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# Dietary Selenium Supplementation Improves the Antioxidant Capacity and Oxidative Stability of *Camellia oleifera* Seed Oil

Chenhao Zhao <sup>1,2</sup>, Yanzhi Xiong <sup>1,2</sup>, Mengjia Xu <sup>1,2,\*</sup> and Mingquan Guo <sup>1,2,\*</sup>

<sup>1</sup> Zhejiang International Cooperation Base of Biomedical Materials Technology and Application, Zhejiang Engineering Research Center for Biomedical Materials, Ningbo Cixi Institute of Biomedical Engineering, Cixi 315300, China

<sup>2</sup> Ningbo Key Laboratory of Biomedical Imaging Probe Materials and Technology, Laboratory of Advanced Theranostic Materials and Technology, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo 315201, China

\* Correspondence: xumengjia@nimte.ac.cn (M.X.); guomingquan@nimte.ac.cn (M.G.)

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**Abstract:** The use of plant-based biofortification techniques presented only a limited increase in selenium enrichment in *Camellia oleifera* seed oil (COSO). Direct selenium enrichment represents a useful approach to increase both the selenium concentration and the nutraceutical value of COSO. Besides, the antioxidant properties of selenium-enriched COSO are not yet sufficiently characterized. Here, the total phenolic contents and total flavonoid contents of the COSO samples from different geographical origins were determined. Furthermore, DPPH radical scavenging, ABTS decolorization, and FRAP assays were used to evaluate the association between selenium concentration and the antioxidant capacity. The antioxidant assays showed that the ABTS radical cation scavenging capacity is mainly driven by the phenolics, while the DPPH radical scavenging capacity involves both the phenolics and polyphenolic contents. The supplementation of selenoprotein and Se-methylselenocysteine (SeMC) not only enhanced overall antioxidant capacity, but also conferred protection against lipid oxidation. Besides, the selenium-fortified samples in general kept the characteristic flavor profile, although a measurable reduction in overall aroma intensity was observed. Altogether, selenoprotein and SeMC demonstrated promise as selenium-fortifying agents for COSO, and direct selenium fortification of COSO is a technically feasible and nutritionally effective intervention to elevate its selenium concentration and enhance its nutraceutical functionality. These results support the antioxidant capacity of COSO and show the essential contribution of selenium to its functionality as a dietary supplement with nutraceutical properties.

**Keywords:** *Camellia oleifera* seed oil; selenium fortification; antioxidant capacity; oxidative stability; flavor profile; dietary selenium supplement

## 1. Introduction

*Camellia oleifera* C. Abel (*C. oleifera*), a member of the genus *Camellia* within the Theaceae family, is a highly important woody oil plant in China and ranks among the world's four major woody edible oil sources [1]. It is widely cultivated and mainly distributed in southern China. *C. oleifera* seed oil (COSO) is obtained from the seeds of this plant. Because of its nutritional value and health benefits, COSO is known as the “Oriental olive oil” and “Longevity oil” [2]. Especially, COSO is characterized by a high amount of oleic acid and linoleic acid, but the oleic acid is predominant [3]. Besides, the content of saturated fatty acids is lower than that of other edible oils,



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even lower than that of olive oil [3]. In addition, numerous studies have proved that it has abundant bioactive components and has positive effects on health, such as lowering cholesterol, anti-inflammatory, scavenging free radicals, decreasing blood pressure, and enhancing immune function [4]. These results highlight the potent antioxidant properties of COSO in the relief of chronic conditions related to oxidative stress, and these are primarily ascribed to the cooperative actions of CO's natural antioxidant components, such as flavonoids, oleic acid, polyphenolic, squalene and tocopherols compounds [5].

Selenium is an essential trace element which is widely distributed in human cells, tissues and organs, and is very important for maintaining physiological functions and overall human health. It exerts diverse biological functions, for example, antioxidant activity, anti-cancer, protection against cardiovascular disease, and protection of the human body against heavy metal toxicity [6]. It has been proposed that selenium supplementation in human diets is a strategy to prevent or to address selenium deficiency, such as cardiovascular diseases, Kashin-Beck disease and Keshan disease [7]. The Chinese Nutrition Society recommends a daily dietary selenium intake of 50 micrograms for people aged 7 and above, and an average requirement of 60 micrograms per day for the Chinese general population [8]. However, the widespread selenium deficiency in China has led to a compromised dietary selenium chain from soil and crops to livestock, and finally to humans, which is a substantial public health concern. Therefore, the strategic advance of selenium-biofortified foods is of critical importance. COSO is one of the promising functional foods owing to its diverse nutritional value. But *Camellia oleifera* is widely cultivated and mainly distributed in southern China, where the soil is deficient in selenium. Therefore, the incorporation of selenium into COSO will further enhance the efficacy and quality of COSO as well as promote human health.

