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A Study on the Antioxidant Effect of Resveratrol on Milk Thistle Seed Oil at Frying Temperature

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Abstract The purpose of this research is to observe the changes in physical and chemical indicators of milk thistle seed oil (MTO) and milk thistle seed oil with resveratrol (MTOR) at the frying temperature, and to explore the antioxidant properties of resveratrol added milk thistle seed oil. The results show the antioxidant effect of resveratrol at the frying temperature. During the heating process, the polyunsaturated fatty acids of MTO is 56.98-52.37(area%) and MTOR is 62.63-56.10(area%) at fresh (0 h) and after heating at frying temperature for 16h , but the degradation rate of γ-linolenic acid of MTO is 0.835 faster than that of MTOR(0.615), and resveratrol can effectively inhibit the degradation of linoleic acid. There is no significant difference between the three aldehydes groups measured by 1H NMR between MTO and MTOR, which is almost consistent with the trend of thiobarbituric acid reactive substances (TBARs). However, the conjugated trienes (K270) content of MTOR is much lower than that of MTO. Resveratrol is a common natural antioxidant, which can be used for antioxidation of milk thistle seed oil. This study can provide for the antioxidant properties of resveratrol in milk thistle seed oil.

Keywords: *milk thistle seed oil, resveratrol, antioxidant, aldehydes*

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1. Introduction

Milk thistle (*Silybum marianum L. Gaertn.*) is an essential medicinal plant from the family Asteraceae [1,2]. Milk thistle oils are available on the market and appeal to consumers because of their healthy properties as cold-pressed oils [3]. Milk thistle seed oil is extracted from milk thistle seeds, with a large number of unsaturated fatty acids (especially linoleic and oleic acid), phenolics, vitamin E, and phytosterols [4,5,6,7]. However, the oil contact with oxygen and food at high temperatures will lead to oxidation, hydrolysis, and other complex reactions, thus producing harmful substances in the oil, and reducing its nutritional value [8]. Therefore, to ensure the nutrition and safety of food, reducing the deterioration of the oils is crucial. Silymarin is the primary active ingredient in thistle seed oil, which is also an antioxidant, but silymarin has a low solubility in oil and does not have an antioxidant effect [9]. Generally, synthetic and natural antioxidants are often used to inhibit lipid oxidation. Natural antioxidants are considered a good substitute for synthetic antioxidants due to their potential health benefits and safety.

Resveratrol (*trans*-3,4',5-trihydroxystilbene), a non-flavonoid polyphenol, is an antitoxin produced by plants that can be found in grapes, causes, and peanuts[10]. It has antioxidant, anti-tumor, anti-inflammatory, and other biological activities [11,12,13]. Hang et al. studied that resveratrol can inhibit the oxidation of high oleic acid peanut oil [14]. Oh et al. studied that resveratrol derivatives can inhibit the production of oxidation products in bulk oil [15]. However, the protective effects of resveratrol on milk thistle seed oil are still unclear.

In this study, resveratrol was used as an addition to milk thistle seed oil. Firstly, this research aims to assess the antioxidant effects of resveratrol on milk thistle seed oil and to monitor the physicochemical and chromatic characteristics during 16 h of continuous heating at frying temperature (180°C). Furthermore, electronic nose and 1H NMR were used to monitor the odor and related oxidation products of milk thistle seed oil.

2. Materials and Methods

2.1. Materials

Milk thistle seeds came from crops in Liaoning province (China) and were supplied by Tongrentang

(Group) Co., Ltd, Resveratrol purchased from Aladdin (≥ 99.5%, Shanghai, China), All the chemicals and HPLC-grade solvents were obtained from Aladdin.

2.2. Extraction of Milk Thistle Seed Oil

The 10 Kg seeds were pressed under a constant pressure of 25 MPa using a hydraulic press (Jiangsu Longxu Heavy Industry Machinery Co., Ltd, Jiangsu, China) for 30 min at ambient temperature and cycle 4 times. The collected oils were centrifuged at 6000×g for 15 min to obtain clear oil without suspended substances.

2.3. Thermal Treatment of Milk Thistle Seed Oils

The milk thistle seed oil (MTO) and the milk thistle seed oil with resveratrol (MTOR) were heated at frying temperature. Before treatment, 1 g resveratrol was dispersed in 1 mL anhydrous ethanol, then vortexed for 1 min and solution was added to milk thistle seed oil to a final concentration of 0.04% (v/v). One-liter portions of MTO and MTOR were heated at 180°C for 16 h and electric blast furnace (DZL, De Mars, Foshan, Guangdong, China),at light and without cover. Samples were taken 50 mL at 2-h intervals for later analysis. Three samples of each type of oil were heated. Three samples of each oil were taken during heating. Moreover, 24 h and under Freeze at -20°C were the samples stored until analyses.

