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Effect of phosphatidylethanolamine and phosphatidylserine on antioxidant capacity, oxidative stability and color reversion of camellia seed oil

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SUMMARY: Non-hydratable phospholipids as pro-oxidants are likely to cause a decrease in the quality of vegetable oils. The influence of phosphatidylethanolamine (PE) and phosphatidylserine (PS) on the oxidative stability, antioxidant capacity and color reversion of refined camellia seed oil (RCSO) was evaluated in this work. The PE/PS addition could improve the oxidative stability and antioxidant capacity, but was not a key factor in the color reversion of RCSO. The results clearly showed that PE and PS were not prooxidants but antioxidants in camellia seed oil, and the findings of the present study would be useful for extending the shelf-life of camellia seed oil and for retaining phospholipids during moderate refining.

KEYWORDS: *Antioxidant capacity; Color reversion; Oxidative stability; Phosphatidylethanolamine (PE); Phosphatidylserine (PS); Refined camellia seed oil (RCSO).*

RESUMEN: ***Efecto de la fosfatidiletanolamina y la fosfatidilserina sobre la capacidad antioxidante, la estabilidad oxidativa y la reversión del color del aceite de semilla de camelia.*** Es probable que los fosfolípidos no hidratables, como prooxidantes, causen una disminución en la calidad de los aceites vegetales. En este trabajo se ha evaluado la influencia de la fosfatidiletanolamina (PE) y la fosfatidilserina (PS) sobre la estabilidad oxidativa, la capacidad antioxidante y la reversión del color del aceite de semilla de camelia refinado (RCSO). La adición de PE/PS pudo mejorar la estabilidad a la oxidación y la capacidad antioxidante, pero no fue un factor clave en la reversión del color de RCSO. Los resultados mostraron claramente que PE y PS no eran prooxidantes sino antioxidantes en el aceite de semilla de camelia, y los resultados obtenidos en el presente estudio serán útiles para extender la vida útil del aceite de semilla de camelia y para retener los fosfolípidos, tanto como sea posible, durante el refinado moderado.

PALABRAS CLAVE: *Aceite de semilla de camelia refinado (RCSO); Capacidad antioxidante; Estabilidad oxidativa; Fosfatidiletanolamina (PE); Fosfatidilserina (PS); Reversión del color.*

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1. INTRODUCTION

Phospholipids, a low-level compound in vegetable oil, are usually present in the form of hydratable phospholipids (HP) and non-hydratable phospholipids (NHP). HP, mainly including phosphatidylcholine (PC) and phosphatidylinositol (PI), are removed after hydration and degumming, and what remains in the vegetable oil is NHP, including phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidic acid (PA) (Oybek *et al*., 2009). The concentration and composition of phospholipids which are endogenous to foods are dependent on the origin of the food and how it is processed. At the same time, phospholipids have a very significant effect on the oxidative stability of lipids as antioxidant, prooxidant, or oxidation substrates themselves (Cui and Decker, 2016; Rajesh, *et al*., 2021). Previous studies focused on the adverse effects of phospholipids as prooxidant on the quality of bulk edible oil, including changes in the oil's appearance such as color reversion and apparent turbidity (Zamora *et al*., 2004), producing bad flavor (likely rancid) and a large amount of foam and black deposits during cooking (Hafidi *et al*., 2005), and the negative effects on the storage stability of oil (Bo *et al*., 2006). However, more and more studies have demonstrated that phospholipids, as an antioxidant or antioxidant synergists, can maintain or improve the quality of canola oil (Jiyeun and Eunok, 2009) and Virgin olive oil (VOO) (Olivera *et al*., 2008), decreasing the intensity of VOO bitterness (Olivera *et al*., 2009), and interfere with the extraction of hydrophilic phenols in VOO (Olivera *et al*., 2010). The high-value phospholipid products used as functional food and nutraceutical ingredients have been exploitated from waste coming from the seed oil refining industry (Chiara *et al*., 2021; Christine *et al*., 2020).

Camellia seed oil (CSO), which is extracted from the seeds of *Camellia oleifera* Abel, has been used extensively for over two thousand years as edible oil and medicine in China, and has been labeled "Oriental Olive Oil" due to the more than 90% unsaturated fatty acids (mainly oleic acid and linoleic acid) and high levels of endogenous biophenols which are rich in quantity and diversity (Haiyan *et al*., 2007). Based on Chinese eating habits for the pursuit of characteristic flavor and nutrition, the current production of CSO in China is based on a physical pressing process after oilseed pre-treatment (including roasting or sun-dried), and then physical degumming (hydration or winterization degumming). Therefore, the prepared CSO contains a certain amount of NHP. To the best of our knowledge, few literature reports focused on the effects of NHP on the quality of CSO, which leads to a lack of practical theoretical basis for the practice of physical degumming of CSO. Therefore, research about NHP (PE and PS) and their effects on the antioxidant capacity, oxidative stability and color reversion of CSO has been carried out, and the results will have a very important theoretical impact on the development of pressing technology of CSO to preserve flavor and nutrition.

2. MATERIAL AND METHODS

2.1. Chemicals

The authentic standards (≥ 98.5%) and chromatographic grade organic solvent (≥ 99.9%) used in this work were all obtained from Sigma-Aldrich. Other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China.

2.2. Preparation of refined camellia seed oil and phospholipids

Refined camellia seed oil (RCSO) was obtained from pressed camellia seed oil by a laboratory-scale refining processes according to our previous report (Bo *et al*., 2016).

One thousand grams of the physically- pressed CSO were twice stirred with 5000 mL of *n*-hexane to collect crude phospholipids. The resulting *n*-hexane in crude phospholipids was removed by a rotary evaporation (RV 10 digital, 104IKA, Germany). About 5 g of crude phospholipids were used to separate PE and PS on a silica gel chromatographic column (3.0 × 50 cm). A solvent of light petroleum (65-75 °C)/isopropanol/water (1:1:0.175, v:v:v) was selected to elute and PE and PS was collected, evaporated and stored at -20 °C (Zheng *et al.*, 2005).

The phospholipids were spotted onto preparared silica gel TLC plates (TLC, silica gel GF UV-254, thickness 2 mm, 10 × 20 cm) and developed in the solvent chloroform:methanol:water (42:22:3, v/v/v). PL bands were identified by comparison with authentic standards [PE (Rf = 0.62) and PS (Rf = 0.43)] which were run in parallel. The band only containing PE or PS was scraped off and extracted three time with chloroform:methanol (2:1, v/v). The solvents were removed by nitrogen gas.

2.3. Analysis of fatty acid composition, phosphorus contents, total phenols and moisture in RCSO and phospholipids

An analysis of fatty acid composition in RCSO was carried out, and purified PE and PS were methylated and analyzed by the GC-FID according to our previous reports (Bo *et al*., 2016). The analysis of total phenols, phosphorus content and moisture in RCSO was conducted according to our previous report (Haiyan *et al*., 2007).