Currently, numerous studies are engaged in incorporating selenium into oilseed crops to produce selenium-enriched edible oils. In recent years, significant progress has been achieved in the application of plant-based biofortification techniques to oilseed crops. Mrština et al. demonstrated that the foliar spraying of selenium fertilizer on soybeans can significantly increase the selenium content and selenium absorption of soybeans [9]. Ren et al. employed a combined approach of soil cultivation and hydroponic systems for selenium biofortification in rapeseed, resulting in increased selenium content [10]. However, selenium enrichment in COSO using this technique had resulted in only limited increases of selenium content (from 0.0124 mg/kg to approximately 0.020–0.0366 mg/kg), which was lower than the selenium content standard for selenium-enriched edible vegetable oils in Hubei Province (0.1–0.5 mg/kg) [11]. The direct selenium enrichment method refers to increasing the selenium content in food by adding selenium compounds directly, which can usually be achieved through the incorporation of organic or inorganic selenium. The selenium source directly affects the stability and flavor characteristics of the COSO product. Currently, inorganic selenium is still mainly employed to increase the content of selenium in selenium-enriched foods, among which sodium selenite is the most common. However, inorganic selenium exhibits a series of limitations, including low absorption and bioavailability, a narrow safety range between the toxic dose and the physiological requirement [12]. Therefore, its application is subject to stringent regulatory limitations. In contrast, organic selenium presents a higher bioavailability, lower toxicity, and greater biological activity, making it a preferred selenium supplement for human consumption [13]. Precisely, organic selenium is actively transported through the intestinal wall, while inorganic selenium is absorbed by passive diffusion [14]. The permitted sources of organic selenium fortification compounds include selenoprotein, Se-methylselenocysteine (SeMC), seleniumized carrageenan, and selenium-enriched yeast [15]. China has set up clear regulations regarding the above-mentioned selenium sources and also has developed corresponding technical standards and management protocols, which explicitly define permissible selenium concentration ranges [16]. Specifically, for selenium-enriched edible oils, the regulatory limit for selenium content is 0.02–1.00 mg/kg, and exceeding this upper limit may pose potential health risks.

In this work, we first investigated the variability in total polyphenols and total flavonoids content of the COSO samples from different origins. Then, selenoprotein and SeMC were chosen as selenium-fortifying agents to evaluate their influence on antioxidant activity and protection capacity on lipid oxidation. It was evidenced that the total polyphenols and total flavonoids were positively related to the antioxidant activity, and the selenium content also contributed to the enhanced antioxidant capacity. The supplementation of selenium improved the oxidative stability of COSO without compromising the native flavor profile of the oil. Overall, selenoprotein and SeMC show promise as selenium-fortifying agents, and the direct selenium enrichment could be an effective approach to improve the selenium content and health value of camellia oil.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

JX1 COSO sample was obtained from Jiangxi Yezhu Forestry Development Co., Ltd. (Shangrao, China). HB COSO sample originated from Longzhu high mountain was collected from Enshi Xinxin E-commerce Co., Ltd. (Enshi, China). Selenium-enriched COSO sample (JX2) was obtained from Jiangxi Xihai Oil Co., Ltd. (Yichun, Jiangxi, China). ZJ COSO sample was obtained from Zhejiang Shengtai Tea Oil Technology Co., Ltd. (Changshan, Zhejiang, China). Selenoprotein (500 ppm) was purchased from Shaanxi Ruimao Biotechnology Co., Ltd. (Xi'an, Shaanxi, China). SeMC (98%) was purchased from Zhengzhou Yukong Biotechnology Co., Ltd. (Zhengzhou, Henan, China). DPPH, ABTS, and Folin-Ciocalteu reagent were purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Hexahydrate ferric chloride, gallic acid, aluminum chloride, rutin, and ferrous sulfate were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Hydrochloric acid and anhydrous sodium carbonate were purchased from Shanghai Hushi Laboratory Equipment Co., Ltd. TPTZ was obtained from Shanghai Yongchuan Biotechnology Co., Ltd. (Shanghai, China).

### 2.2. Preparation of Dietary Selenium-Supplemented COSO Samples

Dietary selenium-supplemented working solutions were prepared by separately dissolving 98 mg of selenoprotein and 108 µg of SeMC in 10 mL and 1 mL of 70% (v/v) aqueous ethanol, respectively. Subsequently, each working solution was added to a separate 100 g of ZJ COSO sample. Then, the mixtures were sonicated for 10 min, followed by placing in a 40 °C water bath with continuous stirring until complete evaporation of ethanol. The theoretically calculated selenium contents in selenoprotein-supplemented and SeMC-supplemented COSO were 0.490 mg/kg and 0.494 mg/kg, respectively.

