in SO and RO samples, we measured

Table 5 PVs (mmol/kg) of SO samples with different concentrations and forms of tocopherols and sterols during storage at 65 °C

Samples	Content/(%) (T + S)	0	2	4	8	10	12
SO-T0S0	0.004 + 0.03	3.18 ± 0.103	18.76 ± 1.442^{e}	32.15 ± 2.754^{e}	84.05 ± 4.154^{f}	118.83 ± 8.828^{g}	282.09 ± 6.116^{g}
SO-T1S1	0.04 + 0.35	0.79 ± 0.051	3.74 ± 0.151^{c}	7.71 ± 0.151^{b}	33.79 ± 2.421^{c}	52.62 ± 3.020^{d}	84.05 ± 4.154^{c}
SO-T3S1	0.14 + 0.35	0.83 ± 0.042	1.32 ± 0.089^{a}	1.75 ± 0.064^{a}	3.10 ± 0.001^{a}	18.20 ± 0.141^{b}	44.19 ± 3.836^{b}
SO-αT3S1	0.16 + 0.35	2.22 ± 0.022	3.51 ± 0.318^{c}	11.02 ± 0.743^{c}	58.59 ± 0.797^{d}	81.19 ± 6.621^{e}	119.76 ± 2.593^{d}
SO-αT4S1	0.28 + 0.35	1.92 ± 0.025	5.49 ± 0.929^{d}	20.07 ± 3.020^{d}	78.90 ± 4.443^{e}	106.83 ± 6.216^{f}	150.52 ± 11.202^{f}
SO-T3S3	0.14 + 1.29	0.75 ± 0.001	2.52 ± 0.218^{a}	1.70 ± 0.050^{a}	3.13 ± 0.001^{a}	5.20 ± 0.113^{a}	14.21 ± 0.219^{a}
SO-αT3S3	0.16 + 1.29	1.98 ± 0.016	2.83 ± 0.205^{b}	6.90 ± 0.487^{b}	24.17 ± 2.302^{b}	41.43 ± 2.677^{c}	73.39 ± 3.235^{c}
SO-αT4S3	0.28 + 1.29	2.08 ± 0.010	5.24 ± 0.361^{c}	10.68 ± 1.331^{c}	57.37 ± 4.193^{d}	78.39 ± 0.686^{e}	134.72 ± 2.419^{e}

Values was shown as the means ± SDs of three replicates; the superscript letter corresponded to the significant analysis results and the different letter in the same column indicated a significant difference ($p < 0.05$)

the PVs during storage at 65 °C for SO samples supplemented with α-tocopherol (Table 5). Compared with SO-T0S0, increasing the content of α-tocopherol alone significantly improved the oxidative stability and storage stability of SO ($p < 0.05$). However, the antioxidant effect of α-tocopherol was not as strong as the mixture of γ-and δ-tocopherols (7:3, mass ratio). At the same tocopherol and sterol concentrations, the PVs of samples containing mixture of γ-and δ-tocopherols were much lower than those of samples containing added α-tocopherol alone ($p < 0.05$). Furthermore, PVs increased significantly as the α-tocopherol content increased for both the S1 and S3 groups ($p < 0.05$). However, the synergistic effects of sterols were clear in the presence of α-tocopherol. Finally, increasing the sterol content significantly decreased the PV of oil at the same α-tocopherol concentration ($p < 0.05$).

Discussion

Oils are susceptible to oxidative processes in the presence of light, heat, metals, and other stimuli initiated by the formation of free radicals (Kamal-Eldin et al. 2010). Once free radicals were formed, they react in a chain to convert the material to a hydroperoxide (Kinen et al. 2000). The anti-oxidant mechanism of tocopherol occurs through donation of a hydrogen atom to a peroxyl radical (LOO•) of an unsaturated lipid molecule, forming a hydroperoxide (LOOH) and a tocopheroxyl radical (TO•), which has a lower capacity to propagate lipid peroxidation compared with LOO•. TO• reacts with other LOO• radicals or TO•, forming more stable products. Below the specified PV of 5 mmol/kg oil in the CODEX Standard for vegetable oils (CODEX STAN 210-1999), increasing the content of tocopherol from 0.004 to 0.14% would significantly delay the autoxidation of SO samples; however, in RO samples, adding tocopherol did not significantly influence the stability of the sample. Our results also suggested that at a concentration of 0.14% or higher, the antioxidant activity of α-tocopherol was lower than that in the mixture of γ- and δ-tocopherols (7:3, mass ratio). These observations may be related to the different characteristics of various types of tocopherols in SO and RO samples.

