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Study on the antioxidant activity of β-sitosterol and stigmasterol from Sacha Inchi oil and *Prinsepia* oil added to walnut oil

Qin CHENG[1], Yuejiang ZHANG[1], Qi LIN[1], Yang TIAN[1]*, Yuanyuan BAO[1*]

Abstract

The qualitative and quantitative sterol compositions of *Sacha Inchi* oil and *Prinsepia* oil were analysed by high-performance liquid chromatography (HPLC). The sterol compounds were mixed with citric acid, VE and other natural antioxidants and added to walnut oil, oxidation resistence was determined by the peroxide value and acid value. Gas chromatography/mass spectrometry (GC/MS) analysis was used to measure the fatty acids. The results showed that the content of β-sitosterol and stigmasterol in *Sacha Inchi* oil was 156.46 mg/100 g and 68.74 mg/100 g, *Prinsepia* oil was 176.26 mg/100 g and 38.29 mg/100 g, respectively. After 12 days of accelerated oxidation, the antioxidant effect was TBHQ > compound antioxidants > BHA > sterols of *Sacha Inchi* oil > sterols of *Prinsepia* oil > 30% *Prinsepia* oil > citric acid > VE. After accelerated oxidation for 48 h, the total unsaturated fatty acids in blank walnut oil showed a decreasing trend, and the antioxidant group inhibited the decrease in total unsaturated fatty acids. In conclusion, the antioxidant treatment groups can delay the oxidative cycle of walnut oil and effectively prolong the shelf life of walnut oil, and the compound groups can significantly enhance the antioxidant effect of walnut oil. This study provides a technical reference for the storage stability of walnut oil.

Keywords: walnut oil; *Sacha Inchi* oil; *Prinsepia* oil; sterol; oxidation.

Practical Application: Sterol compounds have a variety of biological activities, and the sterols of green prickly fruit oil and Myosterol oil contain abundant β-sitosterol and stigmasterol. When added to walnut oil, it was found that they could significantly inhibit the oxidation of walnut oil and prolong the storage period of walnut oil.

1 Introduction

Walnut (Juglans regia L.) belongs to the family Juglandaceae, is a very popular tree nut and has high economic value, especially for the food industry, because of its various phytochemical compounds and nutritional properties (Shah et al., 2018). Walnut has a good fatty acid composition (the ratio of linolenate acid to linoleate acid is close to 1:4) and rich active substances, which have a high calorie level, because of its specific compounds, such as polyunsaturated fatty acids (Gharibzahedi et al., 2014). Walnuts are rich in fat, protein, vitamins, zinc, chromium, manganese and other essential mineral elements for human body. In addition to a large number of common nutrients, walnut oil also contain many functional components, such as sterols, polyphenols, flavonoids, squalene, tocopherol, carotene and so on. Walnut oil has many health benefits. Batirel found that walnut oil can affect tumour growth and metastatic potential in oesophageal cancer cells and can suppress the adhesion, migration and colony formation of the cells (Batirel et al., 2018). Gencoglu found that supplementation could alleviate the adverse impacts of both HCD and HFD in rats, and intake can modulate carbohydrate metabolism and increase antioxidant capacity (Gencoglu et al., 2020). Esselun found that Decreased Aβ formation and enhanced ATP levels might enhance neurite growth, making walnut oil a potential agent to enhance neuronal function and to prevent the development of Alzheimer's disease (Esselun et al., 2022). Jost investigated the effects of walnut oil high in PUFAs on spatial cognition and fecal corticosterone metabolite concentrations under non-stressed conditions in rats, and revealed that learning performance was improved in PUFA-supplemented rat (Jost et al., 2022). Therefore, the oxidative stability of walnut oil is an important feature due to the undesirable changes that can occur during storage (Vicente et al., 2018). Walnut oil is prone to oxidative reactions in the storage process, and artificial antioxidants have safety risks, so adding natural antioxidants has become an inevitable trend.

Sacha inchi of the family Euphorbiaceae is also known as sacha peanut, mountain peanut, Inca nut or Inca peanut (Wang et al., 2018). Its seeds are a potential source of natural oil rich in essential polyunsaturated fatty acids and have various nutraceutical and health benefits (Nguyen et al., 2020). *Sacha inchi* oil has many nutritional properties due to its richness in unsaturated fatty acids, such as omega-3 fatty acids and alpha-linolenic acid, and bioactive substances, such as tocopherols and phytosterols (Minh & Nga, 2019; Ramos-Escudero et al., 2021). Fourteen sterols were identified, and β-sitosterol, stigmasterol, and campesterol were the major components (Ramos-Escudero et al., 2019). Studies have also shown that the oil has a certain scavenging ability for free radicals, strong antioxidant properties in vitro, and can improve the stability of oil. *Prinsepia utilis* Royle, which belongs to the

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[1] *College of Food Science and Technology, Yunnan Agricultural University, Kunming, Yunnan, China*
*Corresponding author: 380984741@qq.com; tianyang1208@163.com

Rosaceae family, is a deciduous perennial shrub (Zhang et al., 2018). *Prinsepia* contains a wild woody oil that yields edible oil. The high content of unsaturated fatty acids has been proven to possess particular benefits for human health and medical therapy (Wang et al., 2013). The flavonoids, polyphenols, VE, sterols and other substances contained in *Prinsepia* oil can delay oxidation, and *Prinsepia utilis* Royle has antibacterial, anticancer, hypoglycaemic, hypolipidaemic, antiplatelet aggregating, and antioxidant activity.

Stigmasterol and β-sitosterol are phytosteroids (Lee et al., 2021). Stigmasterol is a steroid derivative having a hydroxyl group in position C-3 of the steroid skeleton, unsaturated bonds in position C-5/C-6 of the B ring, and alkyl substituents in position C-22/C-23. β–Sitosterol is one of the main dietary phytosterols belonging to the class of organic compounds known as stigmastanes (Sharma et al., 2021). These are sterol lipids with a structure based on the stigmastane skeleton, which consists of a cholestane moiety bearing an ethyl group at the C-24 position (Marahatha et al., 2021). Phytosterols, as components of the human diet, have received much attention because of their cholesterol-lowering and antioxidant properties (Lengyel et al., 2012). And phytosteroids could be useful for the treatment of inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis, asthma, and cardiovascular diseases. Sterols could effectively prevent gastric cell damage and suggest the potential application of LPSs as bioactive ingredients for healthy foods (Zhang et al., 2021a). The incorporation of gallic acid into typical phytosterols (β-sitosterol and *stigmasterol*) and galloyl β-sitosterol had slightly better antioxidant activity at ambient temperature and better cholesterol-reducing activity (Wang et al., 2019). Vasanth found that Lupeol and β-sitosterol reduced cell viability in a dose-dependent manner showcasing increased G phase cell accumulation while reducing other cell cycle phases (S and G /M) and significant lowering of intracellular lipid accumulation (Vasanth et al., 2022).

Sterols can be used as antioxidants in plants and animals (Liu et al., 2016). Knowledge of sterol content and composition is becoming increasingly important, both in terms of understanding vegetal biochemistry and as a basis for sterolomic studies (Simonetti et al., 2021).The molecular structure of sterols is close to the molecular structure of cholesterol, and the common phytosterols are stigmasterol and β-sitosterol (Chen et al., 2017), which have antioxidant effects (Zhao, 2016). In this study, sterol compounds were extracted and purified from native Sacha Inchi oil and Prinsepiaoil from Yunnan Province. In order to improve the oxidative stability of walnut oil, the sterols in the oil of Sacha Inchi and Prinsepia were added, then the sterols in the oil were mixed with citric acid and vitamin E. The sterol compounds were mixed with citric acid, VE and other natural antioxidants and added to walnut oil. The effects of the sterol compounds on the stability of walnut oil were studied by measuring the acid value and peroxide value of walnut oil as well as the changes in fatty acids. To seek the best natural antioxidant formula for walnut oil production and further development and utilization of technical support. Sacha Inchi and Prinsepia oil has the good oxidation stability, this may be due to the effect of sterol, and there are few reports about adding sterol to walnut oil. This study confirmed that sterols in oils and fats had antioxidant effects and synergistic effect with natural antioxidants (vitamin E, citric acid), providing reference for the study of natural compound antioxidants and the preservation of oils and fats such as walnut oil.

2 Materials and methods

2.1 Materials

Sacha Inchi was purchased in Pu'er. Walnut procurement Dali and *Prinsepia* were purchased in Lijiang Yunnan. These materials were squeezed by a spiral cold press, centrifuged and filtered to obtain the clarified oil, and the oils were sealed at low temperature in the dark for later use.