### 2.3. Quantitative Determination of Baseline Selenium Content in COSO Samples without Selenium Supplementation

COSO samples were weighed (0.25 g) and transferred into a microwave digestion tube, then 6 mL of HNO<sub>3</sub>, 1 mL of H<sub>2</sub>SO<sub>4</sub>, and 1 mL of H<sub>2</sub>O<sub>2</sub> was added. The mixture was heated at 130 °C for 30 min for pre-digestion. After cooling to room temperature, microwave-assisted digestion process was carried out under the following temperature program: (i) heating from 0 °C to 120 °C at a ramp rate of 24 °C/min (5 min), followed by a 10-min hold; (ii) heating from 120 °C to 150 °C at a ramp rate of 6 °C/min (5 min), followed by a 5-min hold; and (iii) heating from 150 °C to 180 °C at a ramp rate of 6 °C/min (5 min), followed by a 10-min hold. The mixture was then transferred to a glass bottle for acid evaporation and finally diluted to 2.5 mL before analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7850, United States). Selenium standard solutions (0.5–2 µg/L) were prepared sequentially to plot the calibration curve.

### 2.4. Determination of Total Phenolic Content (TPC)

The TPC values of COSO samples were determined according to the Folin–Ciocalteu method reported in a previous study [17]. Briefly, the mixture containing the diluted sample (20 µL), Folin–Ciocalteu (20 µL with ultrapure water, 25%, v/v), Na<sub>2</sub>CO<sub>3</sub> (100 µL, 1 M) and deionized water (20 µL) was incubated in the dark at room temperature for 1 h. After incubation, the absorbance at 760 nm was measured. Gallic acid standard solutions (5–40 µg/mL) were prepared sequentially to establish a calibration curve. TPC values were reported as milligrams of gallic acid equivalents per gram of COSO sample (mg GAE/g).

### 2.5. Determination of Total Flavonoid Content (TFC)

The TFC values of COSO samples were quantified using an aluminum chloride colorimetric assay as previously reported [18]. Briefly, the mixture of sample solution (60 µL), methanol (360 µL) and NaNO<sub>2</sub> solution (20 µL, 5%, w/v) was incubated at room temperature for 6 min. Then, AlCl<sub>3</sub> solution (40 µL, 10%, w/v) was introduced into each tube and mixed uniformly for 6 min. Thereafter, 40 µL of 10% AlCl<sub>3</sub> was incorporated and incubated for an additional 6 min. Subsequently, with the addition of NaOH solution (120 µL, 4%, w/v), the absorbance was measured at 510 nm after 15 min reaction. TFC values were reported as milligrams of rutin per gram of COSO sample (mg RE/g sample).

## 2.6. Antioxidant Capacity Evaluation

### 2.6.1. DPPH Free Radical Scavenging Capacity

The antioxidant capacity of COSO samples toward DPPH free radicals was assessed [19]. Firstly, COSO sample in each group (10  $\mu$ L) was mixed with DPPH solution (190  $\mu$ L, 100  $\mu$ M) and kept for half an hour in the dark under ambient conditions. Subsequently, the absorption value at 517 nm was recorded. DPPH free radical scavenging capacity was quantified as the percentage inhibition (%) according to the formula:

$$\text{Scavenging inhibition rate (\%)} = \frac{A_c - A_s}{A_c} \times 100\%$$

where  $A_c$  and  $A_s$  denote the absorption values of the control (without sample) and the sample-containing reaction mixture, respectively.

### 2.6.2. ABTS Free Radical Scavenging Capacity

A minor adaptation of the protocol described previously was applied to conduct ABTS radical cation scavenging assays of COSO samples [20]. The working solution (ABTS<sup>+</sup>) was prepared by mixing an equal-volume mixture of 7 mM ABTS aqueous solution and 4.9 mM potassium persulfate aqueous solution and standing in the dark for 12–16 h. To achieve a target absorbance of  $0.700 \pm 0.100$  at 734 nm, the ABTS<sup>+</sup> solution was properly diluted with methanol. Then, 190  $\mu$ L of ABTS<sup>+</sup> solution was mixed with 10  $\mu$ L of samples, and the absorption values at 734 nm were recorded after incubation in the dark for 30 min. The results of ABTS scavenging activity were determined according to the aforementioned formula, where  $A_c$  and  $A_s$  denote the absorption values of the control (without sample) and the sample-containing reaction mixture, respectively.

### 2.6.3. Assessment of Ferric Ion-Reducing Antioxidant Capacity (FRAP)

The FRAP assay was performed according to the protocol reported in prior work [21]. The FRAP working solution was freshly prepared through a mixture of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub>, combined in a volume ratio of 10:1:1. Subsequently, the mixture of diluted test solutions (10  $\mu$ L), deionized water (30  $\mu$ L) and FRAP working solution (260  $\mu$ L) was incubated at 37 °C for 10 min. The absorption value of the mixture was measured at 593 nm. FeSO<sub>4</sub> standard solutions (31.25–1000  $\mu$ M) were adopted to plot a standard concentration-response curve.

## 2.7. Stability Assessment of COSO Samples

### 2.7.1. Homogeneity Assessment

The homogeneity of dietary selenium-supplemented COSO samples was observed under daylight by taking pictures at 5-day intervals to monitor the amount of precipitation.