The hydrogen atom-donating capacity of α-tocopherol is thought to be higher than that of γ-tocopherol (Huang et al. 1995), indicating that α-tocopherol may be a more potent antioxidant than γ-tocopherol. However, contradictory findings (Elisia et al. 2013; Kinen et al. 2000) on the roles of tocopherol isoforms in stabilizing lipids have been reported. For example, α-tocopherol has been reported to exhibit higher antioxidant activity than γ-tocopherol in a methyl linoleic model system (Kinen et al. 2000), whereas study showed that the antioxidant capacity of tocopherols follows the order of δ-tocopherol > γ-tocopherol > α-tocopherol at high concentrations in fish oil (Elisia et al. 2013). These contradictory findings may be largely dependent on the concentration of the component. At levels below 0.05%, α-tocopherol is more stable and a more effective antioxidant than γ-tocopherol, whereas at levels above 0.10%, γ-tocopherol is a more effective antioxidant than α-tocopherol (Huang et al. 1995). Fuster et al. (1998) reported that α-tocopherol is a better antioxidant than γ-tocopherol in purified sunflower triacylglycerols at concentrations lower than 0.04% but was a poorer antioxidant at concentrations above 0.20%. Furthermore, this study showed that increasing the content of α-tocopherol from 0.14 to 0.30% decreased the PV in SO samples. This result was consistent with the findings of Huang et al. (1995), who showed that there is a relative increase in hydroperoxide formation parallel to consumption of α-tocopherol at levels above 0.10%. Therefore, for SO,

application of moderate refining processes may provide better storage stability through retention of tocopherol than simple addition of α-tocopherol.

Sterols are thought to have anti-oxidative activity because of their capacity for formation of allylic free radicals and isomerization to other relatively stable free radicals (Hidalgo et al. 2009; Wang et al. 2002). During the storage time, when the PV was below 5 mmol/kg oil, the sterol content did not influence the PV of SO significantly at each tested concentration of tocopherol. Moreover, from day 10, when the PV exceeded 5 mmol/kg oil, further increases in sterols significantly decreased the PVs of SO samples at tocopherol contents of 0.14 and 0.22%. Similar effects of sterols were also found in SO samples supplemented with α-tocopherol. Therefore, based on these results, we hypothesize that sterols may act synergistically with tocopherols; when the content of tocopherols is decrease due to consumption during anti-oxidative processes, sterols could donate their hydrogen atoms to TO• and then regenerate tocopherols.

Notably, the concentration-dependent effects of tocopherols and sterols in RO samples differed compared with those in SO samples. The main tocopherol forms in RO were γ-and α-tocopherols (mass ratio of 2:1). When the tocopherol content only was increased from 0.06 to 0.12% or even to 0.18%, corresponding to 0.04 and 0.08% α-tocopherol, respectively, changes in PVs and p-AVs were subtle in ROs. Moreover, the increase in sterols did not delay the autoxidation of RO. However, the PV was increased when the tocopherol content reached 0.18%. At lower concentrations of tocopherol, increasing sterol levels did not influence PVs or p-AVs of RO samples. Tocopherols and TO• may also participate in reactions with oxygen or other substances that could donate hydrogen atoms (Huang et al. 1995; Kamal-Eldin and Budilarto 2015). Because α-tocopherol has a higher hydrogen atom-donating capacity than γ-tocopherol (Huang et al. 1995), the subtle concentration-dependent effects of tocopherols in RO samples may be explained by reaction between α-tocopherol or α-tocopheroxyl radicals and γ-tocopherol, which could counteract the antioxidant effects of γ-tocopherol. Furthermore, the synergistic effect of sterols may be a double-edged sword; that is, when the α-tocopherol content was high enough (e.g., 0.08%) in the RO-T3 groups (0.18%), the hydrogen atom donated by sterols may react with LOO• instead of TO• to produce LOOH, leading to increases in PVs.