2.4. Accelerated oxidation experiment

0.2 g, 0.5 g, 1.0 g, 1.5 g, and 2.0 g of PE and PS were weighed and put in a test tube filled with 100 g RCSO, Then the tubes were placed in a water bath at 50°C with constant stirring to dissolve phospholipids, and the oil sample was cooled to 4°C. Then the Schaal oven method was used to heat continuously at 63°C for 20 d, and the oil samples were collected by taking out three separate test tubes every 4 d. The collected oil samples were stored in a refrigerator at 4°C for later use. Three replicates for each sample and RCSO without phospholipids were used as the control group.

2.5. Determination of oxidative stability of RCSO

Acid value (AV, expressed as mg KOH/g of oil), peroxide value (POV, expressed as the mass fraction of peroxide equivalent to iodine with g/100g), *p*-anisidine value (*p*-AV), and the induction period (IP) of RCSO were determined according to our previous report (Bo *et al*., 2018).

The Totox value was calculated as twice POV plus *p*-AV (Bo *et al*., 2018).

In order to evaluate the rate of changes in the oxidative stability of RCSO, the ΔAV, ΔPOV or Δ*p*-AV was calculated as follows:

$$\Delta = \frac{(X_T - X_C)}{X_C}\%$$

Where, and represents the data (AV, POV and *p*-AV) of samples with and without phospholipid addition on the same heating days.

2.6. Antioxidant capacity test of RCSO

The free-radical scavenging capacity (FRSC), including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and oxygen radical absorbance capacity (ORAC), were detrmined according to our previous report (Bo *et al*., 2018). The results of the DPPH, ABTS and ORAC tests were expressed as μmol of Trolox equivalent g^{-1} oil (μmol TE/g).

2.7. Color determination

The color value for RCSO was assayed using a colorimeter (Minolta CR-10 Plus, Konica Minolta (China) Investment Ltd.) according to the manufacturer's instructions.

$$\Delta E = \sqrt{(\triangle L^*)^2 + (\triangle a^*)^2 + (\triangle b^*)^2}$$

Where, ΔE: total color difference; ΔL*: brightness difference between the treated and control samples; Δa*: red / green difference between the treated and control samples; Δb*: yellow / blue difference between the treated and control samples.

2.8. Statistical analysis

All data were evaluated using analysis of variance (ANOVA) and significant differences among the means of three replicates ($p < 0.05$) were determined by Turkey's test using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). All figures were drawn with OriginPro 8.0 (OriginLab Corporation, Northampton, MA 01060, USA).

3. RESULTS AND DISCUSSION

3.1. Chemical composition analysis of RCSO, purified PE and PS

In present work, the fatty acid profile of RCSO was palmitic acid (PMA, C16:0) at 8.7%, stearic acid (SA, C18:0) at 1.52%, oleic acid (OA, C18:1) at 71.31%, linoleic acid (LA, C18:2) at 10.25%, and linolenic acid (LLA, C18:3) at 0.86% (Table 1), and the unsaturated fatty acid (UFA) content in RCSO was 89.87 (Table 1). The fatty acid profile and content in RCSO was consistent with our previous reports (Haiyan *et al*., 2007). The fatty acid profiles of PE and PS from CSO were as follows: PMA (20.87, 19.81%), SA (2.39, 3.15%), OA (48.88, 50.69%), LA (27.56%, 25.72%), and LLA (1.16 and 0.96%) (Table 1). The UFA contents in PE and PS were 77.28 and 76.41%, respectively (Table 1). The proportions and profiles of fatty acids in PE and PS were con-

Table 1. Analysis of chemical profiles of RCSO, purified PE and PS

Samples	C16:0	C18:0	C18:1	C18:2	C18:3	∑SFA	∑UFA	∑PUFA	Phosphorus Contents (mg/kg oil)	Total phenols (μg/g caffeic acid)	Moisture (%)
PS	19.81±2.21	3.15±0.39	50.69±6.13	25.72±4.92	0.96±0.3	22.87±2.08	76.41±2.01	26.68±1.94			
PE	20.87±0.04	2.39±0.03	48.88±0.07	27.56±0.04	1.16±0.04	23.26±0.08	77.28±0.08	28.72±1.33			
RCSO	8.70±1.03	1.52±0.52	71.31±8.02	10.25±2.01	0.86±0.22	10.21±0.23	89.87±1.36	12.57±0.15	ND	ND	≤0.05

Note: ND, not detected. Values are means ± SD of triplicate determinations.

sistent with that of RCSO. The phosphorus and total phenols were not detected in RCSO; the moisture of RCSO was less than 0.05% (data not shown).

The above results indicated that the main fatty acid profiles of RCSO, PE and PS were PMA, SA, OA, LA, and LLA. The proportions and profiles of fatty acids in PE and PS were consistent with that of RCSO. Refining had no significant influence on the fatty acid profiles or contents of CSO and polyphenols were removed very effectively.

3.2. Antioxidant capacity analysis of purified PE and PS

As expected, the key factor in determining whether phospholipids play a role in prooxidants or antioxidants in foods is closely related to their physical environment (Cui and Decker, 2016). The results in the present study indicated that PE and PS both have a certain DPPH scavenging capacity, and the scavenging capacity was positively related to the added concentration of PE and PS (Figure 1). For example, when the addition amount was 2.0%, the DPPH scavenging rate of PE and PS was 15 and 14%, respectively (Figure 1). Our results also matched the findings of Espín *et al.* (2000).

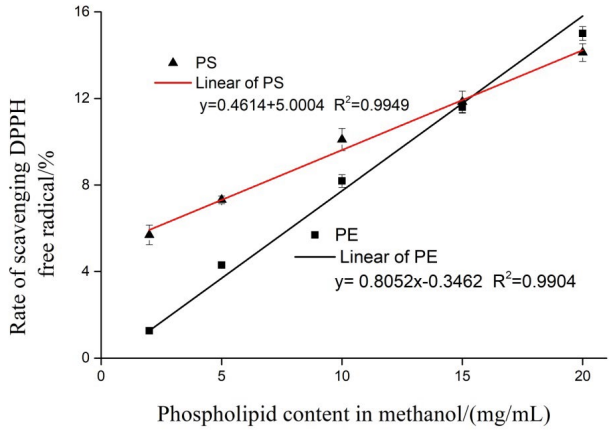

Figure 1. DPPH scavenging effect of PE and PS methanol chloroform solution (means ± SD of triplicate determinations)

The above results indicated that PE and PS obtained from CSO themselves played a role in antioxidants, and the antioxidant capacity of PS and PE depended on the concentration added.

3.3. Effect of PE/PS addition on antioxidant capacity of RCSO

DPPH, ABTS, and ORAC were used to evaluate the antioxidant capacity of RCSO, PE and PS in the present work. The values of DPPH, ABTS, and ORAC for RCSO without phospholipid (PE and PS) addition were 52.23 μmol TE/g, 63.45μmol TE/g, and 146.87μmol TE/g, respectively (Table 2). The results indicated that RCSO itself has a certain antioxidant capacity, which may be due to the more than 70% oleic acid in the RCSO (Haiyan *et al*., 2007).