### 2.7.2. Oxidation Stability Assessment

The lipid oxidation process was accelerated using the Schaal oven method [22]. The dietary selenium-supplemented COSO samples were incubated at 60 °C and aliquots were collected at 10-day intervals. Oxidation stability of samples was assessed using a commercially available peroxide value assay kit (Jiangsu Edison Biotechnology Co., Ltd., Jiangsu, China). Briefly, the mixture of sample (50  $\mu$ L), methanol (900  $\mu$ L), working solution 1 (10  $\mu$ L) and working solution 2 (10  $\mu$ L) was incubated at room temperature for 5 min. Next, 200  $\mu$ L of the mixture was transferred to a 96-well plate and the absorbance was measured at 500 nm.

## 2.8. Sensory Flavor Assessment of COSO Samples

Sensory flavor evaluation of dietary selenium-supplemented COSO samples and conventional COSO samples sourced from four provinces was conducted by electronic nose (PEN3, Airsense, Germany) and electronic tongue (SA402B, Insent, Japan) analysis. In brief, 15 mL of supernatant was collected after centrifugation and 5 mL of sample was taken and then stored at 37 °C for 30 min before testing. All the tests were carried out in triplicate. Ten distinct flavor sensors integrated into an electronic nose system were utilized to detect the flavor. In addition, the primary taste-sensing electrodes employed were aftertaste-A, aftertaste-B, astringency, bitterness, richness, saltiness, sourness and umami.



## 2.9. Statistical Analysis

All experiments were performed in triplicate and results are expressed as mean  $\pm$  standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) and Tukey's multiple-range test. The  $p$  value  $< 0.05$  indicated a significant difference.

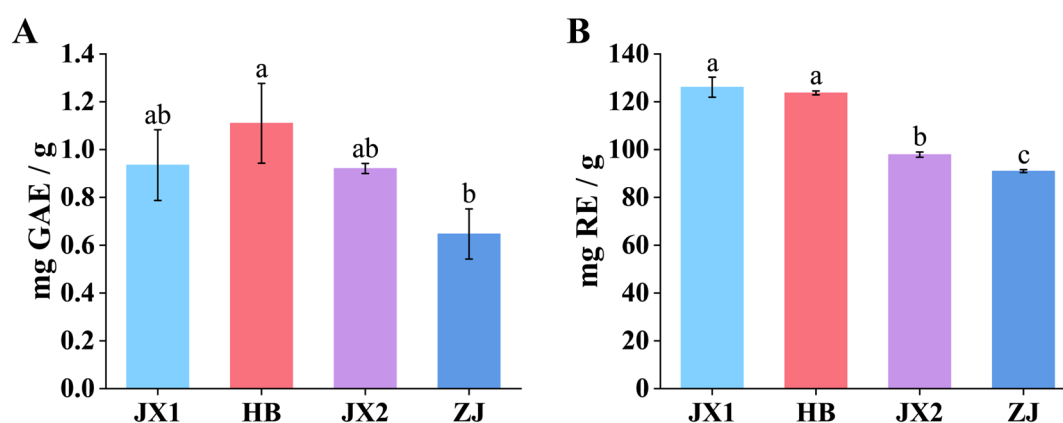
## 3. Results and Discussion

### 3.1. Selenium Content Analysis of COSO Samples

Among all the unsupplemented COSO samples, only the JX2 COSO sample which originated from a selenium-rich region complied with the regulatory selenium concentration criteria for selenium-enriched foods (up to 0.11 mg/kg). The selenium concentrations in the remaining samples were too low to be detectable. It is worth noting that compared with plant-based biofortification approaches which have yielded only modest selenium increases in COSO, the direct selenium enrichment strategy employed here provided a notable substantial elevation in selenium concentrations (both were above 0.50 mg/kg). This finding was consistent with the higher bioavailability and more efficient incorporation of organic selenium compounds into lipid matrices, as previously reported for selenomethionine and selenocysteine derivatives [23,24]. Based on the recommended dietary allowance for selenium (60  $\mu$ g/day for Chinese adults), a typical daily intake of 10–15 g of fortified oil would contribute approximately 10–20% of the daily requirement.

### 3.2. TPC and TFC Values of COSO Samples

Antioxidant phytochemicals, particularly polyphenols and flavonoids, function as the primary endogenous cellular protectors against free radicals by neutralizing reactive oxygen species before interacting with biomolecules, thereby mitigating oxidative damage [25]. COSO contains abundant polyphenols, and their levels vary substantially depending on the plant's growth phases and the specific extraction methodology employed. Flavonoids represent another prominent class of bioactive phytochemicals present in COSO. As shown in Figure 1, the HB COSO sample exhibited the most abundant content of phenolics ( $1.11 \pm 0.167$  mg GAE/g), which was approximately twice the concentration observed in the unsupplemented ZJ COSO sample, and the JX1 and JX2 COSO samples presented the TPC values of  $0.935 \pm 0.148$  and  $0.921 \pm 0.021$  mg GAE/g, respectively. Besides, the TFC values in different COSO samples revealed a parallel pattern. Specifically, the JX1 COSO sample showed the highest value of  $126.1 \pm 4.2$  mg RE/g, remarkably higher relative to that of the ZJ COSO sample ( $91.0 \pm 0.6$  mg RE/g), while the HB COSO sample presented a comparative value to the JX1 COSO sample of  $123.7 \pm 0.86$  mg RE/g, and the JX2 COSO sample displayed an intermediate value of  $97.9 \pm 1.1$ . These results supported the further investigation of the antioxidant activities of COSO samples and the regulatory effect of dietary selenium supplementation on these properties.