2.4. Determination of Fatty Acid Composition

The fatty acids of oil samples were methylated following the BF3-methanol method as described in the standard of ISO:5509 [16]. The fatty acid composition of the oil was analyzed by gas chromatography (GC) using GC-2010 plus chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with a flame ionization detector (FID Supelco SP SP-2560 gas capillary column (100 m×0.25 mm, 0.2 μm, Supelco, Bellefonte, PA, USA) was used to separation. It was got with the fixed conditions for the oven temperature program as 140°C for 5 min. raised to 240°C at a rate of 3°C/min and then kept at 240°C for 30 min. Injector and detector temperatures were fixed at 250°C and 260°C, respectively. Supelco 37 component fatty acid methyl ester (FAME) mix was used as an external standard for the FAME identification and quantification.

2.5. Determination of Color Parameters

A hand-held chromatic aberration meter (NR110, Sanenshi Technology Co., Ltd.,Shenzhen China) was used to determine the color parameters of heated MTO and MTOR.The *L**, *a**, *b**, reffered to CIELab color space were measured and the total color difference (Δ*E*) was calculated.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

2.6. Acid Value Determination

The official American Oil Chemists' Society (AOCS) Cd 3d-63 method [2] was used to determine acid value (AV). An aliquot of 1.00 g of oil was mixed with 15 mL ethanol-toluene solution (1:1, *v*:*v*) and it was titrated with potassium hydroxide (0.25 M) solution using phenolphthalein as a color indicator, which causes pink coloring. The AV was calculated by the formula (2):

$$\mathrm{AV}(\mathrm{mg/g}) = (\mathrm{V}\times\mathrm{C}\times 56.1)/\mathrm{W} \quad (2)$$

where V is the volume of KOH used for titration of the oil samples, C is KOH concentration, and W is the weight of the oil sample in grams.

2.7. Iodine Value Determination

The iodine value (IV) of oils was determined following the AOCS official methodCd 1d-92 [17]. Accurately 3 g of oil was weighed into 500 mL iodine flask,15 mL of solvent (cyclohexane/glacial acetic acid 1:1, *v*/*v*) was added and mixture was shaken to dissolve completely. Further addition 25 mL Wijs solution in a dark place for 1 h and added 20 mL KI (10%) solution and 100 mL distilled water. Titrate with 0.1 M $Na_2S_2O_3$ solution, after the yellow disappears, added 1 mL starch indicator, titrate until the blue disappears, and blank the sample without the oil samples. The IV was calculated by the following formula:

$$\mathrm{IV}(\mathrm{mg/g}) = [(\mathrm{B\text{-}S})\times\mathrm{M}\times 12.69]/\mathrm{W} \quad (3)$$

where B is the volume of titrant of blank (mL); S is the volume of titrant of sample (mL); M is the molality of $Na_2S_2O_3$ solution and W is the sample weight (g).

2.8. Saponification Value Determination

The saponification value (SV) of oils was determined according to the method of AOCS Cd 3d-25 [17]. Mass (mg) of potassium hydroxide required for saponification of saponifiable matter in 1g oil.

2.9. Peroxide Value Determination

Peroxide value (PV) was determined using the official AOCS method Cd 8b-90 [2]. Weigh about 5 g of oil into the flask, further added glacial acetic acid/chloroform (3:2, *v*:*v*, 30 mL) and a saturated potassium iodide solution. Then react the mixed solution in the dark for 3 min, adding distilled water and saturated starch solution. The PV was calculated by the formula (4).

$$\mathrm{PV}\ (\mathrm{g}/100\ \mathrm{g}) = (\mathrm{V}\times 0.01\times 1000)/\mathrm{W} \quad (4)$$

where V is the volume of the sodium thiosulfate and M is the sample weight.

2.10. Determination of Content of Thiobarbituric Acid Reactive Substances

The content of thiobarbituric acid reactive substances (TBARs) in oils was determined according to the method described by Pegg RB. [18]. In 100 mL 0.25 M hydrochloric acid 15 g of 3-chloroacetic acid was

dissolved and afterward, 0.375 g of thiobarbituric acid was added to that mixture to obtain the thiobarbituric acid solution. The TBA value was obtained by heating a mixture of 2.5 mg sample, 2.5 mL 2-thiobarbituric acid reagent, and 3 mL n-butanol in a water bath at 95 °C for 30 min. After cooling to room temperature (25°C), After centrifugation at 3000 g for 10 min (High speed centrifuge TDZ5-MS, Xiangyi Laboratory Instrument Development Co., LTD., Hunan, China), the absorbance was measured at 532 nm UV-spectrophotometer (UV-1700 Shimadzu, Tokyo, Japan) . Absorbance in terms of TBARS, The standard test curve of 1,1,3,3 tetraethoxypropane(TEP) is calculated.