2.2 Extraction and quantification of sterols

Sterol extraction

To obtain the crude extract of sterols, refer to Zheng Gao and Xixi Zhao's method and modify it slightly, the saponification time and temperature were 60 min and 85 °C, respectively. The concentration of the NAOH-ethanol solution was 2 mol/L, and the ratio of solid to liquid was 1:5 g/mL.

Determination of sterol content

Chromatographic conditions: The chromatographic column was a SyncronIS-C18 (4.6 mm × 250 mm, 5 μm). Mobile phase: methanol, wavelength: 205 nm, column temperature: 30 °C, injection volume: 10 μL, flow rate: 1 mL/min

(1) Sterol content of *Prinsepia* oil

Concentrations of 0.05 mg/mL, 0.1 mg/mL, 0.15 mg/mL, 0.2 mg/mL, and 0.25 mg/mL were mixed with linear standards, and a standard curve was prepared. A 10 g/L sterol crude extract solution/methanol solution was prepared. Stigmasterol and β-sitosterol mixed standards were used for qualitative determination of sterols, followed by the use of an external standard method.

(2) Sterol content of *Sacha Inchi* oil

According to the experimental results and published articles of our research group, the content of sterol of *Sacha Inchi oil* is known (Zhang et al., 2021b).

2.3 Testing of antioxidative activity

All oils were determined by the Schaal oven method. The oils were placed in a constant temperature drying oven at 60 ± 1 °C to accelerate oxidation, and samples for analyses were measured once every 2 days for 12 days. The antioxidant activity was evaluated by determining the peroxide value (GB/T5538-2016) and acid value (GB/T5530-2016). Walnut oil was added at mass ratios of 5%, 10%, 15%, 20% and 30% of *Sacha Inchi* oil and *Prinsepia* oil, VE, TBHQ, BHA and citric acid. The compounds were named HO-5%-30% (*Sacha Inchi* oil), HO-5%-30% (*Prinsepia* oil), HO-VE, HO-TBHQ, HO-BHA, and HO-citric acid; and Walnut oil was supplemented with sterols of *Sacha Inchi* oil (β-Sitosterol : stigmasterol 2:1) and

sterols of *Prinsepia* oil(β-Sitosterol : stigmasterol 4:1), compound antioxidants (200 g/kg sterol of *Sacha Inchi* oil, 163.63 mg/kg citric acid, 174.66 mg/kg Vitamin E, and 200 g/kg sterol of *Prinsepia* oil, 163.63 mg/kg citric acid, 174.66 mg/kg Vitamin E), The compounds were named HO-Sterols of *Sacha Inchi oil*, HO-Sterols of *Prinsepia oil*, HO-compound sterol of *Sacha Inchi* oil(HO-compound antioxidants (M)), HO-compound sterol of *Prinsepia* oil (HO-compound antioxidants (M)), the amount of compound antioxidant was referred to the literature (Zhang et al., 2021b); the stability of the *Sacha Inchi* oil, *Prinsepia* oil, and walnut oil was determined by MO, QO and HO samples, respectively.

2.4 Fatty acid composition

The relationship between fatty acids and oxidation stability was analysed by measuring the changes in fatty acids in samples after accelerated oxidation at 0 h and constant temperature drying oven temperature for 48 h.

GC conditions

The samples were separated by gas chromatography/mass spectrometry (GC/MS) with an Agilent DB-WAX capillary column (30 m × 0.25 mm ID × 0.25 μm). Programmed temperature: the initial temperature was 50 °C, which was maintained for 3 min, and the temperature was raised to 220 °C at 10 °C/min for 5 min. The carrier gas was helium, and the carrier gas flow rate was 1.0 mL/min. A QC sample was analysed for a certain number of experimental samples at intervals in the sample queue to test and evaluate the stability and repeatability of the system. An Agilent 7890/5975C was used for GC/MS analysis. The injector temperature was 280 °C, the ion source temperature was 230 °C, and the transmission line temperature was 250 °C. Electron ionisation (EI) source, selected ion monitoring (SIM) scanning mode, and electron energy of 70 eV were used.

Standard preparation and sample handling

The mixed standard solution of 40 fatty acid methyl esters was diluted into ten mixed standard concentration gradients (0.5 mg/L, 1 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L). Five hundred microlitres of mixed standard sample was measured, 25 μL of 500 ppm methyl nonanoate was added as the internal standard. The mixture was fully mixed and placed in a sample bottle for GC/MS analysis. Then, to a 200-μL oil sample, 1 mL chloroform methanol solution was added, the mixture was ultrasonicated for 30 min. The supernatant was collected, and 2 mL 1% sulfuric acid-methanol solution was added; 500 μL of supernatant was collected, and 25 μL of methyl salicylate was added as the internal standard, and mixed well. The sample volume was 1 μL, the split ratio was 10:1, and the split injection was performed in the GC/MS analysis.

Sample preparation

All oil samples were oxidised for 0 h, and accelerated oxidation for 48 h, and all sample preparation is shown in the Table 1.

Data processing

The chromatographic peak area and retention time were extracted by MSD ChemStation software. The fatty acid content in the samples was calculated by drawing the standard curve.

2.5 Statistical analysis

All measurements were repeated at least three times. The experimental data are expressed as the mean ± standard deviation, and Duncan (D) in SPSS software was used for significance analysis. $P < 0.05$ indicated a significant difference, and Excel software was used for processing and plotting.

3 Results

3.1 Qualitative sterol compounds

The sterol content in Prinsepia oil and Sacha Inchi oil

Blank methanol solution did not interfere with the determination of sterol content in *Prinsepia* oil (Figure 1). β-Sitosterol and stigmasterol are the main components of sterols in *Prinsepia* oil

Table 1. Sample preparation.

Sample	oxidised for 0 h	After accelerated oxidation for 48 h
Walnut oil	HO	HE
Sacha Inchi oil	MO	ME
Prinsepia oil	QO	QE
30% of *Sacha Inchi*	HO-30% (M)	HE-30% (M)
30% of *Prinsepia* oil	HO-30% (Q)	HE-30% (Q)
Sitosterol of *Sacha Inchi*	HO-sitosterol of *Sacha Inchi*	HO-sitosterol of *Sacha Inchi*
Sitosterol of *Prinsepia*	HO-sitosterol of *Prinsepia*	HO-sitosterol of *Prinsepia*
Citric acid	HO-citric acid	HO-citric acid
VE	HO-VE	HO-VE
Complex sterols of *Sacha Inchi* oil	HO-complex sterols of *Sacha Inchi* oil	HE-complex sterols of *Sacha Inchi* oil
Complex of sterols of and *Prinsepia* oil	HO-complex sterols of *Prinsepia* oil	HE-complex sterols of *Prinsepia* oil

Sitosterol of Sacha Inchi oil (β-Sitosterol : stigmasterol 2:1); Sterols of Prinsepia oil (β-Sitosterol : stigmasterol 4:1); Complex sterols of Sacha Inchi oil (200 g/kg Sitosterol of Sacha Inchi oil, 163.63 mg/kg citric acid, 174.66 mg/kg Vitamin E); Complex of sterols of and Prinsepia oil (200 g/kg Sitosterol of Sacha Inchi oil, 163.63 mg/kg citric acid, 174.66 mg/kg Vitamin E; HO: Walnut oil oxidized for 0h ; HE: Walnut oil oxidized for 24h ;MO: Sacha Inchi oil oxidized for 0h ;ME: Sacha Inchi oil oxidized for 24h ;QO: Prinsepia oil oxidized for 0h; QE: Prinsepia oil oxidized for 24h; HO-30% (M): M, Sacha Inchi oil; HO-30% (Q): Q, Prinsepia oil; HO-VE: The VE group was oxidized for 0h).

(Figure 2). The peak elution time of stigmasterol and β-sitosterol standard product was 25.93 min and 29.6 min, respectively, and the peak elution time of stigmasterol in the crude extract of *Prinsepia* oil was approximately 25.9 min, and β-sitosterol of crude extract was approximately 29.6 min. The peak areas of stigmasterol and β-sitosterol in *Prinsepia* oil were 12.1022 ± 0.15 mAU.min and 50.3554 ± 1.15 mAU.min, respectively, so the content of stigmasterol in the crude extract was 38.2992 ± 0.48 mg/100 g. β-Sitosterol in the crude extract was 176.2678 ± 4.03 mg/100 g (Figure 3).