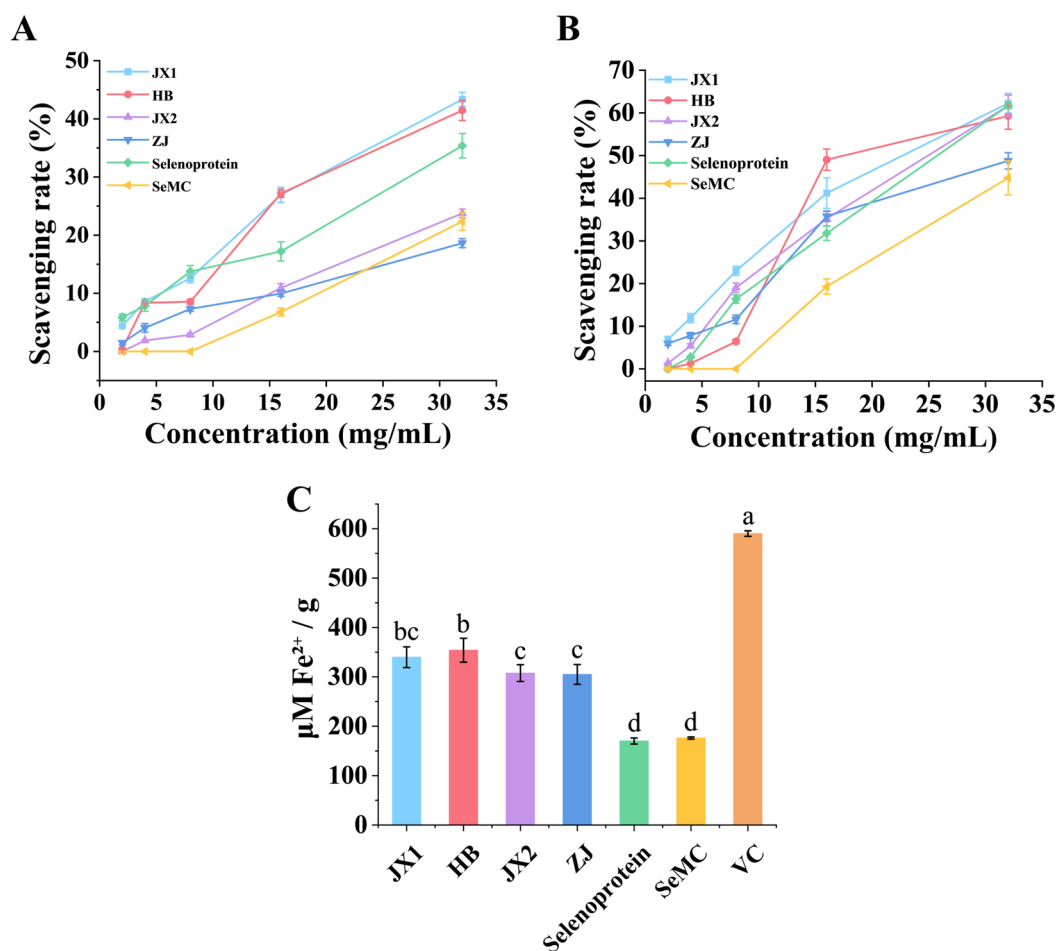


**Figure 1.** TPC (A) and TFC (B) values of COSO samples sourced from four provinces in China. GAE: gallic acid equivalent; RE: rutin equivalent. Significant differences ( $p < 0.05$ ) among the various oil sources were indicated by corresponding lowercase letters (a, b and c).

### 3.3. Antioxidant Activities of COSO Samples

Given the chemical complexity of COSO and the multifaceted nature of antioxidant mechanisms, relying on a single assay to evaluate its antioxidant capacity is limited. To ensure a comprehensive and robust assessment,