2.11. Determination of Vonjugated Diene and Triene Content

The content of conjugated dienes (K232) and trienes (K270) was determined according to the method described by Lolis *et al*. [19]. 0.25 g sample was added to a 10 mL mixture of cyclohexane, the absorbance (UV-1700 Shimadzu, Tokyo, Japan) was measured at a wavelength of 232 nm and 270 nm. The contents of K232 and K270 (g/100g) were calculated by the formulas (5) and (6), respectivly.

$$K232 = A232 / (b \times c \times 115) \quad (5)$$

$$K270 = A270 / (b \times c \times 920) \quad (6)$$

where A232 and A270 are the absorbance values at 232 and 270 nm, respectivly, b is the light path, and c is sample concentration.

2.12. Odor Analysis by Electronic Nose

Electronic nose instrument (PEN3, AIRSENSE Analytics GmbH, Schwerin, Germany) equipped with 10 sensors (W1C: aromatic, W5S: broadrange, W3C: aromatic, W6S: hydrogen, W5C: arom-aliph, W1S: broad-methane, W1W: sulphur-organic, W2S: broad-alcohol, W3S: methane-aliph) was used for odor analysis. The E-nose system was preheated 24 h before the experiment. Briefly, 3±0.01 g of sample were weighed into a 20 mL headspace bottle, capped with plastic polytetrafluorethylene silicone septa, and equilibrated at 60°C for 10 min. After balanced, a 2.5 mL syringe (Hamilton, USA) was inserted to adsorb the balanced headspace gas and quickly injected to the E-nose system. Pure dry air was used as carrier gas with a flow rate of 400 mL/min to clean the sensor array with its signal response restored to zero. The total acquisition time was 90 s with the acquisition interval set to 5 s and the acquisition delay time of 200 s. Then, the signal processing system converted the analog signal into digital signal, and the output results were obtained after the computer analysis. Finally, the response records were analyzed by PCA for the pattern recognition.

2.13. 1H NMR Analysis

1H NMR analysis of oils was carried out using a Bruker Avance 500 spectrometer (BRUKER, Switzerland) at 500.13 MHz. Each oil was weighed and mixed with 600 μL of CDCl3 containing 0.03% tetramethylsilane as an internal standard. After vortexing for 1 min, the mixture was introduced into a matched nuclear magnetic tube (5 mm diameter, 8'' lengths). The parameters were as follows: spectral width 65,536 Hz, pulse width 10 ppm, relaxation delay 1 s, number of scans 16, acquisition time 3.2768 s. The test temperature was 25℃. The content of the oxidation product in the sample is calculated by the internal standard deuterated chloroform (CDCl 3). The calculation formula is:

Internal standard content (CIS)

$$C_{Is} = \frac{M_{IS}}{MW_{IS}} = \frac{p \times v \times 0.2\%}{MW_{IS}}$$

Sample content (Cs)

$$C_S = \frac{A_S \times C_{IS}}{A_{IS}}$$

Sample Molality (bs)

$$b_s = \frac{C_S}{m_s}$$

MW IS = 119.38 (Molar weight, g/mol); ms (Sample Weight, mg); MIS (Internal standard Weight, mg); CS (Sample Content, mol); CIS (Internal standard Content, mol); AS (Sample Area); AIS = 1 (Internal standard Area); bS (Sample Molality, mmoL/kg); q (1.500, 25 ?C) (Internal standard Density, g/mL);

2.14. Statistical Analysis

Data were analysed by two-way analysis of variance (ANOVA) using the SPSS 22.0 program for Windows. Duncan's multiple range test was used to determine significant differences at the 5% level.