The relationship between peak area and concentration of stigmasterol and β-sitosterol standard product was established as a standard curve. In the range of 0.05~0.25 mg/mL, stigmasterol: $Y = 31.746x - 0.0545$, $R^2 = 0.9949$, β-sitosterol: $Y = 28.471x + 0.1721$, $R^2 = 0.9916$. The RSDs of stigmasterol and β-sitosterol of the standard product were 1.09% and 1.11%, respectively. These results indicate that the precision of the apparatus was good. The average recoveries of stigmasterol and β–sitosterol from *Prinsepia* oil were 87.3% (RSD 0.53%) and 113% (RSD 0.08%), respectively. Therefore, the detection results are accurate and reliable.

We know from the research and published articles of this research group, the content in Sacha Inchi oil of stigmasterol in the crude extract was 68.7434 ± 0.46 mg/100 g. β-Sitosterol was 156.4668 ± 0.46 mg/100 g (Zhang et al., 2021b).

The ratio of stigmasterol to β -sitosterol is about 1:4 from *Prinsepia* oil, and the ratio of stigmasterol to β -sitosterol is about 1:2 from Sacha Inchi oil, which results in different oxidation stability of the *Prinsepia* oil and Sacha Inchi oil, and different antioxidant effects.

3.2 Oxidation stability of all samples

Peroxide value

The peroxide value was used to determine the concentration of hydroperoxides and peroxides generated during lipid oxidation, indicating whether the sample deteriorated due to oxidation, and the peroxide value could also reflect the initial oxidation degree of oil (Chen et al., 2014).The peroxide values of all samples showed an upwards trend with the extension of time in the accelerated oxidation process (Table 2). At 12 d, the peroxide values of *Sacha Inchi* oil and *Prinsepia* oil were 52.53 mmol/kg and 12.33 mmol/kg, respectively, and the peroxide value of the blank walnut oil was 79.47 mmol/kg, indicating that the stability of the two oils was stronger than the stability of walnut oil, possibly because *Sacha Inchi* oil and *Prinsepia* oil

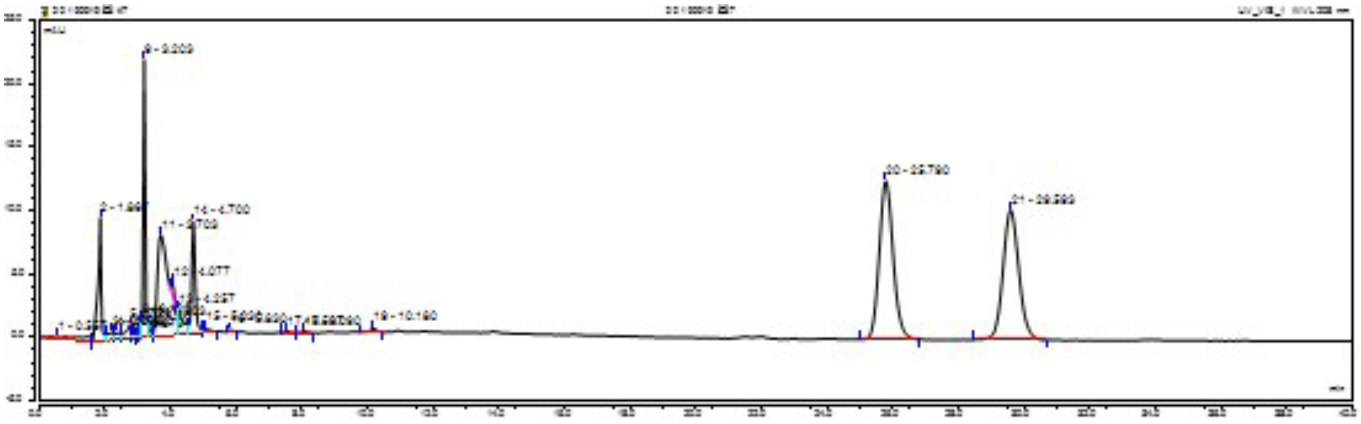

Figure 1. HPLC chromatogram of methanol solution.

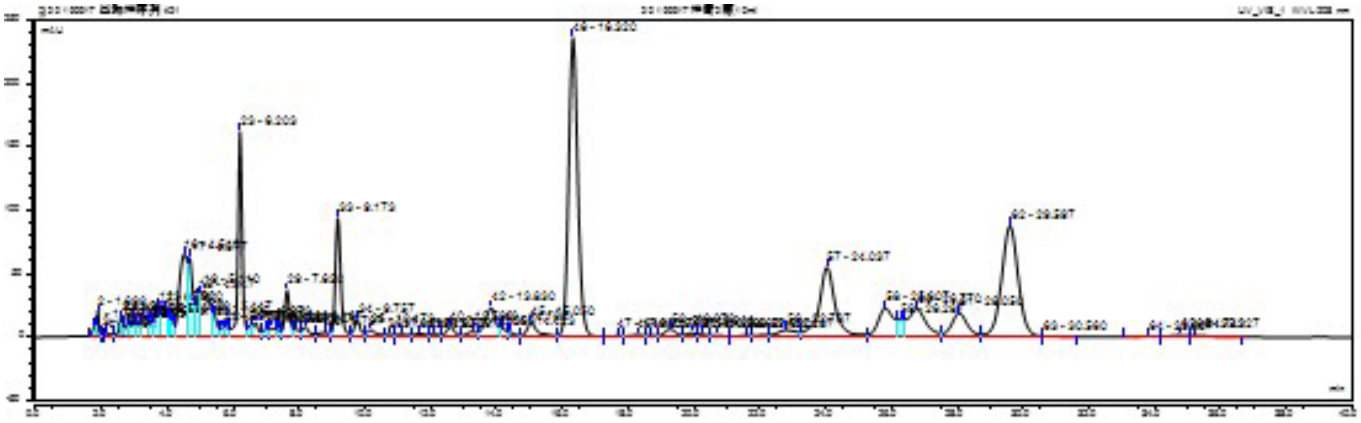

Figure 2. HPLC chromatogram of mixed sterol standards of 0.25 mg/mL stigmasterol and 0.25 mg/mL sitosterol.

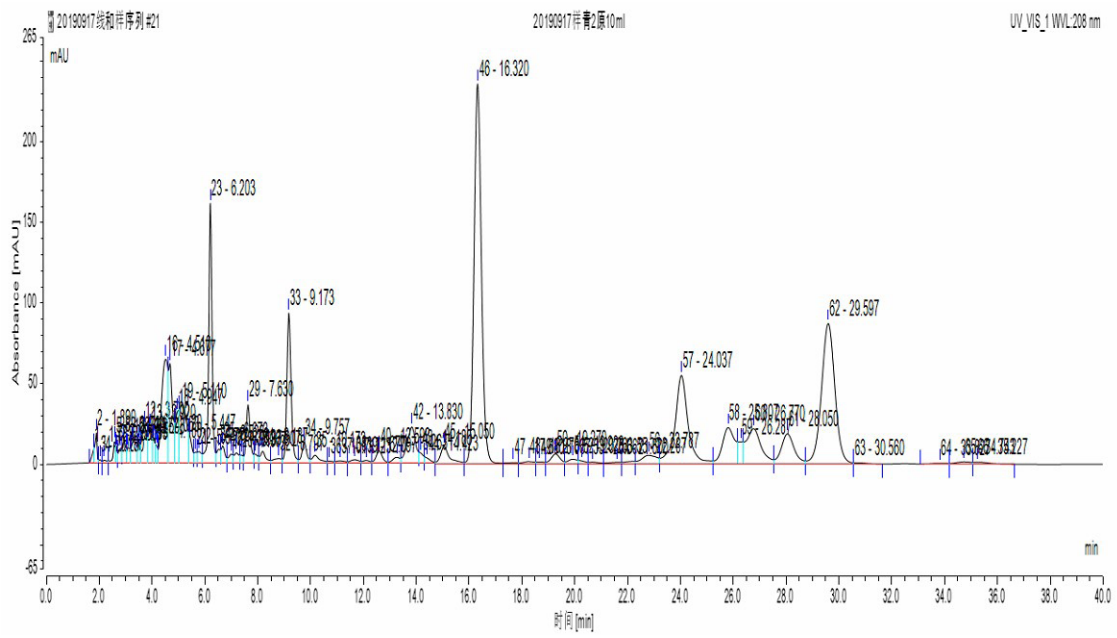

Figure 3. HPLC chromatogram of a 0.1 g/10 mL sample of crude sterol extract of *Prinsepia* oil.

Table 2. The peroxide values of all samples.