The DPPH and ABTS changes in RCSO with PE addition showed an initial y decrease followed by a slightly increasing pattern (Table 2). Compared to RCSO without PE addition, the DPPH and ABTS was first decreased by 35 and 23% (0.2% addition), 17 and 9% (0.5% addition), 24 and 22% (1.0% addition), 9 and 1% (1.5% addition), and then increased by 11 and 5% (2.0% addition), respectively. Interesting, the ORAC changes in RCSO with PE addition always showed an upward trend, which was increased from 165.61μmol TE/g (0.2% addition) to 245.8 μmol TE/g (2.0% addition) (Table 2). DPPH, ABTS and ORAC changes with PS addition showed an upward trend (Table 2). DPPH, ABTS and ORAC were increased by 37, 79, and 29% (0.2% addition), and 54, 114, and 176% (2.0% addition), respectively, compared to RCSO without PS addition (Table 2). The results showed that PE and PS could both significantly improve the antioxidant capacity of RCSO, which was similar to the

Table 2. Antioxidant capacity and oxidative stability of PE and PS on RCSO

Phospholipid	Adding amount (w/v, %)	DPPH (μmol TE/g)	ABTS (μmol TE/g)	ORAC (μmol TE/g)	IP (h)
PE	0.0	52.23±0.47^{e}	63.45±0.69^{b}	146.87±2.96^{a}	3.72±0.02^{a}
	0.2	33.23±0.17^{a}	48.64±0.17^{a}	165.61±3.92^{b}	6.13±0.05^{b}
	0.5	43.37±0.25^{c}	57.56±0.25^{c}	207.84±2.50^{c}	9.11±0.01^{c}
	1.0	39.56±0.67^{b}	49.36±0.67^{c}	194.60±2.90^{c}	10.57±0.04^{c}
	1.5	47.45±0.91^{d}	62.74±0.91^{d}	223.24±4.54^{d}	17.70±0.02^{d}
	2.0	58.04±0.66^{f}	66.32±0.66^{e}	245.80±4.72^{d}	20.05±0.04^{e}
PS	0.0	52.23±0.47^{a}	63.45±0.69^{a}	146.87±2.96^{a}	3.72±0.02^{a}
	0.2	71.73±2.96^{bc}	113.36±2.96^{b}	189.35±6.35^{b}	5.35±0.02^{b}
	0.5	71.84±1.61^{bc}	108.71±1.61^{b}	235.56±4.75^{c}	7.92±0.03^{c}
	1.0	75.94±2.37^{cd}	117.54±2.37^{b}	308.23±6.88^{d}	10.06±0.02^{d}
	1.5	67.97±0.53^{b}	116.79±0.53^{b}	348.07±7.01^{e}	13.20±0.07^{e}
	2.0	80.68±1.57^{d}	135.52±1.57^{c}	405.22±6.28^{f}	16.22±0.05^{f}

Note: Values are means ± SD of triplicate determinations. Different letters in superscript within the same column indicate significant differences among the oil samples (Tukey's test, $p < 0.05$).

results reported for refined olive oil (Hidalgo *et al*., 2006), but contrary to results on perilla oil (Minoru e*t al*., 1991) and virgin olive oil (Olivera *et al*., 2008; Olivera *et al*., 2010).

As expected, the DPPH scavenging capacity of phospholipids was the cause of the changes in the DPPH scavenging capacity of the oil (Jiyeun and Eunok, 2009). The key factor to determine whether phospholipids have a certain DPPH scavenging capacity in RCSO depends on the polar groups of phospholipids and their content (Reis and Spickett, 2012). The one key factor that affects the antioxidant capacity of RCSO with PE/PS addition may be attributed to the stronger hydrophilicity of PS than that of PE in this paper, which could not only enhance the antioxidant activity of some hydrophilic primary oxidation products (likely peroxyl, alkane, alkene and aldehyde derivatives) derived from lipid oxidation (Zheng *et al*., 2005), but also promote their production (Reis and Spickett, 2012). Therefore, the addition of PE to improve the antioxidant capacity of RCSO was mainly attributed to the ORAC of PE, but PS addition to improve the antioxidant capacity of RCSO was attributed to not only ORAC but also to the FRSC of PS in the present work.

The above results in the present study indicated the RCSO itself has a certain antioxidant capacity, and phospholipids (PE and PS) could also significantly improve the antioxidant capacity of RCSO.

3.4. Effect of PE/PS addition on oxidative stability of RCSO

3.4.1. Induction period

The induction period (IP) of RCSO with PE and PS addition has been extended by 2.41 and 1.63 h (0.2%), 5.39 and 4.2 h (0.5%), 6.85 and 6.34 h (1.0%), 13.98 and 9.48 h (1.5%), 16.33 and 12.5 h (2.0%), respectively (Table 2). Our findings were compatible to some previous reports that a high concentration (0.5–2.0%) of lecithin showed obvious auto-oxidation inhibitory activity on VOO (Olivera *et al*., 2008), although contrary to the results reported for perilla oil (Minoru e*t al*., 1991), which may be related to the fatty acid composition and contents of phospholipids themselves (Cui and Decker, 2016). All the results in the present study indicated that PE and PS could improve the IP of RCSO.

3.4.2. Acid value

Acid value (AV) is used to measure the production of free fatty acids in RCSO in the present work. The AV of RCSO without PE/PS addition increased from 0.32 mg/g (0 d) to 0.71 mg/g (20 d), and the

significant changes in the AV of RCSO mainly occurred after 8 days (Figure 2A, 2B, 2a, 2b). Simultaneously, although PE/PS addition could promote an increase in the AV of RCSO, the increase rate of the AV in RCSO was inhibited (Figure 2B, 2b). For example, the AV of RCSO without PE/PS addition increased by 122% on the 20th day, and the AV of RCSO with the addition of PE/PS increased by 84/60.44% (0.2%), 95/47% (0.5%), 71/67% (1.0%), 38/36% (1.5%), and 33/26% (2.0%) compared to no added PE/PS (Figures 2A, 2a), respectively.

In general, thermal processing could result in hydroperoxides producing in primary oxidation processes and increasing the level of free fatty acid in heat-treated oils (Fozia *et al*., 2006). The results from this work demonstrated that the AV of RCSO did not change significantly before heating for 8 days due to the high-stability oleic acid with a content of more than 70% in RCSO (Table 1). The addition of PE and PS could inhibit the hydrolysis of RCSO, reduce the generation of free fatty acids and then slow down the rise of its AV, which may be ascribe to the antioxidant capacity of PE/PS (McDonnell *et al*., 1995). The inhibitory effect of PS on the AV of RCSO was more effective than that of PE, which was consistent with the report of Peng *et al*. (2020). The reason may be attributed to the FRSC of PS, which is stronger than that of PE (Figure 1 and Table 2), or the speed of antioxidant (including peroxyl, alkane, alkene, and aldehyde derivatives) production from the oxidation of PE, which is slower than that of PS (Reis and Spickett, 2012).