3. Results and Discussion

3.1. Fatty Acid Composition of Oils

The fatty acid compositions of MTO and MTOR are shown in Table 1. Compared with before and after heating, the unsaturated fatty acid content of MTO and MTOR showed a decreasing trend, but the decreasing rate of MTRO was slower. But resveratrol had no inhibitory effect on trans fatty acid. Min et al. show that the oxidative rates of oleic acid, linoleic acid, and linolenic acid are 1:12:25 [20], this was almost consistent with the fatty acid oxidation trend of MTO. While the linolenic acid oxidation rate of MTOR was slower. Resveratrol first removes the hydrogen of the 4'-phenol hydroxyl group, and then removes the 3,5 phenol hydroxyl groups, providing electrons to lipid oxidation radicals to form stable compounds, blocking the transfer of oxidation chains, and thus inhibiting the oxidation of fatty acids [21]. Therefore, based on this fact, it should be observed that MTOR has higher oxidative stability compared to MTO.

3.2. Color of Oils

The *L**, *a**, *b** and Δ*E* values of MTO and MTOR during heating are shown in Table 2. With the extension of heating time, the color of MTO and MTOR gradually deepens, because the color change is related to the oxidation of triglycerides and the oxidation of tocopherols and tocotrienols [22]. In addition, compared with MTO, the Δ*E* value of MTOR increases faster, which is related to the yellow color of resveratrol after oxidation.

3.3. Acid Value

The fatty acid value is relaxed to fatty acid content, glycerol, and free fatty acids produced by lipase hydrolysis. In addition, the acid value usually increases before the physical properties show a change in quality, so an increase in acid value may harm the edible quality of fats and oils [23,24]. In this research, as the heating time increases, the acid value of MTO and MTOR shows an upward trend (Table 3). But there is no significant difference between MTO and MTOR (p>0.05). The result indicates that resveratrol does not inhibit thermal degradation.

Table 1. Fatty acid composition (g/100 g total fatty acids) of milk thistle seed oil (MTO) and milk thistle seed oil with resveratrol (MTOR) fresh (0 h) and after heating at frying temperature for 8 h and 16 h

Fatty acid	MTO			MTOR		
	0 h	8 h	16 h	0 h	8 h	16 h
C16:0	0.77±0.14^{a}	0.16±0.01^{c}	0.41±0.02^{b}	0.63±0.03^{A}	0.00±0.00^{C}	0.41±0.02^{B}
C18:0	12.35±0.62^{b}	15.08±0.75^{a}	15.23±0.76^{a}	13.14±0.66^{C}	19.92±1.00^{A}	17.36±0.87^{B}
C20:0	0.40±0.02^{b}	0.12±0.01^{c}	0.89±0.05^{a}	0.47±0.02^{B}	0.05±0.01^{C}	0.61±0.03^{A}
C18:2*n*6*t*	0.98±0.05^{b}	0.16±0.01^{c}	1.19±0.06^{a}	0.47±0.02^{B}	0.05±0.01^{C}	0.89±0.04^{A}
C18:3*n*3	0.40±0.02^{b}	0.58±0.03^{a}	0.54±0.03^{a}	0.9±0.05^{A}	0.38±0.02^{C}	0.57±0.03^{B}
C18:3*n*6	4.44±0.22^{a}	3.33±0.17^{b}	2.77±0.14^{c}	3.34±0.17^{A}	3.28±0.16^{A}	2.11±0.11^{B}
C18:1*n*9*c*	28.95±1.45^{a}	18.22±0.91^{b}	30.32±1.52^{a}	22.611.13^{AB}	20.52±1.03^{B}	24.87±1.24^{A}
C18:2*n*6*c*	51.16±2.56^{b}	62.16±3.11^{a}	47.87±2.39^{b}	57.93±2.9^{A}	55.68±2.78^{B}	52.54±2.63^{A}
C22:0	0.55±0.04^{b}	0.58±0.03^{b}	0.74±0.04^{a}	0.52±0.03^{B}	0.11±0.01^{C}	0.65±0.03^{A}
ΣSFA	14.07±0.70^{b}	15.94±0.80^{a}	17.31±0.87^{a}	14.76±0.74^{B}	20.08±1.00^{A}	19.03±0.95^{A}
ΣPUFA	56.98±2.85^{b}	65.84±3.29^{a}	52.37±2.61^{b}	62.63±3.13^{A}	59.4±2.97^{AB}	56.1±2.81^{B}
ΣMUFA	28.95±1.45^{a}	18.22±0.91^{b}	30.32±1.52^{a}	22.611.13^{AB}	20.52±1.03^{B}	24.87±1.24^{A}
ΣTFA	0.98±0.05^{b}	0.16±0.01^{c}	1.19±0.06^{a}	0.47±0.02^{B}	0.05±0.01^{C}	0.89±0.04^{A}

ΣSFA, sum of saturated fatty acid; ΣPUFA, sum of polyunsaturated fatty acid; ΣMUFA, sum of monounsaturated fatty acid, ΣTFA, sum of trans fatty acid.