	Peroxide values (mmol/kg)						
Oxidation time	0 d	2 d	4 d	6 d	8 d	10 d	12 d
HO	0.76 ± 0.11^{c}	2.03 ± 0.15^{k}	8.67 ± 0.31^{g}	19.40 ± 0.20^{k}	32.53 ± 0.12^{j}	52.93 ± 0.23^{i}	79.47 ± 4.00^{h}
MO	2.47 ± 0.12^{a}	4.87 ± 0.12^{l}	12.47 ± 0.12^{f}	23.87 ± 0.23^{i}	29.67 ± 0.58^{k}	40.53 ± 0.46^{l}	52.53 ± 0.92^{m}
QO	0.80 ± 0.10c^{d}	1.67 ± 0.12^{c}	2.20 ± 0.20^{o}	3.41 ± 0.02^{s}	5.20 ± 0.35^{r}	8.53 ± 0.23^{t}	12.33 ± 0.92^{s}
HO-5% (*Sacha Inchi* oil)	0.61 ± 0.02^{de}	6.93 ± 0.12^{a}	19.03 ± 0.61^{d}	43.33 ± 1.51^{f}	67.33 ± 1.15^{a}	92.03 ± 2.67^{a}	141.27 ± 5.43^{a}
HO-10% (*Sacha Inchi* oil)	0.73 ± 0.12^{b}	5.13 ± 0.12^{d}	19.07 ± 0.12^{d}	39.73 ± 0.46^{c}	62.27 ± 1.50^{c}	87.20 ± 0.72^{b}	106.13 ± 3.35^{b}
HO-15% (*Sacha Inchi* oil)	0.79 ± 0.12^{c}	6.93 ± 0.12^{b}	19.17 ± 0.06^{c}	38.07 ± 0.46^{e}	57.07 ± 0.92^{d}	83.87 ± 1.80^{c}	102.60 ± 2.42^{c}
HO-20% (*Sacha Inchi* oil)	0.79 ± 0.02^{c}	5.73 ± 0.12^{c}	20.07 ± 0.12^{b}	39.20 ± 1.06^{d}	62.60 ± 0.35^{b}	83.50 ± 0.50^{d}	101.27 ± 2.19^{d}
HO-30% (*Sacha Inchi* oil)	1.17 ± 0.06^{b}	7.13 ± 0.23^{a}	21.47 ± 0.92^{a}	41.07 ± 0.12^{b}	56.93 ± 0.23^{d}	79.50 ± 0.50^{e}	85.40 ± 1.22^{f}
HO-5% (*Prinsepia* oil)	0.37 ± 0.06^{g}	3.63 ± 0.06^{h}	12.41 ± 0.02^{f}	30.47 ± 0.61^{f}	52.33 ± 0.58^{e}	72.83 ± 2.02^{g}	92.73 ± 1.10^{e}
HO-10% (*Prinsepia* oil)	0.43 ± 0.02^{fg}	4.67 ± 0.58^{f}	13.87 ± 0.23^{e}	28.47 ± 0.31^{g}	51.33 ± 0.58^{f}	75.50 ± 1.80^{f}	82.93 ± 1.01^{g}
HO-15% (*Prinsepia* oil)	0.35 ± 0.09^{g}	3.87 ± 0.12^{g}	11.57 ± 0.15^{g}	26.53 ± 1.12^{h}	44.33 ± 0.58^{g}	57.20 ± 1.66^{h}	64.20 ± 0.35^{i}
HO-20% (*Prinsepia* oil)	0.53 ± 0.05^{ef}	1.97 ± 0.21^{k}	9.23 ± 0.06^{i}	21.07 ± 0.12^{j}	37.07 ± 1.01^{h}	50.07 ± 0.12^{j}	60.07 ± 0.12^{j}
HO-30% *Prinsepia* oil)	0.42 ± 0.03^{g}	2.87 ± 0.12^{i}	9.72 ± 0.16^{h}	19.13 ± 0.42^{l}	34.67 ± 0.58^{i}	48.07 ± 1.50^{k}	56.07 ± 0.12^{k}
HO-Sterols of *Sacha Inchi* oil	0.68 ± 0.16c^{d}	2.06 ± 0.21^{k}	5.26 ± 1.04^{m}	12.77 ± 1.79^{p}	18.60 ± 4.03^{o}	29.87 ± 1.98^{p}	45.57 ± 6.03^{o}
HO-Sterols of *Prinsepia* oil	0.67 ± 0.16c^{d}	2.20 ± 0.44^{g}	5.27 ± 1.15^{m}	15.60 ± 2.07^{o}	19.50 ± 4.56^{n}	31.37 ± 3.79^{o}	49.03 ± 3.95^{n}
HO-Citric acid	0.75 ± 0.05^{c}	2.90 ± 0.36^{i}	6.80 ± 0.72^{l}	16.17 ± 1.85^{n}	23.30 ± 2.5^{m}	37.13 ± 1.96^{a}	52.30 ± 4.00^{m}
HO-VE	0.78 ± 0.11^{c}	2.80 ± 0.22^{i}	8.20 ± 0.88^{k}	16.63 ± 1.73^{m}	27.07 ± 2.07^{l}	39.67 ± 4.03^{m}	53.67 ± 3.40^{l}
HO-TBHQ	0.13 ± 0.06^{i}	0.17 ± 0.058^{p}	0.27 ± 0.058^{r}	0.47 ± 0.058^{u}	0.55 ± 0.05^{t}	0.67 ± 0.058^{u}	1.533 ± 0.06^{t}
HO-BHA	0.22 ± 0.03^{hi}	0.67 ± 0.058^{n}	1.43 ± 0.12^{q}	2.60 ± 0.346^{t}	4.43 ± 0.84^{s}	8.80 ± 0.70^{s}	15.70 ± 0.61^{r}
HO-compound antioxidants (M)	0.22 ± 0.09^{gh}	1.15 ± 0.004^{m}	2.80 ± 0.12^{n}	7.49 ± 0.19r	11.86 ± 0.31^{p}	15.96 ± 0.24^{r}	25.81 ± 1.12^{p}
HO-compound antioxidants (Q)	0.33 ± 0.07^{hi}	0.29 ± 0.12^{o}	1.62 ± 0.12^{p}	5.37 ± 0.44q	9.72 ± 0.67^{q}	12.48 ± 0.09^{q}	32.27 ± 0.32^{q}

Lowercase letters mean there's difference between groups, same letters means there's no difference, different means there's difference. HO-TBHQ, It represents TBHQ in walnut oil; HO-BHA, It represents BHA in walnut oil.

contained high levels of sterol compounds, and their fatty acid composition and content were different. The addition of 5-30% *Sacha Inchi* oil and 5-10% *Prinsepia* oil promoted an increase in the peroxide value of walnut oil and the oxidation of walnut oil, which may be due to the increase in some trace oxidation substances in the mixed oil or the change in fatty acid content in the mixed oil. The peroxide values of 15%, 20%, 30% *Prinsepia* oil were 64.20 mmol/kg, 60.07 mmol/kg and 56.07 mmol/kg, respectively, which were significantly lower than the peroxide value of the blank walnut oil ($P < 0.05$), indicating that *Prinsepia* oil inhibited the increase in the peroxide value of walnut oil. The 30% Prinsepia oil had the best effect, which might be due to the addition of endogenous antioxidants in *Prinsepia* oil, thereby improving the oxidative stability of walnut oil. The peroxide values of sterols of *Sacha Inchi* oil and *Prinsepia* oil were 45.57 mmol/kg and 49.03 mmol/kg, respectively, significantly lower than the peroxide values in the blank group ($P < 0.05$), suggesting that sterols of *Sacha Inchi* oil and Prinsepia oil could effectively delay the generation of hydroperoxide in walnut oil. The inhibition of the increase in the peroxide value of walnut oil may be caused by the increase in the content of sterols in walnut oil. The peroxide value of the compound antioxidants (M and Q) were 25.81 ± 1.12 and 32.27 ± 0.32, respectively, and the antioxidant effect of compound antioxidants was the best, possibly because the compound with VE and citric acid increased the antioxidant effect. Adding antioxidant treatment groups could delay the oxidative rancidity of walnut oil and effectively extend the shelf life of walnut oil, but the effect was smaller than the effect of TBHQ and BHA, which may be due to the sterol heat and oxygen, and the role of the metal ions and other factors, leading to a weaker sterol effect. The sterol content of the *Sacha Inchi* oil and *Prinsepia* oil groups was greater than the VE and citric acid groups. The antioxidant effect was TBHQ > BHA > compound antioxidants (M) > compound antioxidants (Q) > sterols of *Sacha Inchi* oil > sterols of *Prinsepia* oil > VE and citric acid > 30% *Prinsepia* oil.