this study applied three complementary methods to comparatively rank the antioxidant profiles of the original and post-supplemented COSO samples. Differential responses across DPPH radical scavenging, ABTS decolorization, and FRAP assays were observed. Figure 2 demonstrated that the antioxidant activities of all the COSO samples increased in a concentration-dependent manner. JX1 and HB COSO samples displayed the strongest scavenging effect in all three antioxidant assays. Specifically, JX1 COSO sample showed inhibition rates of  $43.34 \pm 1.21\%$  and  $62.27 \pm 2.28\%$  at the COSO concentration of 32.0 mg/mL of DPPH and ABTS radical scavenging assays, respectively, while HB COSO sample presented inhibition rates of  $41.45 \pm 1.75\%$  and  $59.29 \pm 3.14\%$  at the same concentration. In contrast, the inhibition rate of ZJ COSO sample toward DPPH and ABTS free radicals assays ranked the lowest level of  $18.63 \pm 0.77\%$  and  $48.78 \pm 1.91\%$ , respectively. JX1 and HB COSO samples also showed the most prominent iron-reducing ability among the four sources, demonstrating FRAP values of  $339.80 \pm 21.00$  and  $353.9 \pm 24.14 \mu\text{M Fe}^{2+}/\text{g}$ . The JX2 COSO sample exhibited relatively higher antioxidant activities in the three assays. Moreover, the antioxidant activities of selenium-supplemented ZJ COSO samples were also assessed. Notably, the addition of selenoprotein significantly enhanced the scavenging effect on DPPH and ABTS free radical assays, whereas SeMC supplementation yielded only marginal improvements.



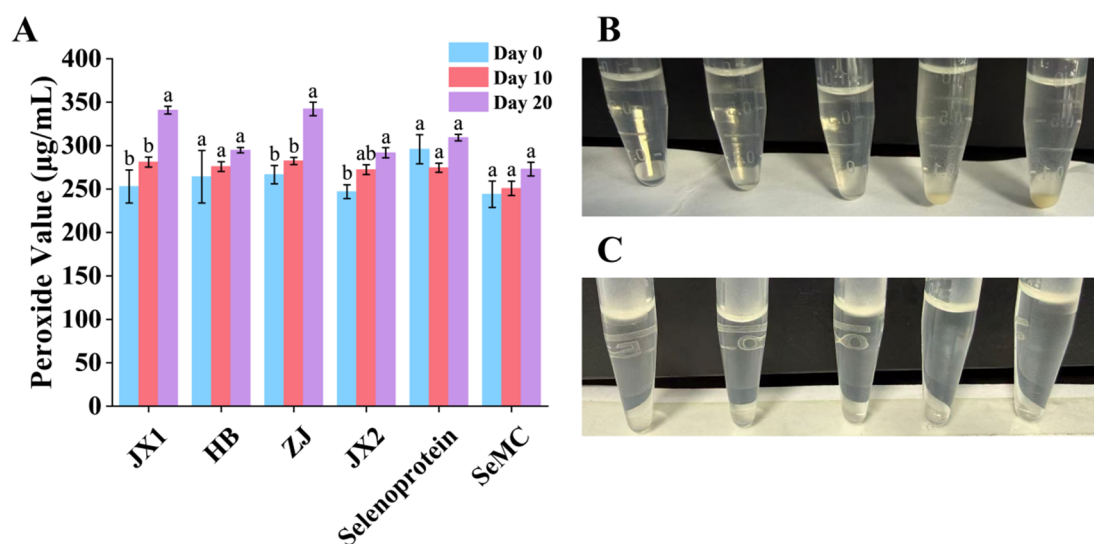
**Figure 2.** Antioxidant activity of COSO samples sourced from four provinces in China and two selenium-supplemented COSO samples. (A) The inhibition rates of DPPH radical scavenging assay, (B) The inhibition rates of ABTS radical scavenging assay, (C) ferric-ion-reducing antioxidant power (FRAP) assay. All experiments were performed in triplicate. DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Significant differences ( $p < 0.05$ ) among the various oil sources were indicated by corresponding lowercase letters (a, b, c and d).

ABTS radical scavenging activity was predominantly driven by total phenolic content, whereas DPPH inhibition appeared to involve contributions from both phenolic and flavonoid compounds. This mechanistic divergence aligns with previous reports that ABTS is more sensitive to hydrophilic chain-breaking antioxidants, while DPPH better reflects combined lipophilic and hydrophilic radical quenching [26]. Importantly, beyond the phenolic and flavonoid contributions, selenium content independently correlated with improved antioxidant capacity

in all three assays. This suggests that selenium-containing species in COSO may either directly scavenge free radicals or indirectly enhance the redox activity of other antioxidants, possibly through synergistic interactions [27]. Specifically, organoselenium compounds (e.g., SeMC) can directly scavenge free radicals through their redox-active selenol (–SeH) groups, which donate hydrogen atoms or electrons to neutralize reactive oxygen species (ROS). This direct chemical quenching is concentration-dependent and occurs independently of enzymatic activity [27]. Moreover, selenium is an essential catalytic cofactor for glutathione peroxidases (GPx) and thioredoxin reductases, which catalyze the reduction of lipid hydroperoxides and hydrogen peroxide, thereby protecting cell membranes and lipoproteins from oxidative damage. In lipid-based matrices such as edible oils, selenoproteins may also display GPx-like activity, reducing primary and secondary oxidation products [27].