Table 2. Color parameters of of milk thistle seed oil (MTO) and milk thistle seed oil with resveratrol (MTOR) fresh (0 h) and after heating at frying temperature for 8 h and 16 h

Oil	Heating time (h)	*L**	*a**	*b**	Δ*E*
MTO	0	82.31±0.01^{c}	-0.24±0.01^{f}	3.69±0.00^{q}	9.43±0.01^{p}
	2	79.67±0.03^{g}	-0.47±0.00^{i}	7.32±0.01^{p}	13.74±0.02^{n}
	4	80.24±0.02^{f}	-0.43±0.00^{h}	8.03±0.02^{o}	13.81±0.00^{n}
	6	80.38±0.03^{e}	-0.86±0.02^{k}	12.76±0.03^{m}	17.38±0.04^{m}
	8	79.06±0.00^{i}	-1.37±0.02^{m}	21.19±0.06^{k}	25.38±0.06^{k}
	10	78.22±0.04^{k}	-1.67±0.01^{p}	26.54±0.01^{i}	30.61±0.02^{i}
	12	76.78±0.01^{l}	-1.01±0.00^{l}	36.23±0.00^{g}	40.16±0.01^{g}
	14	75.35±0.00^{m}	-0.06±0.00^{e}	39.98±0.01^{e}	44.17±0.01^{e}
	16	73.32±0.01^{o}	1.81±0.00^{d}	44.56±0.00^{d}	49.21±0.00^{d}
MTOR	0	82.83±0.01^{b}	-0.32±0.01^{g}	3.46±0.00^{r}	8.87±0.01^{q}
	2	83.90±0.01^{a}	-0.82±0.00^{j}	9.21±0.00^{n}	12.51±0.01^{o}
	4	81.09±0.01^{d}	-1.44±0.00^{n}	17.78±0.00^{l}	21.44±0.00^{l}
	6	79.45±0.00^{h}	-1.93±0.02^{q}	26.39±0.00^{j}	30.03±0.00^{j}
	8	78.37±0.00^{j}	-1.54±0.01^{o}	32.28±0.01^{h}	35.92±0.01^{h}
	10	76.77±0.01^{l}	-1.00±0.00^{l}	38.97±0.01^{f}	42.76±0.02^{f}
	12	73.59±0.00^{n}	2.81±0.00^{c}	47.65± 0.01^{c}	52.10±0.01^{c}

	14	72.93±0.08^{p}	4.38±0.00^{b}	54.06±0.00^{b}	58.49±0.02^{b}
	16	70.24±0.01^{q}	7.46±0.01^{a}	57.08±0.00^{a}	62.50±0.00^{a}

MTO is represent milk thistle seed oil; MTOR is milk thistle seed oil with resveratrol. The difference is Significant (p<0.05)

Table 3. The SV, IV, K232 and K270 of oils samples MTO and MTOR by 0 to 16 hours frying

Oil	Time(h)	AV (mg/g)	SV (mg/g)	IV (g/100 g)	K232 (g/100 g)	K270 (g/100 g)
	0	0.54±0.01^{ij}	516±1^{a}	127±9^{a}	7.51±0.01^{i}	13.52±0.03^{q}
	2	0.73±0.03^{h}	535±13^{a}	119±4^{abcd}	12.2±0.14^{h}	23.00±0.00^{m}
	4	0.93±0.00^{g}	531±18^{a}	122±0^{ab}	15.99±0.12^{g}	27.47±0.05^{h}
	6	1.12±0.00^{ef}	532±14^{a}	122±0^{ab}	19.96±0.00^{f}	29.51±0.08^{e}
MSO	8	1.32±0.00^{d}	533±9^{a}	122±2^{ab}	23.09±0.06^{e}	30.86±0.05^{d}
	10	1.50±0.01^{c}	541±5^{a}	112±3^{bcde}	24.89±0.02^{d}	30.42±0.01^{e}
	12	1.67±0.02^{bc}	484±29^{ab}	110±1de	27.70±0.11^{c}	31.51±0.00^{c}
	14	1.83±0.01^{ab}	491±27^{ab}	113±1^{bcde}	30.40±0.03^{a}	32.92±0.00^{a}
	16	1.95±0.01^{a}	443±16^{b}	111±2^{cde}	29.66±0.02^{b}	31.87±0.05^{b}
	0	0.46±0.04^{j}	511±17^{a}	121±0^{abc}	7.20±0.00^{ij}	13.80±0.04^{p}
	2	0.55±0.04^{ij}	539±10^{a}	120±2^{abcd}	7.28±0.00^{ij}	17.12±0.01^{o}
	4	0.66±0.13^{hi}	541±23^{a}	107±1^{e}	7.11±0.01^{j}	20.87±0.01^{n}
	6	0.76±0.06^{h}	544±8^{a}	110±2de	6.20±0.00^{kl}	24.30±0.01^{l}
MSOR	8	1.05±0.04^{fg}	501±11^{ab}	111±4^{cde}	6.14±0.00^{l}	24.97±0.06^{k}
	10	1.26±0.03^{de}	505±2^{ab}	112±0^{bcde}	6.55±0.36^{k}	26.71±0.00^{i}
	12	1.29±0.11^{de}	547±36^{a}	104±4^{e}	6.19±0.01^{kl}	26.38±0.21^{j}
	14	1.67±0.04^{bc}	498±7^{ab}	114±2^{bcde}	7.38±0.01^{ij}	29.38±0.01^{f}
	16	1.81±0.04^{ab}	520±22^{a}	105±1^{e}	6.47±0.10^{kl}	29.03±0.04^{g}