Acid value

In the process of accelerating oxidation, the oil is decomposed into free fatty acids, resulting in an increasing trend of acid value. The acid value of all samples showed a rising trend with increasing time (Table 3). Walnut oil may decompose into oxides during storage, resulting in oil deterioration in hydrolysis and rancidity, and free fatty acids are continuously generated (Xie et al., 2018). After 12 days of accelerated oxidation, the acid value of

Table 3. Acid values of all samples.

	Acid values mg/g						
Oxidation time	0 d	2 d	4 d	6 d	8 d	10 d	12 d
HO	0.35 ± 0.01^{g}	0.35 ± 0.01^{j}	0.35 ± 0.01^{j}	0.43 ± 0.02^{k}	0.59 ± 0.07^{h}	0.77 ± 0.02^{h}	1.06 ± 0.14^{k}
MO	0.23 ± 0.06^{l}	0.44 ± 0.01^{a}	0.45 ± 0.01^{fg}	0.53 ± 0.05^{f}	0.55 ± 0.01^{i}	0.72 ± 0.01^{j}	0.75 ± 0.05^{r}
QO	0.32 ± 0.06^{g}	0.37 ± 0.05^{hi}	0.41 ± 0.04^{i}	0.39 ± 0.02^{l}	0.40 ± 0.04^{k}	0.47 ± 0.03^{p}	0.55 ± 0.06^{s}
HO-5% (*Sacha Inchi* oil)	0.37 ± 0.01^{e}	0.38 ± 0.02^{gh}	0.38 ± 0.0i^{c}	0.62 ± 0.01^{c}	0.88 ± 0.01^{c}	1.19 ± 0.01^{a}	1.47 ± 0.01^{a}
HO-10% (*Sacha Inchi* oil)	0.39 ± 0.01^{d}	0.43 ± 0.05^{d}	0.47 ± 0.02^{j}	0.61 ± 0.02^{d}	0.82 ± 0.01^{d}	1.18 ± 0.01^{b}	1.46 ± 0.01^{b}
HO-15% (*Sacha Inchi* oil)	0.36 ± 0.04^{f}	0.40 ± 0.01^{ef}	0.45 ± 0.02^{fg}	0.56 ± 0.02^{e}	0.79 ± 0.01^{e}	1.16 ± 0.01^{c}	1.45 ± 0.01^{c}
HO-20% (*Sacha Inchi* oil)	0.35 ± 0.05^{g}	0.39 ± 0.01^{fg}	0.45 ± 0.01^{fg}	0.56 ± 0.03^{e}	0.78 ± 0.02^{e}	1.16 ± 0.01^{c}	1.43 ± 0.01^{d}
HO-30% (*Sacha Inchi* oil)	0.48 ± 0.01^{b}	0.61 ± 0.03^{a}	0.54 ± 0.08^{c}	0.56 ± 0.03^{e}	0.90 ± 0.02^{b}	1.12 ± 0.02^{d}	1.40 ± 0.01^{e}
HO-5% (*Prinsepia* oil)	0.32 ± 0.03^{g}	0.36 ± 0.03^{ij}	0.38 ± 0.02^{c}	0.53 ± 0.02^{f}	0.67 ± 0.01^{f}	0.79 ± 0.04^{g}	1.05 ± 0.09^{l}
HO-10% (*Prinsepia* oil)	0.33 ± 0.05^{i}	0.40 ± 0.01^{ef}	0.44 ± 0.01^{h}	0.56 ± 0.04^{e}	0.68 ± 0.04^{f}	0.76 ± 0.06^{i}	1.09 ± 0.07^{i}
HO-15% (*Prinsepia* oil)	0.33 ± 0.01^{i}	0.36 ± 0.0ij	0.46 ± 0.04^{f}	0.52 ± 0.04^{g}	0.63 ± 0.02^{g}	0.79 ± 0.03^{g}	1.07 ± 0.04^{j}
HO-20% (*Prinsepia* oil)	0.34 ± 0.03^{h}	0.41 ± 0.03^{e}	0.45 ± 0.01^{fg}	0.50 ± 0.04^{h}	0.59 ± 0.03^{h}	0.67 ± 0.02^{l}	1.03 ± 0.01^{n}
HO-30% (*Prinsepia* oil)	0.37 ± 0.02^{e}	0.43 ± 0.03^{d}	0.49 ± 0.02^{d}	0.48 ± 0.07^{i}	0.59 ± 0.01^{h}	0.66 ± 0.06^{m}	1.03 ± 0.02^{m}
HO-Sterols of *Sacha Inchi* oil	0.217 ± 0.028^{m}	0.205 ± 0.008^{l}	0.226 ± 0.007^{m}	0.290 ± 0.009^{m}	0.357 ± 0.028^{m}	0.556 ± 0.038^{o}	0.907 ± 0.038^{q}
HO-Sterols of *Prinsepia* oil	0.204 ± 0.005^{n}	0.214 ± 0.144^{l}	0.250 ± 0.048^{l}	0.289 ± 0.010^{n}	0.385 ± 0.037^{l}	0.575 ± 0.032^{n}	0.915 ± 0.043^{p}
HO-Citric acid	0.233 ± 0.029^{kl}	0.236 ± 0.006^{k}	0.273 ± 0.031^{k}	0.453 ± 0.068^{n}	0.051 ± 0.050^{p}	0.660 ± 0.072^{m}	1.120 ± 0.052^{f}
HO-VE	0.237 ± 0.012^{k}	0.247 ± 0.023^{k}	0.267 ± 0.031^{k}	0.383 ± 0.011^{m}	0.483 ± 0.029^{g}	0.707 ± 0.015^{k}	1.103 ± 0.096^{g}
HO-TBHQ	0.133 ± 0.029^{o}	0.157 ± 0.012^{m}	0.162 ± 0.008^{o}	0.160 ± 0.010^{p}	0.163 ± 0.006^{o}	0.168 ± 0.008^{r}	0.173 ± 0.006^{u}
HO-BHA	0.137 ± 0.032^{o}	0.157 ± 0.006^{m}	0.187 ± 0.006^{n}	0.240 ± 0.005^{o}	0.267 ± 0.029^{n}	0.340 ± 0.052^{q}	0.367 ± 0.058^{t}
HO-compound antioxidants (M)	0.49 ± 0.01^{a}	0.49 ± 0.03^{c}	0.62 ± 0.01^{a}	0.83 ± 0.01^{b}	0.89 ± 0.02b^{c}	0.94 ± 0.03^{f}	0.97 ± 0.01^{o}
HO-compound antioxidants (Q)	0.47 ± 0.01^{c}	0.51 ± 0.02^{b}	0.55 ± 0.02^{b}	0.96 ± 0.03^{a}	0.97 ± 0.03^{a}	1.03 ± 0.02^{e}	1.04 ± 0.02^{m}

Lowercase letters mean there's difference between groups, same letters means there's no difference, different means there's difference.

walnut oil increased from 0.35 ± 0.01 mg/g to 1.06 ± 0.14 mg/g, and the oil values of MO and QO were 0.75 ± 0.05 mg/g and 0.55 ± 0.06 mg/g, respectively, which were significantly lower than the oil values of blank walnut oil ($P < 0.05$). These results indicated that the stability of *Sacha Inchi* oil and *Prinsepia* oil was stronger than the stability of walnut oil, which may be due to the higher sterol compounds in *Sacha Inchi* oil and *Prinsepia* oil and the different composition and content of fatty acids. The acid value of 5-30% *Sacha Inchi* oil was significantly higher than the acid value of blank walnut oil ($P < 0.05$), indicating that the addition of *Sacha Inchi* oil increased the acid value of walnut oil and promoted the oxidation of walnut oil, and the results were consistent with the peroxide value. There was no significant effect of the acid value of walnut oil and 5-30% of *Prinsepia* oil, Sterols of *Sacha Inchi* oil, sterols of *Prinsepia* oil, compound antioxidants (Q), compound antioxidants (M), VE and citric acid. Then, the acid values of TBHQ and BHA were significantly lower than the acid value of blank walnut oil, and the results were consistent with the peroxide value.