### 3.4. Stability Assessment of COSO Samples

During a 20-day lipid oxidation period, peroxide values increased progressively across all COSO samples, with the JX1 and ZJ COSO sample variants exhibiting the highest levels at the endpoint (Figure 3A). The supplementation of organic selenium in the ZJ COSO sample positively influenced oxidative stability. The oxidation stability assessment indicated that selenoprotein- and SeMC-fortified ZJ COSO samples exhibited reduced peroxide value increase compared with the non-fortified control at day 20 ( $309.26 \pm 3.7006$  and  $273.01 \pm 7.87$   $\mu\text{g/mL}$ , respectively). Moreover, SeMC-enhanced ZJ COSO sample presented the lowest peroxide values throughout the oxidation period, indicating the antioxidant activity of selenium. This improved stability is likely attributable to the role of selenium in reducing primary and secondary lipid oxidation products, possibly via the GPx-like activity of selenoproteins [28]. Such an effect is particularly relevant for edible oils rich in unsaturated fatty acids, where oxidative rancidity remains a major quality concern.



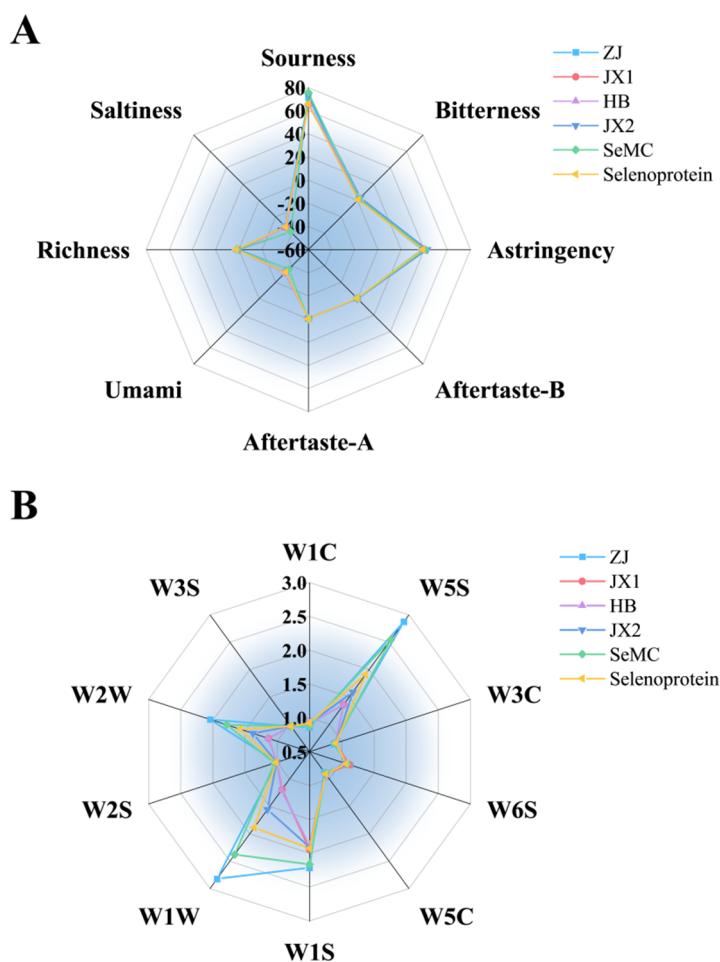
**Figure 3.** Assessment of oxidation stability and homogeneity of COSO samples. (A) Peroxide values of COSO samples sourced from four provinces in China and two selenium-supplemented COSO samples. (B) Homogeneity of ZJ COSO samples supplemented with selenoprotein in a 20-day period. (C) Homogeneity of ZJ COSO samples supplemented with SeMC in a 20-day period. Significant differences ( $p < 0.05$ ) among the different time points were indicated by corresponding lowercase letters (a and b).

Direct fortification is technically simple, cost-effective, and scalable, requiring only standard blending and homogenization equipment, making it attractive for small- to medium-scale oil producers. Figure 3B,C represents the homogeneity of ZJ COSO samples supplemented with selenoprotein and SeMC in a 20-day period. Only the SeMC-fortified group remained homogeneous throughout, whereas visible precipitate formed in the selenoprotein-fortified sample starting on day 15. The observed precipitation of selenoprotein presents a significant formulation challenge for commercial products, as it compromises visual quality, consumer acceptance, and dose uniformity. As large macromolecular proteins (typically containing multiple selenocysteine residues), selenoproteins are highly hydrophilic and tend to undergo phase separation when mixed with hydrophobic oils, leading to precipitation. Potential solutions include the use of nanoemulsification or liposomal encapsulation to improve dispersion stability, the addition of food-grade emulsifiers (e.g., lecithin or polysorbates), or the selection of alternative organic selenium sources with better lipid solubility (e.g., selenized lipids or selenium-enriched yeast

extracts). The supplementation of SeMC remained homogeneous throughout the 20-day observation period, indicating its suitability for commercial application in its current formulation. Overall, the use of selenoprotein and SeMC as selenium-fortifying agents is advantageous due to their high safety profile and regulatory acceptance in food and dietary supplement applications [29]. These compounds are actively transported and metabolized via pathways that avoid the toxicity associated with inorganic selenium. Therefore, the direct selenium enrichment approach represents a feasible and scalable strategy for producing functional COSO with enhanced health-promoting properties. Future optimization studies should focus on stability-enhancing formulation strategies and accelerated shelf-life testing under real-world storage conditions.