The difference is Significant (p<0.05).SV is saponification value; IV is iodine value; K232 is content of conjugated dienes; K270 is trienes; MTO is represent milk thistle seed oil; MTOR is milk thistle seed oil with resveratrol.

3.4. Iodine Value

Iodine value is usually used to measure the degree of unsaturation of oils, and its determination is based on the reactivity of the triglyceride double bond. The iodine values of MTO and MTOR during heating are shown in Table 3. With the extension of heating time, the IV of MTO and MTOR showed a decreasing trend. Besides, we observed that the iodine value of MTO decreases faster than that of MTOR, which indicates that MTO has a faster rate of oxidative rancidity. It also shows that resveratrol protects the double bond of unsaturated fatty acids by blocking oxidation chain transmission.

3.5. Saponification Value

The saponification values of MTO and MTOR are shown in Table 3. There is no significant difference in the saponification value of MTO and MTOR before and after heating (p>0.05). When heating, the oil first undergoes a decomposition reaction to produce a large amount of free fatty acids, which leads to an increase in the saponification value. When certain substances reach a certain amount, the reaction proceeds in reverse and the saponification value begins to decrease [25]. Therefore, the saponification values of MTO and MTOR both increased first and then decreased. The reverse reaction of the MTOR saponification reaction started at 6 h, while the reverse reaction of MTO started at 10 h, indicating that resveratrol can promote the reverse reaction of the saponification reaction.

3.6. PV and TBARs

During continuous heating, the peroxide values of MTO and MTOR show a gradual upward trend (Figure 1). Moreover, in 2-12 h, the peroxide values of MTOR were lower than MTO, while in 14 h, the peroxide value of MTOR was higher than MTO; in 16 h, MTOR peroxide value decreased, and finally was lower than MTO. This is due to resveratrol being able to inhibit the production of peroxides [26], at 12-14 h, resveratrol no longer dominates, and the oil starts to oxidize rapidly, at 14 h, the peroxide value reaches its maximum, Peroxides are unstable at high temperature and quickly degrades into aldehydes, ketones, and other small molecules [27,28]. The content of TBARs of MTOR was lower than that of MTO, and the growth rate of MTOR value increases after 12 hours, which is consistent with the result of the peroxide value. In addition, TBARs shows an "N" upward trend, which is due to the instability of MDA, which forms tertiary oxidation products such as aluminum furans, ketones, and aluminum condensation products through degradation, condensation, and other reactions [29].

3.7. K232 and K270

The determination of the conjugated olefin value of MTO and MTOR can better reflect the rancidity of fat during heating. K232 denotes the content of primary oxidation products produced and K270 represents the content of secondary oxidation products. During the

heating process, the K232 value of MTOR presents an upward trend and then a downward trend, while the K232 value of MTO presents a gradual upward trend (Table 3). After heating, the K232 value of MTO was significantly higher than MTO (p<0.05).

The K270 values of MTO and MTOR show a gradual upward trend, and during the heating process, the rate of increase of the K270 value of MTO was faster than that of MTOR, and the final value of MTO was significantly greater than that of MTOR (p<0.05). The trends of K232 and K270 are the same as those of PV and TBARs. Similarly, this result also confirms that resveratrol has a certain inhibitory effect on the oxidation of milk thistle oil at 180℃.