3.3 Fatty acids

Fatty acid content of three kinds of oils

The HO fatty acid content was in the order linoleate > oleate > α-linolenate > palmitate > stearate. Vaidy found that walnut oil has a shorter oxidation induction period because theα-linolenate content is higher (Vaidya & Eun, 2013). The MO fatty acid content was in the order linoleate > linolenate > oleate > palmitate > stearate. The QO fatty acid content was as follows: oleate > linoleate > palmitate > stearate > linolenate. Three kinds of oils are found in trace behenate: EPA, erucate and DHA. Total fatty acid content of HO, MO, QO was 368865.72 μg/mL, 503989.81 μg/mL, 65137.87 μg/mL, respectively (Table 4), illustrating the total fatty acid content: *Sacha Inchi* oil > walnut oil > *Prinsepia* oil. α-linolenate is polyunsaturated fatty acid,

Table 4. Fatty acid content results of oil samples.

Name	HO	HE	MO	ME	QO	QE
C4:0	NA	NA	NA	NA	NA	NA
C6:0	NA	NA	NA	NA	NA	NA
C8:0	0.40	4.72	0.45	0.35	0.69	0.52
C10:0	0.81	0.79	0.85	0.85	6.76	4.01
C11:0	NA	NA	NA	NA	NA	NA
C12:0	2.05	2.71	2.00	1.47	47.93	38.41
C13:0	0.29	0.49	0.53	0.10	0.80	0.56
C14:0	32.80	50.24	48.68	23.90	389.07	333.09
C14:1N5	2.26	1.51	5.80	6.69	4.63	9.72
C15:0	85.01	102.76	36.87	31.43	69.15	64.76
C15:1N5	9.90	9.71	10.71	10.39	9.79	10.94
C16:0	28803.79	38698.89	22093.47	14843.70	85821.04	75822.48
C16:1N7	686.63	921.70	244.97	135.12	1792.85	1499.73
C17:0	206.26	260.87	443.79	293.31	490.70	436.39
C17:1N7	185.05	231.15	223.68	158.14	188.34	163.69
C18:0	9146.96	12812.28	16111.90	10560.33	52753.22	45363.95
C18:1TN9	1845.01	2552.16	1173.56	848.60	5150.88	3713.20
C18:1N9	86929.61	113544.64	52393.41	35884.40	202573.20	174905.96
C18:2TTN6	2787.99	4096.82	2381.20	1825.54	3678.53	1520.27
C18:2N6	195208.39	233860.29	171646.53	126831.22	198411.17	172825.93
C18:3N6	182.31	243.87	490.60	416.77	174.65	230.99
C18:3N3	41088.64	51597.60	232564.69	168821.95	8348.18	6133.23
C20:0	347.77	459.07	477.96	352.98	3057.21	2672.37
C20:1N9	755.38	1045.23	2274.93	1581.33	647.17	578.68
C20:2N6	121.29	136.63	478.43	350.46	88.40	81.50
C21:0	56.99	62.83	22.99	16.87	34.93	37.65
C20:3N6	38.06	75.48	85.90	58.02	38.64	37.35
C20:4N6	5.84	7.57	50.64	28.35	3.92	4.50
C20:3N3	16.55	20.32	103.43	66.01	20.39	19.31
C22:0	58.12	219.47	265.62	192.10	67.99	60.45
C20:5N3	72.26	84.16	79.64	60.79	364.55	356.24
C22:1N9	40.24	47.73	116.42	66.28	26.60	50.51
C22:2N6	NA	NA	NA	NA	NA	NA
C23:0	39.48	43.90	55.64	36.17	156.44	169.10
C22:4N6	14.95	14.24	9.48	8.67	15.59	17.50
C22:5N6	NA	NA	NA	NA	NA	NA
C24:0	63.66	67.44	71.47	54.70	672.95	715.27
C22:5N3	NA	NA	NA	NA	NA	NA
C24:1N9	NA	NA	NA	NA	NA	NA
C22:6N3	30.95	30.26	23.59	17.93	31.52	42.55

NA means not detected. Unit is ug/mL, same as below.

which is easily oxidized in the accelerated oxidation process. The content of α-linolenate in *Prinsepia* oil is significantly lower than the other two oils, so it has better stability than the other two oils. After accelerated oxidation for 48 h, The increased content of trans oleate and trans linoleate in walnut oil, indicated that some fatty acids were transformed into trans fatty acids, and the changes of oleate, linoleate and α-linolenate acid were 30.61%, 19.80% and 25.58%, respectively, indicating that walnut oil had poor stability. The percentages of oleate, linoleate, γ-linolenate, and α-linolenate in walnut oil increased by 7.2%, 10.48%, 0.02%, and 2.85%, respectively, indicating that the fatty acid composition of walnut oil may affect its oxidation stability.

After accelerated oxidation for 48 h, the saturated fatty acid content of HO increased from 10.53% to 11.44% (Table 5). Unsaturated fatty acids were reduced from 89.47% to 88.56% and were oxidised to saturated fatty acids, which means that the walnut oil was oxidised. The saturated fatty acid content of MO was reduced from 7.86% to 7.26%, and the unsaturated fatty acid content increased from 92.14% to 92.74%. The saturated fatty acid content of QO increased from 25.40% to 25.77%, and the unsaturated fatty acid content decreased from 74.60% to 74.23%. The changes in *Sacha Inchi* oil and *Prinsepia* oil were lower than the changes in walnut oil, showing that the stability of fatty acids was stronger than the stability of walnut oil after oxidation, and the fluctuation range of fatty acids affected the oxidation stability of oils.

Effects of two oils on fatty acids of walnut oil

The total fatty acid content of walnut oil was 368865.72 μg/mL. The total fatty acid content of HO-30% (M) was 416085.2257 μg/mL, and HO-30% (Q) was 428099.8544 μg/ mL (Table 6). These results showed that the fatty acid content of walnut oil increased when *Sacha Inchi* oil and *Prinsepia* oil were added. Because *Sacha Inchi* oil and *Prinsepia* oil are also high in fatty acids themselves. After accelerated oxidation for 48 h, the total fatty acid content of walnut oil was 461307.5424 μg/mL, increasing by 25%, and the total fatty acid content of HE-30% (M) increased by 11.1%, indicating that the stability of walnut oil could be affected by adding 30% (M), the content of fatty acids increased, and the oxidation stability decreased. The total fatty acid content of HE-30% (Q) was 393693.1992 μg/mL, and the total fatty acid content of HE-30% (Q) was lower than the total fatty acid content of walnut oil after oxidation for 48 h, After accelerated oxidation for 48h, the content changes of the changes of oleate, linoleate and α-linolenate acid of HE-30% (Q) were 7.7%, 7.54% and 11.5%, significantly lower than that in blank walnut oil, The content changes of linoleic acid in HE-30% (M) were 19.93%, slightly higher than that in blank walnut oil, indicating that 30% *Sacha Inchi* oil could promote the oxidation of linoleic acid in walnut oil.indicating that the addition of 30% *Prinsepia* oil in walnut oil was more beneficial to the stability of walnut oil in the later stage than the stability of blank walnut oil.

After accelerated oxidation for 48 h, saturated fatty acids increased from 9.75% to 10.28% and decreased by 0.53% of HO-30% (M), unsaturated fatty acid decreased from 90.25% to 89.72%, saturated fatty acid decreased from 14.19% to 14.02% of HO-30% (Q) and unsaturated fatty acids increased from 85.81% to 85.98%, with a change of 0.17% (Table 7). The changes in unsaturated fatty acids in 30% *Sacha Inchi* oil and *Prinsepia* oil were both smaller than the changes in walnut oil, indicating that their addition inhibited the oxidation of unsaturated fatty acids, which was beneficial to the stability of walnut oil after oxidation.

Added sterol fatty acid content

The total fatty acid content of HO-sterol from *Prinsepia* oil and HO-sterol from *Sacha Inchi* oil were 429967.6346 μg/mL and 547048.82 μg/mL, respectively. After oxidation for 48 h, the HO-sitosterol content of *Prinsepia* oil was 483984.3096 μg/mL, an increase of 12.56%. The HO-sitosterol concentration in the *Sacha Inchi* oil was 530979.73 μg/mL, a decrease of 2.57% (Table 8). The variation trend of the two was less than the variation trend of walnut oil (25%). After 48 h of oxidation, the changes of oleate, linoleate and α-linolenate of HO-sterol from *Prinsepia* oil were 15.1%, 10.48% and 12.26%, respectively. The changes of sterol of *Sacha Inchi* oil were 4.77%, 1.55% and 3.61%, respectively, which were significantly lower than that of blank walnut oil.However, the total fatty acid content of both decreased, Indicating that sterols had a certain retarding effect on lipid oxidation, and sterols of *Sacha Inchi* oil have a better inhibition effect on the increase of total fatty acid content.