Conclusion

Although the retention of nutritional tocopherols and sterols in vegetable oils was observed, these increases did not necessarily lead to corresponding increases in the stabilities of vegetable oils. Indeed, the concentrations of tocopherols and sterols corresponding to the greatest stability differed according to the tocopherol forms within the oils. In SO, 0.14 and 1.09% tocopherols and sterols, respectively, were optimal, whereas those in RO samples were 0.06 and 1.14–2.90%, respectively. Furthermore, owing to the storage stabilities of these compounds, retaining more tocopherols and sterols in SO samples by monitoring and improving the refining process may be much more effective than adding back α-tocopherol.

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References

Afinisha Deepam LS, Sundaresan A, Arumughan C (2011) Stability of rice bran oil in terms of oryzanol, tocopherols, tocotrienols and sterols. J Am Oil Chem Soc 88:1001–1009

Anna-Maija L, Leena K, Afaf KE, Piironen V (1999) Antioxidant activities of α- and γ-tocopherols in the oxidation of rapeseed oil triacylglycerols. J Am Oil Chem Soc 76:749–755

CODEX standard for named vegetable oils. CODEX STAN 210-1999

Colombo ML (2010) An update on vitamin E, tocopherol and tocotrienol—perspectives. Molecules 15:2103–2113

Dijkstra AJ, van Duijn G (2016) Vegetable oils: oil production and processing. Elsevier, Oxford

Dunford NT (2004) Oil- and oilseed-based bioactive compounds and their health effects. AOCS, Illinois

Elisia I, Young JW, Yuan YV, Kitts DD (2013) Association between tocopherol isoform composition and lipid oxidation in selected multiple edible oils. Food Res Int 52:508–514

Fang B, Wei C, Wang Y, Liu J, Duan Z, Luan X (2014a) Influence of refining process on minor components in soybean oil. China Oils Fats 39:8–11 **(Chinese)**

Fang B, Wei C, Wang Y, Luan X (2014b) Effect of refining on anti-oxidant activities of soybean oil and rapeseed oil. China Oils Fats 39:45–48 **(Chinese)**

Farhoosh R, Einafshar S, Sharayei P (2009) The effect of commercial refining steps on the rancidity measures of soybean and canola oils. Food Chem 115:933–938

Fuster MD, Lampi AM, Hopia A, Kamal-Eldin A (1998) Effects of α- and γ-tocopherols on the autoxidation of purified sunflower triacylglycerols. Lipids 33:715–722

Hidalgo FJ, León MM, Zamora R (2009) Effect of β-sitosterol in the antioxidative activity of oxidized lipid–amine reaction products. Food Res Int 42:1215–1222

Huang SW, Frankel EN, German JB (1995) Effects of individual tocopherols and tocopherol mixtures on the oxidative stability of corn oil triglycerides. J Agric Food Chem 43:2345–2350

Johnson LA (2008) Recovery, refining, converting, and stabilizing edible fats and oils. CRC, New York

Kamal-Eldin A, Budilarto E (2015) Tocopherols and tocotrienols as antioxidants for food preservation. Elsevier, Oxford

Kamal-Eldin A, Makinen M, Lampi AM (2010) Lipid oxidation pathways. AOCS, Urbana

Kinen M, Kamal-Eldin A, Lampi AM, Hopia A (2000) Effects of α- and γ-tocopherols on formation of hydroperoxides and two

decomposition products from methyl linoleate. J Am Oil Chem Soc 77:801–806

Moreau RA, Whitaker BD, Hicks KB (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res 41:457–500

Wang T, Hicks KB, Moreau R (2002) Antioxidant activity of phytosterols, oryzanol and other phytosterols conjugates. J Am Oil Chem Soc 79:1201–1206

Winkler-Moser JK, Hwang HS, Bakota EL, Palmquist DA (2015) Synthesis of steryl ferulates with various sterol structures and comparison of their antioxidant activity. Food Chem 169:92–101

Yi J, Andersen ML, Skibsted LH (2011) Interactions between tocopherols, tocotrienols and carotenoids during autoxidation of mixed palm olein and fish oil. Food Chem 127:1792–1797