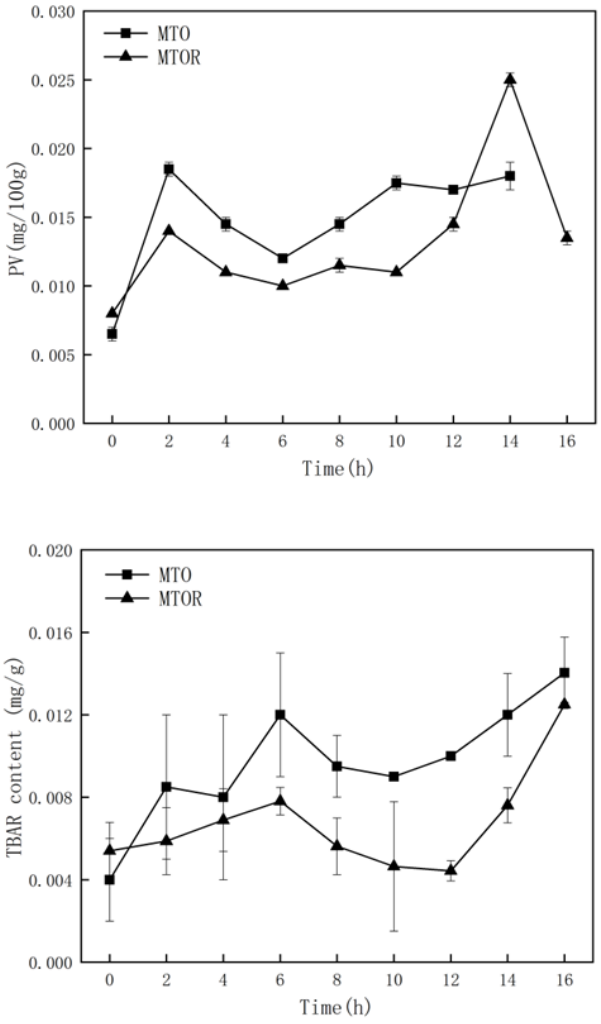

Figure 1. PV and TBARs of MTO and MTOR during heating at 180℃

Peroxide value (PV); The content of thiobarbituric acid reactive substances (TBARs); MTO is represent milk thistle seed oil; MTOR is milk thistle seed oil with resveratrol.

3.8. Odor of Oils

The response values of 10 sensors of the electronic nose analysing MTO and MTOR after different heating times (0 h, 8 h, and 16 h) are shown in Figure 2. The response intensity of the W2S sensor (broad- ahcohol) only on the MTOR heated for 8 h was different from other samples. To further verify the distinguishing effect of the electronic nose on MTO and MTOR with different heating times, the data obtained is subjected to PCA, and the result is shown in Figure 3. The results show that the total contribution of the two principal components (PCs) reaches 99.20%, of which the contribution rate of PC1 to the model is 88.91%, the contribution rate of PC2 is 10.29%, and the sample heated by MTOR for 8 hours (MTOR8) is very different from other samples on PC1. This is related to the content of primary oxidation products because both PV and K232 were found to be close to the minimum values during this period. The specific mechanism needs to be further studied.

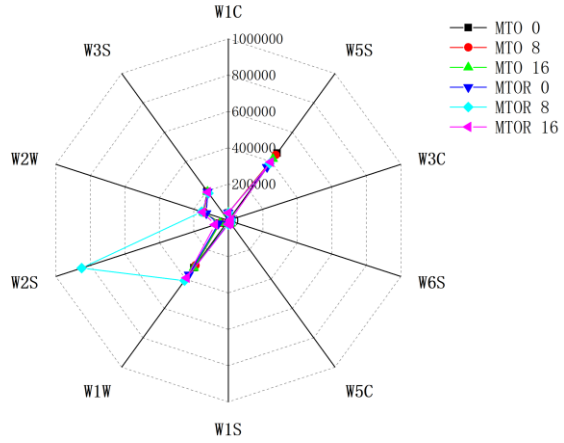

Figure 2. Odor characteristic fingerprints of MTO and MTOR at different heating times

MTO and MTOR represent milk thistle seed oil and milk thistle seed oil with resveratrol, respectively, and the number represents heating time (h). ; W1C: aromatic, W5S: broadrange, W3C: aromatic, W6S: hydrogen, W5C: arom-aliph, W1S: broad-methane, W1W: sulphur-organic, W2S: broad-alcohol, W3S: methane-aliph