### 3.5. Sensory Flavor Assessment of COSO Samples

The electronic tongue is an analytical platform designed to mimic human taste perception, enabling rapid, selective identification, quantification, and discrimination of non-volatile compounds in complex aqueous matrices [30]. Eight tastes, including aftertaste-A, aftertaste-B, astringency, bitterness, richness, saltiness, sourness and umami were measured, respectively. A positive sensor response indicates the presence of the corresponding taste attribute in the sample, with signal intensity increasing in proportion to the attribute intensity. As shown in Figure 4A, the taste sensors of bitterness, saltiness, richness, umami, aftertaste-A and aftertaste-B in all COSO samples were not positive, while the response values of the sourness and astringency taste sensors were all greater than 0. Specifically, the sourness taste sensor exhibited the greatest value around 60–80 in all COSO samples, indicating the samples presented a predominantly sour taste profile. The concentration of free fatty acids in edible oils exhibits a direct, dose-dependent relationship with the output signal intensity of electronic sourness sensors [31]. In addition, the supplementation of SeMC enhanced the sour flavor in the COSO sample originating from Zhejiang, and exerted negligible effects on the other flavors.



**Figure 4.** Sensory evaluation of taste and flavor profiles in two selenium-supplemented COSO samples and conventional COSO samples sourced from four provinces by electronic tongue analysis (A) and electronic nose analysis (B).

Figure 4B displayed the flavor profiles of the COSO samples, which were detected by an electronic nose equipped with ten distinct metal-oxide gas sensors. Notably, the W1C, W3C, and W5C sensors exhibited high responsiveness toward aromatic compounds and alkanes, while the W5S, W6S, W1S, W2S, and W3S sensors exhibited selectivity for nitrogen-containing species and oxygenated volatiles, and W1W and W2W were primarily specific to terpenoids and sulfur-bearing compounds. The measurements showed that the response values of W1W, W5S, W2W and W1S were relatively high, indicating that the odor components in all COSO samples are primarily composed of sulfides, nitrogen oxides, aromatic compounds, organic sulfur compounds and methyl (alkyl) compounds. However, the remaining six electronic nose sensors showed relatively low responsive values near 1, indicating that the odor composition of COSO samples contained trace amounts of aromatic benzene compounds, ammonia-like aromatic components, hydrocarbons, short-chain alkane aromatics, alcohols, aldehydes and ketones, as well as long-chain alkanes. The formation of these odor characteristics may be related to the genetic identity of COSO and the edaphoclimatic conditions of the production region. Notably, ZJ COSO sample represented the strongest flavor profiles, whereas organic selenium supplementation significantly attenuated the overall aroma intensity of COSO.

#### 4. Conclusions

This study confirmed that direct selenium enrichment with selenoprotein and SeMC significantly improved the antioxidant capacity and oxidative stability of COSO. The enhanced antioxidant activity is not solely dependent on phenolic and flavonoid compounds but also on the selenium content itself, which exerts an independent contribution. These findings support the development of selenium-fortified COSO as a functional nutraceutical product, particularly for populations at risk of selenium deficiency. Selenium-fortified COSO also improved the oxidative stability and kept the characteristic flavor profile of the original oil. A limitation of this study is that the specific chemical forms of selenium retained in COSO after direct enrichment have not been characterized. In the absence of speciation analysis (e.g., HPLC-ICP-MS), the identities and relative abundances of individual selenium species (e.g., SeMC, selenoproteins, selenomethionine, other potential selenocompounds) cannot be resolved, either in the fortified product or following storage-induced transformations. This ambiguity directly affects the interpretation of both antioxidant mechanisms and bioavailability, given that distinct selenocompounds exhibit differing redox potentials and radical-scavenging kinetics, while organic selenium species such as SeMC are absorbed via distinct active transport pathways, whereas selenoproteins may undergo partial proteolytic degradation in the gastrointestinal tract. Future studies should therefore prioritize speciation analysis to identify the dominant selenium species retained in the fortified oil to correlate specific chemical forms with functional activity, and to assess their stability under thermal processing and gastrointestinal digestion. Additionally, assessment of *in vivo* bioefficacy should also be the principal focus.

#### Author Contributions

C.Z.: investigation, software, collection of resources and data analysis, writing—original draft preparation; Y.X.: software, visualization, validation. M.X.: conceptualization, resources, project administration, validation, writing—original draft preparation, review and final editing; M.G.: conceptualization, supervision, resources, project administration, funding acquisition, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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#### Institutional Review Board Statement

Not applicable.

#### Informed Consent Statement

Not applicable.

#### Data Availability Statement

Applicable if requested.

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## Conflicts of Interest

The authors declare no conflict of interest.

## Use of AI and AI-Assisted Technologies

Grammarly was used to improve grammar, spelling, and clarity. All edits were reviewed and approved by the authors, who take full responsibility for the final manuscript.

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