After 48 h of oxidation, the change ratio of total fatty HO sterols in *Prinsepia* Oil was 0.46% and the change ratio of HO sterols in *Sacha Inchi* oil was 0.06% (Table 9). They have oxidation stability different from walnut oil, which shows that the latter is more stable, but the changes of both were lower than the walnut oil (0.91%). Adding sterol promoted the oxidative stability of walnut oil fatty acids.

Different antioxidants on walnut oil fatty acid content

The total fatty acid content of HO-VE was 481413.04 μg/mL; after 48 h of oxidation, the total fatty acid content was

Table 5. Content of saturated fatty acids and unsaturated fatty acids in oil samples.

Samples	Saturated (ug/mL)	Unsaturated (ug/mL)	Saturated ratio (%)	Unsaturated ratio (%)
HO	38844.40	330021.31	10.53	89.47
HE	52786.47	408521.07	11.44	88.56
MO	39632.21	464357.59	7.86	92.14
ME	26408.27	337176.66	7.26	92.74
QO	143568.88	421568.99	25.40	74.60
QE	125719.00	362201.80	25.77	74.23

Table 6. Fatty acid composition results of walnut oil samples with two oils.

Name	HO-30% (Q)	HE-30% (Q)	HO-30% (M)	HE-30% (M)
C4:0	NA	NA	NA	NA
C6:0	NA	NA	NA	NA
C8:0	0.42	0.80	0.43	1.41
C10:0	1.59	1.49	0.71	0.99
C11:0	NA	NA	NA	NA
C12:0	9.19	8.05	1.96	2.63
C13:0	0.47	0.25	0.35	0.73
C14:0	92.81	74.26	34.12	54.57
C14:1N5	2.18	1.89	3.34	1.79
C15:0	86.34	74.94	73.26	90.83
C15:1N5	8.64	8.84	8.45	9.10
C16:0	42043.69	38455.96	28843.85	36993.13
C16:1N7	922.43	785.87	599.99	815.70
C17:0	265.38	222.35	238.09	320.28
C17:1N7	197.62	169.99	187.30	240.92
C18:0	17070.79	15359.41	10760.01	14450.24
C18:1TN9	2375.21	2590.52	1833.95	2533.38
C18:1N9	115346.62	106463.37	84553.06	106823.75
C18:2TTN6	3115.86	3108.86	3127.44	4129.18
C18:2N6	205354.40	189865.31	196018.77	235098.66
C18:3N6	179.98	219.69	244.89	260.58
C20:0	38613.52	34172.64	382.80	489.59
C20:1N9	797.60	698.94	1190.86	1373.04
C20:2N6	861.63	772.87	161.95	213.62
C21:0	120.95	96.52	43.73	57.36
C20:3N6	56.17	41.53	41.84	49.18
C20:4N6	6.79	7.65	14.70	18.03
C20:3N3	20.29	21.01	33.67	38.48
C22:0	55.42	81.72	99.16	143.60
C20:5N3	126.77	101.50	65.58	81.79
C22:1N9	35.56	33.84	40.89	55.08
C22:2N6	NA	NA	NA	NA
C23:0	65.68	46.63	34.26	48.43
C22:4N6	12.72	11.22	8.44	12.26
C22:5N6	NA	NA	NA	NA
C24:0	181.75	131.11	55.66	70.56
C22:5N3	NA	NA	NA	NA
C24:1N9	NA	NA	NA	NA
C22:6N3	31.05	23.52	27.87	31.34

Table 7. The saturated fatty acids and unsaturated fatty acids of walnut oil samples with two oils.

Samples	Saturated (ug/mL)	Unsaturated (ug/mL)	Saturated ratio (%)	Unsaturated ratio (%)
HO-30% (Q)	60727.29	367372.56	14.19	85.81
HE-30% (Q)	55197.43	338495.77	14.02	85.98
HO-30% (M)	40568.39	375516.83	9.75	90.25
HE-30% (M)	52724.35	459974.15	10.28	89.72

547165.12 μg/mL and increased by 13.6% (Table 10). However, walnut oil increased by 25%, indicating that the antioxidant effect

Table 8. The results of adding 200 mg/kg sterol to walnut oil fatty acids.

Name	HO-sterol of *Prinsepia* oil	HO-sterol of *Sacha Inchi* oil	HE-sterol of *Prinsepia* Oil	HE-sterol of *Sacha Inchi* oil
C4:0	NA	NA	NA	NA
C6:0	NA	NA	NA	NA
C8:0	0.45	0.32	3.02	2.97
C10:0	0.64	0.67	0.90	0.86
C11:0	NA	NA	NA	NA
C12:0	2.33	3.21	2.77	3.14
C13:0	0.71	0.61	0.43	0.54
C14:0	39.83	62.10	53.86	67.35
C14:1N5	1.39	2.20	1.50	2.63
C15:0	91.05	116.10	105.26	120.39
C15:1N5	9.31	9.92	8.85	9.31
C16:0	34691.27	45920.62	40249.65	44629.99
C16:1N7	806.10	1093.40	979.53	1101.96
C17:0	226.30	315.52	269.95	313.12
C17:1N7	208.34	273.97	241.18	282.51
C18:0	11201.66	16289.74	13466.36	15335.21
C18:1TN9	2608.96	2779.28	2649.65	2719.47
C18:1N9	102861.11	136752.56	118396.64	130216.18
C18:2TTN6	3923.50	5317.12	4447.17	4730.15
C18:2N6	222187.51	271852.89	245484.05	267632.13
C18:3N6	212.99	213.49	212.59	199.57
C18:3N3	49009.98	63523.30	55020.82	61229.40
C20:0	401.35	559.96	478.78	537.07
C20:1N9	922.05	1255.24	1147.61	983.91
C20:2N6	121.62	158.39	155.19	177.37
C21:0	56.91	81.88	64.58	78.87
C20:4N6	9.62	11.67	8.69	12.31
C20:3N3	20.63	23.28	25.66	25.60
C22:0	59.80	79.76	157.07	168.37
C20:5N3	72.10	95.00	83.05	96.95
C22:1N9	47.29	58.10	54.06	46.33
C22:2N6	NA	NA	NA	NA
C23:0	37.08	51.09	42.91	54.39
C22:4N6	11.19	11.86	14.07	14.34
C22:5N6	NA	NA	NA	NA
C24:0	59.16	73.78	66.16	79.40
C22:5N3	NA	NA	NA	NA
C24:1N9	NA	NA	NA	NA
C22:6N3	25.65	22.72	28.15	34.74

of VE was not ideal. The total fatty acid content of HO-citric acid was 522128.89 μg/mL; after 48 h of oxidation, the total fatty acid content was 518382.85 μg/mL, and it decreased by 0.71%, so the addition of citric acid could delay the oxidation of walnut oil. The changes of oleate, linoleate and α-linolenate of VE were 16.23%, 11.80% and 14.42%, respectively, and those in citric acid were 0.042%, 0.72% and 2.76%, respectively, which were significantly lower than those in blank walnut oil group, indicating they could inhibit the changes of oleic acid, linoleic acid and linolenic acid in walnut oil.

After oxidation for 48 h, the HE-VE fatty acid content changed by 0.44%, the HE-citric acid content increased and

Table 9. Adding sterols to walnut oil saturated fatty acids and unsaturated fatty acids results.

Samples	Saturated (ug/mL)	Unsaturated (ug/mL)	Saturated ratio (%)	Unsaturated ratio (%)
HO-sterols of *Prinsepia* Oil	46868.55	383099.08	10.90	89.10
HE-sterols of *Prinsepia* Oil	54961.70	429022.61	11.36	88.64
HO-sterols of *Sacha Inchi* oil	63555.37	483493.46	11.62	88.38
HE-sterols of *Sacha Inchi* oil	61391.68	469588.05	11.56	88.44

Table 10. Results of different antioxidants on the content of fatty acids in walnut oil.