The results in the present work indicated PE and PS could inhibit the hydrolysis of RCSO to generate free fatty acids, and play a role in delaying the increase in the acid value (AV) of RCSO.

3.4.3. Peroxide value

The peroxide value (POV) of RCSO without PS/PE addition significantly increased from 0.11 g/100 g (4 d) to 1.02 g/100 g (20 d) (Figures 2C, 2D, 2c, 2d), respectively. The PE/PS addition could inhibit the POV increase in RCSO. For example, the POV of RCSO with 2.0% PS/PE addition increased by 0/200% (4 d), 100/500% (8 d), 167/733% (12 d), 333/ 1767% (16 d), 467/3300% (20 d) compared to no added PE/PS (Figure 2D, 2d), respectively. At the same time, PE/PS addition could significantly inhibit the increase rate of POV in RCSO. The rate of POV in RCSO with PE/ PS addition was increased by 49/-11% (0.2%), 25/-36% (0.5%), 15/-75% (1.0%), 5/-78% (1.5%), 0/-83% (2.0%) compared to without PE/PS addition (Figures 2C,2c), respectively.

The generation of oxidative free radicals occurred in the induction period (early period), and then a series of hydroperoxides and new free radicals were generated in the propagation period (late storage period) (McDonnell *et al*., 1995). So, the POV increase mainly occurred in the late heating period due to the automatic oxidation of RCSO (Figures 3A and 3C). The inhibition of PE/PS addition on the increase of POV in RCSO attributed to the improvement in the ORAC in RCSO in the present work (Table 2). At the same time, the inhibitory effect of PE was weaker than that of PS, which was contrary to previous reports (Jiyeun and Eunok, 2009), maybe due to the fact that the antioxidant capacity of PS was stronger than that of PE (Figure1 and Table 2). Other possible reasons included the following: the speed of antioxidant (including peroxyl, alkane, alkene, and aldehyde derivatives) production from the oxidation of PE, which is slower than that of PS (Reis and Spickett, 2012), or/and the hydrophilicity of PS, which is greater than that of PE (Reis and Spickett, 2012).

The results in the present work indicated that PE/PS could delay the POV increase in RCSO as they inhibited the oxidation of RCSO, the inhibitory effect of PS was significantly stronger than that of PE.

3.4.4. **p-***Anisidine value*

The *p*-anisidine value (*p*-AV) increased slowly before 12 days in the present work. PE/PS had an inhibitory effect on the increase in *p*-AV, and it was positively correlated with the amount of PE/PS addition (Figures 2E, 2F, 2e, 2f). In terms of PE addition, when the amount of PE addition was less than 1.0%, the inhibition of *p*-AV was not obvious, but the *p*-AV of RCSO with PE addition (1.0, 1.5 and 2.0%) showed a decreasing-increasing trend. For example, the *p*-AV of RCSO with a 0.2% PE addition increased by 18 (16 d) and 19% (20 d) compared to without PE addition, respectively (Figures 2E, 2F). As far as PS addition was concerned, the *p*-AV of RCSO with PS addition (0.2 and 0.5%) increased slowly before 12 days, then there was a downward trend with the increase in PS addition, such as the *p*-AV of RCSO with PS addition of 1.0, 1.5 and 2.0 decreased by 29, 224, and 268% on the 4th day, and

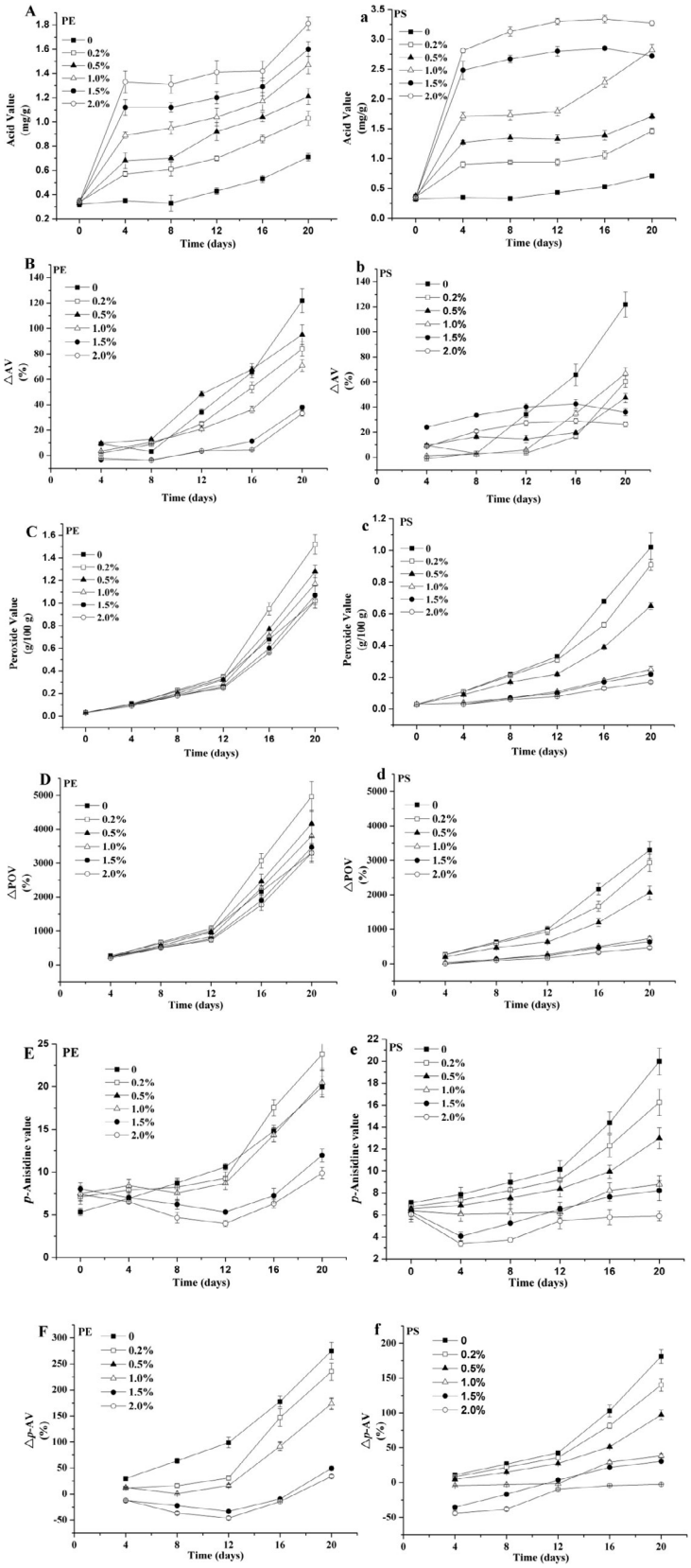

FIGURE 2. Effect of PE and PS on oxidative stability of RCSO during heating (means ± SD of triplicate determinations): (A, B, a, b) AV (mg/g), (C, D, c, d) POV (g/100 g), (E, F, e, f) *p*- AV