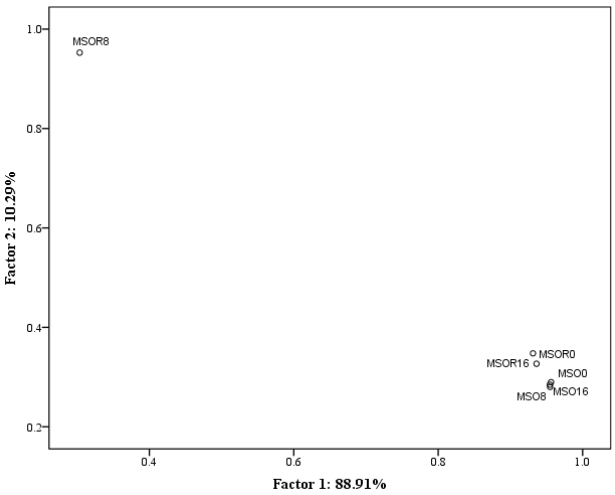

Figure 3. The PCA of MTO and MTOR

MTO and MTOR represent milk thistle seed oil and milk thistle seed oil with resveratrol, respectively, and the number represents heating time (h).

3.9. 1H NMR analysis

1H NMR has been widely used in the food field, and many scholars have shown that 1H NMR can be used to monitor the thermal oxidation process of oils [30]. To further confirm the antioxidant activity of resveratrol, 1H

NMR was used to detect the formation and development of aldehydes. Although the TBARs were used to evaluate secondary oxidation products, they can only detect malondialdehyde generated during lipid oxidation, and malondialdehyde is neither the sole end product nor a substance generated exclusively by lipid oxidation [31]. Therefore, 1H NMR was used to monitor the aldehydes of MTO and MTOR during the heating process to evaluate the effect of resveratrol on milk thistle oil. With the increase in heating time, *n*-alkanals, (*E*,*E*)-alkadienals, and (*E*)-2-alkenals were produced in MTO and MTOR, showing a time-dependent relationship (Figure 4). In addition, D and E signal peaks also appeared between aldehydes, and increased with the increase of heating time. Therefore, it can be inferred that the D and E signal peaks are other aldehydes, and the specific structure needs further study. However, MTO and MTOR are significantly different at the same stage. This is because resveratrol in milk thistle seed oil cannot effectively inhibit the formation of aldehydes.

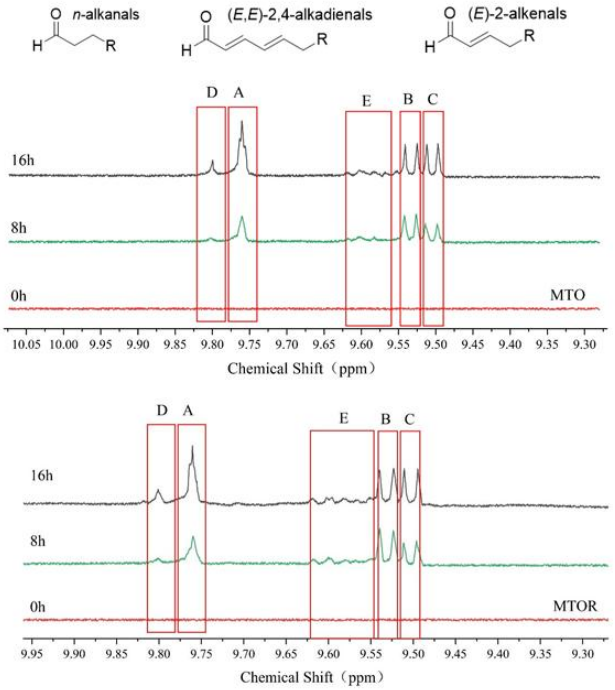

Figure 4. Secondary oxidation products (aldehydes) of MTO and MTOR at different heating time

MTO is represent milk thistle seed oil; MTOR is milk thistle seed oil with resveratrol. A-E: Different signal peak

4. Conclusion

During the heating process, the fatty acid composition, physical and chemical indexes, color parameters, and functional group change of MTO and MTOR were studied. The results showed that resveratrol does not inhibit lipase hydrolysis, but it protects the double bonds. While resveratrol had an effect on the color of the milk thistle seed oil during heating, this does not mean that the milk thistle seed oil added with resveratrol produces more oxidation products and harmful substances, and there is no direct correlation between harmful products and color. Moreover, resveratrol cannot effectively inhibit the production of aldehydes, but resveratrol can inhibit the non-aldehyde secondary oxidation products produced by milk thistle seed oil during heating. Therefore, resveratrol can be used as a natural antioxidant in milk thistle seed oil during cooking.

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Statement of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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