Name	HO-VE	HO-Citric acid	HE-VE	HE-Citric acid
C4:0	NA	NA	NA	NA
C6:0	NA	NA	NA	NA
C8:0	0.71	0.38	3.37	3.90
C10:0	0.81	0.89	0.95	0.79
C11:0	NA	NA	NA	NA
C12:0	2.63	2.88	3.56	3.60
C13:0	0.39	0.66	0.60	0.65
C14:0	50.06	64.58	72.59	69.04
C14:1N5	1.39	1.92	2.01	11.30
C15:0	101.53	117.55	125.72	119.32
C15:1N5	9.02	10.08	10.50	9.54
C16:0	39513.20	43241.89	45976.08	43240.60
C16:1N7	943.12	1071.55	1144.61	1082.79
C17:0	259.11	307.20	334.30	304.24
C17:1N7	234.45	269.45	288.88	268.45
C18:0	13055.87	14795.92	15973.04	14716.65
C18:1TN9	2934.57	2509.89	2334.50	2625.58
C18:1N9	116430.61	126793.84	135336.84	126740.49
C18:2TTN6	4582.12	4447.08	4757.19	4484.68
C18:2N6	245173.20	264099.81	274085.70	262195.94
C18:3N6	217.15	206.93	193.98	198.53
C18:3N3	55737.62	61679.79	63775.60	59972.61
C20:0	457.73	520.88	559.28	536.63
C20:1N9	1105.31	1239.15	1299.39	933.08
C20:2N6	140.04	168.22	177.88	160.57
C21:0	56.88	79.70	83.43	78.87
C20:3N6	38.92	41.78	66.55	78.43
C20:4N6	13.45	11.81	12.56	9.44
C20:3N3	23.35	22.15	24.76	28.30
C22:0	58.09	94.81	158.15	175.34
C20:5N3	80.09	93.15	101.53	95.94
C22:1N9	44.15	52.59	56.31	52.14
C22:2N6	NA	NA	NA	NA
C23:0	41.11	53.09	59.17	54.01
C22:4N6	12.24	12.61	17.32	18.90
C22:5N6	NA	NA	NA	NA
C24:0	65.45	78.15	86.30	78.94
C22:5N3	NA	NA	NA	NA
C24:1N9	NA	NA	NA	NA
C22:6N3	28.67	38.51	42.44	33.60

the unsaturated fatty acid content decreased; the change was 0.09% (Table 11). The changes in fatty acids in walnut oil with different antioxidants were lower than the changes in fatty acids in the blank group (0.91%), indicating that the fatty acid stability of walnut oil with antioxidants was better than the fatty acid stability of the blank group.

Fatty acids in walnut oil by compound sterol

After oxidising for 48 h, the total fatty acid content of blank walnut oil increased by 25%. The HO-compound sterol content of *Sacha Inchi* oil was 511904.46 μg/mL, which decreased by 20.4%, and the HO-compound sterol content of *Prinsepia* oil was

Table 11. Results of different antioxidants on saturated fatty acids and unsaturated fatty acids in walnut oil.

Samples	Saturated (ug/mL)	Unsaturated (ug/mL)	Saturated ratio (%)	Unsaturated ratio (%)
HO-VE	53663.58	427749.46	11.15	88.85
HE-VE	63436.56	483728.56	11.59	88.41
HO-citric acid	59358.59	462770.30	11.37	88.63
HE-citric acid	59382.56	459000.29	11.46	88.54

Table 12. The results of compound sterol antioxidants on walnut oil fatty acids.

Name	HO-compound sterol (4:1)	HO-compound sterol (2:1)	HE-compound sterol (4:1)	HE-compound sterol (2:1)
C4:0	NA	NA	NA	NA
C6:0	NA	NA	NA	NA
C8:0	0.40	0.60	0.87	2.15
C10:0	0.85	0.89	0.69	0.73
C11:0	NA	NA	NA	NA
C12:0	2.56	3.00	2.52	2.20
C13:0	0.54	0.38	0.42	0.36
C14:0	48.30	63.65	49.69	38.05
C14:1N5	1.92	3.03	1.94	2.76
C15:0	99.96	117.64	101.70	89.25
C15:1N5	9.27	9.93	8.67	8.45
C16:0	38353.17	42646.69	36874.28	32927.63
C16:1N7	891.71	1021.90	882.71	780.10
C17:0	254.98	306.92	257.10	220.86
C17:1N7	225.60	271.56	231.28	202.43
C18:0	12784.31	14426.21	12262.14	10575.40
C18:1TN9	2935.50	2401.15	2271.52	2358.55
C18:1N9	112597.10	125114.47	109391.47	98157.42
C18:2TTN6	4964.51	4275.91	3671.63	3440.31
C18:2N6	240935.65	258633.88	232170.20	211408.59
C18:3N6	229.12	190.17	201.56	205.25
C18:3N3	54085.32	59962.96	51552.06	44931.47
C20:0	448.86	518.71	448.13	393.18
C20:1N9	1067.28	1186.01	1048.14	876.21
C20:2N6	132.91	165.60	147.23	117.70
C21:0	60.83	77.68	66.68	54.93
C20:4N6	9.96	11.97	7.00	7.60
C20:3N3	20.12	22.62	21.24	17.18
C22:0	80.34	86.58	103.45	103.57
C20:5N3	74.84	97.10	83.99	71.77
C22:1N9	45.15	43.72	49.94	37.88
C22:2N6	NA	NA	NA	NA
C23:0	38.09	55.87	47.26	37.15
C22:4N6	13.14	19.31	15.48	15.69
C22:5N6	NA	NA	NA	NA
C24:0	61.39	82.06	71.47	61.46
C22:5N3	NA	NA	NA	NA
C24:1N9	NA	NA	NA	NA
C22:6N3	24.36	43.12	33.16	30.52

470541.4232 μg/mL, which was reduced by 3.91% (Table 12). Both can reduce the total fatty acid content of walnut oil, indicating that adding compound sterols has a certain retarding effect on the oxidation process of walnut oil, and adding compound sterols has a better effect than adding the single sterol. The changes of oleate, linoleate and α-linolenate of compound sterol content of *Sacha Inchi* oil was were 21.55%, 18.25% and 25.07%, respectively, And compound sterol content of *Prinsepia* oil were 2.85%, 3.63% and 4.68%, respectively, which were lower than that in blank walnut oil, indicating that adding sterol could inhibit the increase of oleate, linoleate and α-linolenate, which was beneficial to walnut oil storage.

Table 13. The content of saturated and unsaturated fatty acids in walnut oil by compound sterol antioxidants.

Samples	Saturated (ug/mL)	Unsaturated (ug/mL)	Saturated ratio (%)	Unsaturated ratio (%)
HO-compound sterol of *Prinsepia* oil	52234.59	418306.83	11.10	88.90
HE-compound sterol of *Prinsepia* oil	50286.41	401839.48	11.12	88.88
HO-compound sterol of *Sacha Inchi* oil	58386.90	453517.57	11.41	88.59
HE-compound sterol of *Sacha Inchi* oil	44506.92	362735.25	10.93	89.07

After 48 h of oxidation, the saturated fatty acid of HO-sterol of compound *Prinsepia* oil increased from 11.10% to 11.12%, and the unsaturated fatty acid decreased from 88.90% to 88.88%, a change of 0.02% (Table 13). The HO-sterol of the compound *Sacha Inchi* oil had a fatty acid change of 0.48%. In conclusion, the change with compound sterol antioxidant was lower than the change of the blank group (0.91%), showing that the compound sterol antioxidant could delay fatty acid change and enhance the oxidation stability of walnut oil.

4 Conclusion

This paper studied mainly the antioxidant activity of sterol compounds in *Sacha Inchi* oil and *Prinsepia* oil on walnut oil. The oxidative stability of *Sacha Inchi* oil and *Prinsepia* oil was greater than the oxidative stability of walnut oil, and 5-30% *Sacha Inchi* oil and *Prinsepia* oil was added. The 5-30% *Sacha Inchi* oil and 5-10% *Prinsepia* oil could increase the peroxide value and acid value of walnut oil and promote the oxidation of walnut oil. However, 15%-30% *Prinsepia* oil inhibited the increase in the peroxide value and acid value of walnut oil and delayed the oxidative rancidity of walnut oil. The sterol compounds in both *Sacha Inchi* oil and *Prinsepia* oil inhibited the increase in the peroxide value and acid value of walnut oil and inhibited the oxidation of unsaturated fatty acids in walnut oil, which had a better antioxidant effect on walnut oil, but the effect was less than the effect of TBHQ and BHA. Sterol compounds of *Sacha Inchi* and citric acid, VE and natural antioxidants, yield compounds with antioxidants, which can significantly inhibit the increase in the walnut oil peroxide value and acid value, and the inhibition of unsaturated fatty acid oxidative rancidity may

be the coordination effect of citric acid and VE and sterol, thus resulting in a better antioxidant effect, and the antioxidant effect is greater than the antioxidant effect of BHA. The results showed that the antioxidant effect was TBHQ > compound antioxidants > BHA > sterols of *Sacha Inchi* oil > sterols of *Prinsepia* oil > citric acid > VE.

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