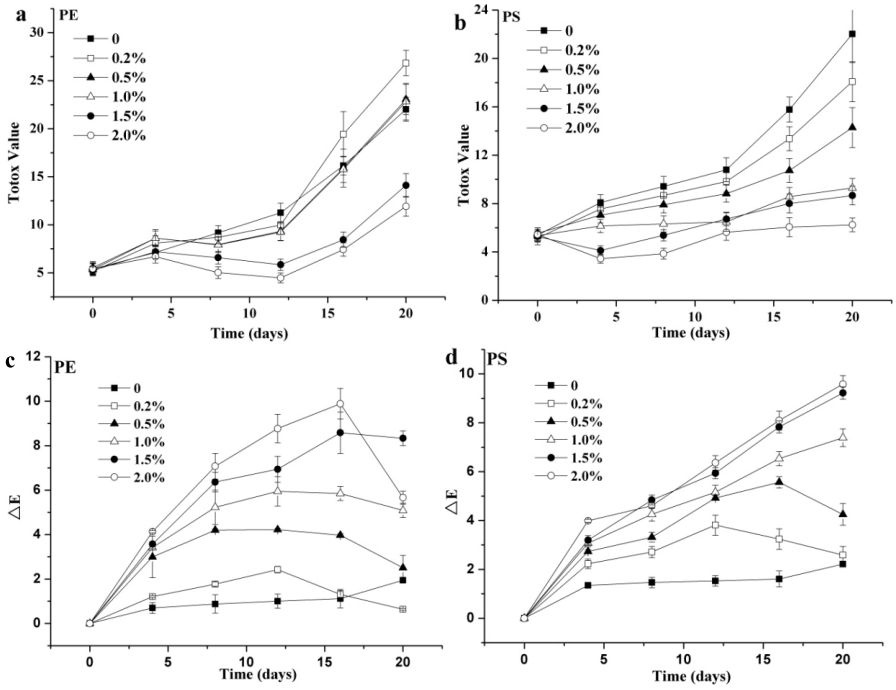

Figure 3. Effect of PE (a, c) and PS (b, d) on total oxidation value and total color difference (∆E) of RCSO during heating (means ± SD of triplicate determinations)

then increased by 29, 22, and -4% on the 16th day compared to no addition of PS (Figures 2e, 2f), respectively.

As expected, with prolonging oxidation, hydrogen peroxide will decompose, and most of the decomposed products cannot react with potassium iodide, so POV is not able to evaluate the oil quality accurately. Some other complementary indicators must be used to measure oil rancidity such as *p*-AV, which reflects the amount of unsaturated aldehydes (secondary oxidation products, including aldehydes, ketones, and quinones) of oils and fats (Seung *et al*., 2010). The more unsaturated the aldehydes, the easier it is to produce small molecules of aldehydes and ketones. In the present work, an increasing trend in *p*-AV in the early stage of heating (Figures 2E, 2F, 2e, 2f) may be attributed to the accumulation of primary oxidation products (Gökhan *et al*., 2010) and products of phospholipid degradation, which have a strong ability to convert primary oxidation products to the corresponding hydroxyl lipids (Xiangqing *et al*., 2010). The results of positive correlation between the inhibitory effect on the increase of *p*-AV and PE/PS addition amount was consistent with the report of (Peng *et al*., 2020). The reason may be due to the strong antioxidant capacity of PE/PS (Figure 1 and Table 2), and/or many antioxidants (including peroxyl, alkane, alkene, and aldehyde derivatives) produced from PE/PS oxidation (Reis and Spickett, 2012).

The results in the present work indicated that PS/PE could inhibit the formation of the secondary metabolites of carbonyl compounds, and the inhibitory effect of PS addition was far better than that of PE.

3.4.5. Totox value

PE addition could slow down the increase in the Totox value in RCSO.The Totox value of RCSO was increased by 22, 5, 4, -36 and -46% on the 20th day compared to no addition of PE (Figure 3a). Regardless of the amount of addition or the duration of heating, PS addition could dramatically decrease the Totox value of RCSO. The otox value of RCSO was decreased by 7% and 18% (0.2%), 13 and 35% (0.5%), 24 and 56% (1.0%), 49 and 61% (1.5%), 57 and 72% (2.0%) on the 4th and 20th days compared to no addition of PS (Figure 3b), respectively.

Besides *p*-AV, Totox value is also an important indicator to measure the oil rancidity acidity and can indicate an oil's overall oxidation state, which means the lower the Totox value, the better the quality of oil (Seung *et al*., 2010). The PE/PS addition could in-

hibit the increase in the Totox value of RCSO, which indicated that PE/PS could maintain the quality of RCSO (Figure 3a, 3b). The reasons for the Totox value change in RCSO may be due to the antioxidant capacity of PE/PS (Figure 1 and Table 2) to inhibit the formation of primary and secondary oxidation products (Figure 3b).

The results regarding changes in the Totox value of RCSO further indicated that PS/ PE addition could improve the oxidative stability of RCSO.

3.4.6. Color value

As shown in Figures 3c and 3d, Tables 3 and 4, the L*, a*, b*, c* and h* indicate the brightness, red-green, yellow-blue, chroma (the degree of color saturation or purity), and hue angle, respectively. In terms of PE addition to RCSO, except for the L* and h*, a*, b* and c* changed significantly. The more PE addition, the greater the a*, b* and c* of RCSO (Table 3). The PE addition caused a trend of first

Table 3. Effect of PE addition on color value of RCSO during heating

Heating (d)	PE (w/v,%)	L*	a*	b*	c*	h*
0	0	47.20±0.17^{a}	-0.57±0.12^{c}	14.27±0.38^{a}	14.28±0.38^{a}	92.28±0.49^{a}
	0.2	47.57±0.29^{a}	-0.54±0.06^{c}	14.77±0.75^{a}	14.78±0.75^{a}	92.09±0.32^{a}
	0.5	47.73±0.06^{a}	-0.54±0.06^{c}	16.07±0.06^{b}	16.08±0.06^{b}	91.91±0.20^{a}
	1.0	47.67±0.15^{a}	-0.44±0.06^{b}	16.67±0.38^{b}	16.68±0.38^{b}	91.50±0.21^{a}
	1.5	46.70±0.17^{a}	-0.40±0.06^{ab}	18.00±0.12^{c}	18.01±0.11^{c}	91.28±0.19^{a}
	2.0	46.63±0.61^{a}	-0.34±0.15^{a}	18.1±0.31^{c}	18.11±0.30^{c}	91.07±0.50^{a}
4	0	46.60±0.17^{b}	-0.57±0.06^{c}	13.93±0.21^{a}	13.94±0.21^{a}	92.33±0.21^{a}
	0.2	46.43±0.00^{b}	-0.53±0.06^{c}	15.20±0.06^{b}	15.21±0.06^{b}	92.15±0.21^{a}
	0.5	46.80±0.46^{b}	-0.40±0.10^{ab}	17.20±0.98^{c}	17.20±0.98^{c}	91.47±0.40^{a}
	1.0	46.43±0.00^{b}	-0.43±0.06^{b}	17.60±0.06^{cd}	17.60±0.06^{c}	91.53±0.19^{a}
	1.5	46.00±0.06^{a}	-0.43±0.06^{b}	17.63±0.10^{cd}	17.64±0.10^{c}	91.53±0.19^{a}
	2.0	45.83±0.00^{a}	-0.37±0.06^{a}	18.16±0.06^{d}	18.17±0.06^{d}	91.27±0.18^{a}
8	0	46.60±0.26^{a}	-0.57±0.06^{f}	13.63±0.32^{a}	13.65±0.32^{a}	92.38±0.23^{b}
	0.2	47.03±0.06^{a}	-0.66±0.06^{e}	16.03±0.10^{b}	16.04±0.10^{b}	92.37±0.22^{b}
	0.5	47.10±0.00^{a}	-0.46±0.06^{d}	18.46±0.06^{c}	18.47±0.06^{c}	91.44±0.18^{ab}
	1.0	46.77±0.32^{a}	-0.36±0.12^{c}	19.46±0.81^{cd}	19.47±0.81^{cd}	91.08±0.39^{ab}
	1.5	46.63±0.25^{a}	-0.26±0.06^{b}	20.60±0.46^{d}	20.60±0.46^{de}	90.74±0.17^{a}
	2.0	46.17±0.47^{a}	-0.10±0.17^{a}	21.23±1.65^{e}	21.23±1.65^{e}	90.29±0.47^{a}
12	0	46.60±0.20^{b}	-0.57±0.06^{c}	13.47±0.25^{a}	13.48±0.25^{a}	92.41±0.26^{b}
	0.2	47.03±0.21^{b}	-0.74±0.12^{d}	16.67±0.26^{b}	16.69±0.26^{b}	92.53±0.43^{b}
	0.5	46.37±0.00^{ab}	-0.70±0.00^{d}	18.40±0.06^{c}	18.42±0.06^{c}	92.19±0.01^{b}
	1.0	45.97±0.30^{a}	-0.54±0.06^{c}	20.07±1.15^{d}	20.08±1.15^{d}	91.54±0.25^{ab}
	1.5	45.43±0.21^{a}	-0.27±0.12^{b}	20.97±0.61^{d}	20.97±0.61^{d}	90.74±0.34^{a}
	2.0	45.57±0.26^{a}	-0.14±0.15^{a}	22.87±0.70^{e}	22.87±0.70^{e}	90.35±0.40^{a}
16	0	47.13±0.21^{bc}	-0.57±0.06^{b}	13.17±0.40^{a}	13.18±0.40^{a}	92.47±0.30^{b}
	0.2	47.26±0.31^{c}	-0.74±0.06^{c}	15.54±0.47^{b}	15.55±0.47^{b}	92.71±0.13^{b}
	0.5	46.96±0.38^{b}	-0.87±0.06^{cd}	18.20±0.72^{c}	18.22±0.72^{c}	92.74±0.26^{b}
	1.0	46.23±0.20^{ab}	-0.67±0.06^{bc}	20.04±0.35^{d}	20.05 ±0.35^{d}	91.92 ±0.19^{ab}
	1.5	46.36±0.21^{ab}	-0.27±0.15^{a}	22.80±0.95^{e}	22.81±0.94^{e}	90.69±0.41^{a}
	2.0	45.60±0.21^{a}	0.00±0.20^{d}	24.00±1.11^{f}	24.00±1.11^{f}	90.02±0.48^{a}
20	0	47.13±0.12^{c}	-0.50±0.00^{a}	12.33±0.06^{a}	12.34±0.06^{a}	92.32±0.01^{b}
	0.2	46.86±0.38^{b}	-0.73±0.06^{b}	13.80±0.40^{b}	13.82±0.40^{b}	93.04±0.17^{c}
	0.5	47.00±0.23^{c}	-0.87±0.10^{bc}	16.73±0.60^{c}	16.76±0.60^{c}	92.98±0.45^{b}
	1.0	46.30±0.15^{b}	-0.93±0.06^{c}	19.27±0.35^{d}	19.29±0.35^{d}	92.77±0.13^{b}
	1.5	45.73±0.35^{a}	-0.50±0.06^{a}	22.47±0.40^{e}	22.47±0.40^{e}	91.28±0.17^{a}
	2.0	49.70±0.15^{d}	-0.97±0.10^{c}	19.33±0.71^{d}	19.36±0.71^{d}	92.87±0.35^{bc}

Note: Values are means ± SD of triplicate determinations. Different letters indicate that there are significant differences between columns (Tukey's test, $p < 0.05$). L*: brightness; a*: red / green; b*: yellow / blue; c*: chroma; h*: hue angle.

increasing and then decreasing in RCSO ΔE during the heating process, and the higher amount of PE addition, the greater the ΔE of RCSO (Figure 3c). Regarding PS addition in RCSO, the brightness (L*) of RCSO decreased, but the a*, b* and c* of RCSO increased with the increase in heating time and PS addition (Table 4). The changing trend in RCSO ΔE with PS addition was similar to that of PE addition, but the effect of PS addition on RCSO ΔE was more obvious than that of PE addition (Figures 3c, 3d).

Table 4. Effect of PS addition on color value of RCSO during heating

Heating (d)	PE (w/v, %)	L*	a*	b*	c*	h*
0	0	47.20±0.17^{a}	-0.57±0.12^{b}	14.27±0.38^{a}	14.27±0.38^{a}	92.23±0.42^{a}
	0.2	46.67±0.42^{a}	-0.47±0.12^{ab}	14.70±0.35^{a}	14.70±0.35^{a}	91.87±0.38^{a}
	0.5	46.20±0.35^{a}	-0.43±0.12^{a}	14.53±0.38^{a}	14.53±0.38^{a}	91.63±0.38^{a}
	1.0	46.47±0.29^{a}	-0.53±0.06^{b}	14.60±0.35^{a}	14.60±0.35^{a}	92.13±0.25^{a}
	1.5	46.93±0.38^{a}	-0.40±0.10^{a}	15.40±0.52^{b}	15.40±0.52^{b}	91.60±0.30^{a}
	2.0	46.50±0.10^{a}	-0.50±0.10^{ab}	15.47±0.06^{b}	15.47±0.06^{b}	91.70±0.36^{a}
4	0	46.60±0.17^{b}	-0.57±0.06^{c}	13.93±0.21^{a}	13.2±0.00^{a}	92.30±0.20^{b}
	0.2	45.63±0.12^{ab}	-0.40±0.10^{b}	15.37±0.76^{b}	15.13±0.15^{b}	91.47±0.38^{ab}
	0.5	45.07±0.06^{a}	-0.30±0.00^{ab}	15.73±0.21^{bc}	15.30±0.10^{b}	91.13±0.12^{ab}
	1.0	44.90±0.17^{a}	-0.37±0.06^{ab}	16.03±0.42^{bc}	15.00±0.00^{b}	91.20±0.17^{ab}
	1.5	44.93±0.15^{a}	-0.30±0.00^{ab}	16.33±0.29^{c}	16.23±0.06^{c}	91.07±0.06^{ab}
	2.0	44.9±0.10^{a}	-0.23±0.12^{a}	17.40±0.66^{d}	17.40±0.66^{d}	90.83±0.38^{a}
8	0	46.60±0.26^{b}	-0.57±0.06^{d}	13.63±0.32^{a}	13.63±0.32^{a}	92.43±0.23^{bc}
	0.2	45.67±0.06^{ab}	-0.47±0.12^{c}	16.23±0.35^{b}	16.23±0.35^{b}	91.60±0.36^{b}
	0.5	45.27±0.12^{ab}	-0.43±0.12^{c}	16.73±0.65^{b}	16.73±0.65^{b}	91.40±0.44^{b}
	1.0	45.53±0.31^{ab}	-0.07±0.06^{b}	18.10±0.82^{c}	18.10±0.82^{c}	90.17±0.25^{a}
	1.5	45.30±0.30^{ab}	0.03±0.21^{a}	18.63±1.19^{c}	18.63±1.19^{c}	89.90±0.72^{a}
	2.0	44.87±0.12^{a}	0.00±0.10^{a}	18.20±0.20^{c}	18.20±0.20^{c}	90.03±0.21^{a}
12	0	46.60±0.20^{c}	-0.57±0.06^{d}	13.47±0.25^{a}	13.47±0.25^{a}	92.33±0.31^{b}
	0.2	46.00±0.17^{bc}	-0.53±0.06^{d}	17.70±0.52^{b}	17.70±0.52^{b}	91.77±0.31^{b}
	0.5	45.37±0.40^{b}	-0.33±0.06^{c}	18.73±0.15^{c}	18.73±0.15^{c}	90.97±0.15^{ab}
	1.0	45.07±0.15^{ab}	0.00±0.00^{b}	18.93±0.31^{c}	18.93±0.31^{c}	90.00±0.10^{a}
	1.5	44.77±0.21^{a}	0.23±0.06^{a}	19.67±0.51^{cd}	19.67±0.51^{cd}	89.33±0.21^{a}
	2.0	44.57±0.12^{a}	0.33±0.23^{a}	20.03±1.12^{d}	20.03±1.12^{d}	89.03±0.64^{a}
16	0	47.13±0.21^{c}	-0.57±0.06^{d}	13.17±0.40^{a}	13.17±0.40^{a}	92.40±0.35^{bc}
	0.2	46.20±0.20^{bc}	-0.87±0.06^{e}	16.97±0.60^{b}	16.97±0.60^{b}	92.90±0.17^{bc}
	0.5	45.90±0.10^{b}	-0.53±0.06^{d}	19.57±0.25^{c}	19.57±0.25^{c}	91.57±0.15^{b}
	1.0	44.97±0.12^{a}	-0.07±0.21^{c}	20.37±0.86^{c}	20.37±0.86^{cd}	89.63±0.45^{ab}
	1.5	44.73±0.06^{a}	0.50±0.10^{b}	21.70±0.26^{d}	21.70±0.26^{d}	88.70±0.20^{a}
	2.0	44.17±0.06^{a}	0.73±0.25^{a}	21.77±0.93^{d}	21.73±0.87^{d}	88.20±0.61^{a}
20	0	47.13±0.12^{e}	-0.50±0.00^{d}	12.33±0.06^{a}	12.33±0.06^{a}	92.43±0.06^{c}
	0.2	46.13±0.15^{d}	-0.90±0.00^{e}	16.07±0.57^{b}	16.07±0.57^{b}	93.17±0.06^{c}
	0.5	45.57±0.21^{cd}	-0.87±0.06^{e}	17.90±0.62^{c}	17.93±0.61^{c}	92.73±0.21^{c}
	1.0	44.90±0.20^{c}	0.17±0.15^{c}	21.27±1.07^{d}	21.27±1.07^{d}	89.63±0.42^{bc}
	1.5	43.57±0.15^{b}	0.80±0.17^{b}	22.73±0.55^{e}	22.73±0.55^{e}	88.00±0.35^{b}
	2.0	42.27±0.84^{a}	1.23±0.25^{a}	22.43±0.25^{e}	22.47±0.25^{e}	86.90±0.56^{a}

Note: Values are means ± SD of triplicate determinations. Different letters indicate that there are significant differences between the columns (Tukey's test, $p < 0.05$). L*: brightness; a*: red / green; b*: yellow / blue; c*: chroma; h*: hue angle.

For example, the ΔE of RCSO reached 3.81 (12th day) and 5.57 (16th day) for 0.2 and 0.5% PS addition (Figure 3d), respectively; while the RCSO ΔE reached 7.39, 9.22, and 9.58 for 1.0, 1.5, and 2.0 PE addition on the 20th day (Figure 3c), respectively.

As expected, refined edible vegetable oil appears light yellow and pale amber, but the color reversion of oils (especially for refined soybean, cottonseed and corn oils) often makes them darker and develop into deep yellow and light red during the transportation, storage and use (Mostafa *et al*., 2014). So far, it has been recognized that precursors to colored substances (such as chroman-5, 6-quinone, γ-tocopherol, γ-tocopherol dimer). Their degradation products, and the oxidation of oils (such as oxidized unsaturated fatty acids) were said to be responsible for the color reversion of oils (Ming-Tain *et al*., 1989). It is worth noting that precursors promote the dark color of oils at the same time, which also promotes or inhibits the oxidation of oils (František *et al*., 2016). The color changes with inconspicuous darkening, reddening, and yellowing in the present work indicated that PE and PS could improve a slightly dark color in RCSO (Figure 3, Tables 3 and 4), which was similar to what was reported by (František *et al*., 2016). PE and PS are amino phospholipids and prone to Maillard reaction to produce a small amount of colored substances (likely pyrroles) during the temperature acceleration process (Reis and Spickett, 2012), which may be a factor to cause some color reversion RCSO.

The results in the present study indicated that PS/PE addition could cause some color reversion in RCSO due to the formation of colored products from the Maillard reaction or/and hydrolysis and oxidation of lipids.

3.5. Correlation of oxidative stability, antioxidant capacity and color reversion of RCSO in terms of addition of PE and PS

3.5.1 Correlation of antioxidant capacity of RCSO in terms of addition of PE and PS

PE addition has an extremely significant correlation with ORAC (0.967, $p < 0.01$) and significant correlations with DPPH (0.787, $p < 0.05$), and ABTS (0.886, $p < 0.05$) (Table 5). However, PS addition has extremely significant correlations with ORAC, DPPH and ABTS, and the Pearson correlation coefficients are 0.931 ($p < 0.01$), 0.897 ($p < 0.01$) and 0.843 ($p < 0.01$), respectively (Table 5). The above results further showed that PS/PE addition can improve the antioxidant capacity of RCSO (Table 3 and 5). PE mainly improved the ORAC in RCSO; while PS not only improved the ORAC of RCSO, but also improved its FRSC (Tables 3 and 5, and Figure 2).

3.5.2 Correlation of oxidative stability of RCSO in terms of addition of PE and PS

As far as the oxidative stability (including AV, POV and *p*-AV) of RCSO was concerned, apart from an extremely significant correlation between PE addition and AV (0.860, $p < 0.01$), an unobvious and negatively significant correlation between PE addition and POV (-0.096), and *p*-AV (-0.434, $p < 0.01$), respectively. These results indicated that PE addition has no obvious inhibitory effect on the primary oxidation of RCSO, leading to an increase in *p*-AV. The positive correlation between PS addition and the AV of RCSO, POV and *p*-AV were extremely significant, and the Pearson correlation coefficients were 0.951 ($p < 0.01$), 0.676 ($p < 0.01$) and 0.629 ($p < 0.01$), respectively (Table 5). These results indicated that PS could inhibit primary and secondary oxidation reactions, and then result in a decrease in the production of primary and secondary oxidation products (Figure 2). An extremely significant correlation between PE/PS addition and the IP of RCSO (0.958, $p < 0.01$)/ (0.966, $p < 0.01$) further indicated that PE and PS could improve the oxidation stability of RCSO (Table 5).

3.5.3 Correlation of color reversion of RCSO in terms of addition of PE and PS

PE/PS addition had significant negative correlations with L*, the Pearson's correlation coefficients were -0.302 ($p < 0.01$) and -0.668 ($p < 0.01$) (Table 5), respectively. However, there was a significant positive correlation between PE/PS addition and color value (a*, b* and ΔE), the Pearson's correlation coefficients between PE/PS addition and a*, b* and ΔE were 0.420/0.506 ($p < 0.05$), 0.323/0.436($p < 0.05$) and 0.417/0.408 ($p < 0.05$) (Table 5), respectively. The above results from the correlation analysis further showed that PE/PS addition can cause some color reversion (Tables 3, 4 and Figure 3).

Based on the analysis of the correlation between color change and oxidative stability indexes (including AV, POV and *p*-AV) of RCSO in terms of PE/PS addition, the POV of RCSO had no correlation with L*, b*, and ΔE, and a significant negative correlation with a*

Table 5. Pearson correlation analysis between PE, PS, and oxidative stability of RCSO

	IP	**ORAC**	**ABTS**	**DPPH**	**AV**	**POV**	**p-AV**	**L***	**a***	**b***	**ΔE**
IP	1	.932**	.635*	.542*	.765**	.886**	.753**	.235*	.156*	.266*	.324*
ORAC		1	0.007	-0.012	.851**	.810**	.798**	.123	.105	0.089	.111
ABTS			1	-0.207	.567**	.154*	.235*	-0.365	-0.214	.156	.212
DPPH				1	.862**	-0.097	-.433**	-0.307	.509**	.836**	.827**
AV					1	.386*	0.008	-0.184	0.131	.781**	.790**
POV						1	.846**	0.142	-.516**	0.005	0.093
p-AV							1	0.246	-.624**	-.432**	-.362*
L*								1	-.536**	-.442**	-.463**
a*									1	.469**	.510**
b*										1	.969**
ΔE											1
PE	.958**	.967**	.886*	.787*	.860**	-0.096	-.434**	-0.302**	.420*	.323*	.417*
IP	1	.932**	753**	.798**	.813**	.857**	.798**	.324*	.361*	.231*	.278*
ORAC		1	0.036	-0.035	.836**	.887**	.813**	.089	.134	0.116	.098
ABTS			1	-0.026	.812**	.668**	.735**	.165	.105	.116	.097
DPPH				1	.936**	-.482**	-.633**	-.709**	.633**	.705**	.633**
AV					1	-0.326	-.497**	-.764**	.753**	.781**	.761**
POV						1	.961**	0.282	-.349*	-0.189	-0.057
p-AV							1	.440**	-.436**	-0.324	-0.196
L*								1	-.837**	-.847**	-.873**
a*									1	.787**	.846**
b*										1	.963**
ΔE											1
PS	.966**	.931**	.843**	.897**	.951**	.676**	.629**	-.668**	.506*	.436*	.408*

Notes: **0.01 level (bilateral) extremely significant.* 0.05 level (bilateral) significant. L*: brightness; a*: red / green; b*: yellow / blue; ΔE: total color difference.

(-0.516, $p < 0.01$) and (-0.349, $p < 0.05$) for PE and PS addition, respectively. In term of PE addition, AV had no correlation with L* (-0.184) and a* (0.013), and extremely significant positive correlation with b* (0.781, $p < 0.01$) and ΔE (0.790, $p < 0.01$). *p*-AV had no correlation with L* (0.246), significant negative correlation with a* (-0.624, $p < 0.01$), b* (-0.432, $p < 0.01$), and ΔE (-0.362, $p < 0.05$). As far as PS addition was concerned, AV had an extremely significant negative correlation with L* (-0.764, $p < 0.01$), and extremely significant positive correlation with a* (0.753, $p < 0.01$), b* (0.781, $p < 0.01$), and ΔE (0.761, $p < 0.01$). *p*-AV had no correlation with b* (-0.324) and ΔE (-0.196), extremely significant positive correlation with L* (0.440, $p < 0.01$), and extremely significant negative correlation with a* (-0.436, $p < 0.01$). These results indicated that the key reason for the color reversion of RCSO added with PE/PS may have been due to the AV changes in RCSO. As expected, the prerequisite for the color reversion is the free fatty acid formation by lipid hydrolysis or further oxidation of the PE/PS to produce some colored substances (causing the oil's yellow value to deepen) (Yuquan *et al*., 2013). In the present work, the possible reasons for the color reversion caused by PE/PS addition may be attributed to the free fatty acid formation or the color of the phospholipid itself (Reis and Spickett, 2012).

4. CONCLUSION

This work demonstrated that PE/PS addition could improve the oxidative stability and antioxidant capacity of RCSO, and the effect of PS addition was far better than that of PE. The possible reason for PE addition to improve the oxidative stability and

antioxidant capacity of RCSO is the inhibiting of the generation of free fatty acids, while there was a comprehensive result (including the inhibition of free fatty acid production, and the generation of primary and secondary oxidation products) for PS addition. Therefore, moderate refining to keep the non-hydratable phospholipids as much as possible can not only improve the functional and nutritional value but also extend the shelf-life of the CSO.

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Conflict of Interest

The authors declare no competing financial interest.

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