

## Effect of Rosemary Extract and TBHQ on the Stability of Radish Seed Oil

Zhao Gongling\*, Li Bing and Guo Yancheng

School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, China.

hnzgl@163.com\*

(Received on 22<sup>nd</sup> October 2015, accepted in revised form 5<sup>th</sup> April 2016)

**Summary:** The effects of rosemary extract (RE) and tert-Butylhydroquinone (TBHQ) on the storage stability of radish seed oil were studied according to the change of the acid value, peroxide value, tocopherol and sulforaphene in radish seed oil. The results showed that under conditions of accelerated oxidation by (60±1) °C, the storage stability of the radish seed oil with antioxidants could be significantly improved, among which TBHQ was better than RE. Besides, RE and TBHQ had a synergistic effect on antioxidation. The compound of 0.01% RE and 0.01% TBHQ had a better antioxidation effect than 0.07% RE and 0.02% TBHQ respectively, which recommended it can be a suitable antioxidant of radish seed oil.

Keywords: Radish seed oil, Rosemary extract, TBHQ, Compound, Storage stability.

### Introduction

The unsaturated fatty acid contained in oils can be easily oxidized, thus results in deterioration of oil. Antioxidant could restrain oil from being oxidized and enhance the stability of oil. Although TBHQ is a wide-accepted high-qualified antioxidant with high safety and stability and already being generally used in food industry [1-3], it is after all artificial, not as safe as natural antioxidants. Hence, finding new natural antioxidants and reducing the addition of artificial antioxidants has become a significant task of food-science researchers [4-5].

Rosemary extract is a kind of recently discovered natural antioxidant with safety, efficiency and other good characteristics, which draws the attention of researchers and becomes a hot point in this field [6-7].

Mature radish seeds, besides its special medical effects on coordinating intestines and stomach, resolving phlegm and improving digestion [8-9], are rich in oil, which proportion is up to 45% [10-11]. Radish seed oil contains plenty of sulforaphene [12-13] and tocopherol [14-15] and unsaturated fatty acids accounts for 90% in its fatty acid composition [16-18]. Thus, radish seeds can be a qualified ingredient of edible oil. The effects of RE and TBHQ on the storage stability of radish seed oil were studied in this paper according to the change of the acid value, peroxide value, tocopherol and sulforaphene and provided theoretical supports for improving the safety of radish seed oil and extending its storage period.

### Experimental

#### *Instruments and Materials*

SHP-160FE biochemical incubator (Shanghai sanfa scientific instruments Co., LTD, Shanghai, China); TU-1801 uv-vis spectrophotometer (Beijing Purkinje General Instruments Co., LTD, Beijing, China); Agilent 6890N gas chromatography (Agilent Technologies Co., Ltd.).

Radish seed oil was self-extracted using solvent method as follows: white radish seed powder was prepared by grinding aliquots of seed in a coffee grinder for 15 s. The radish seed powder (1000 g) was subsequently soaked in 5000 mL of n-hexane for 5 h at 25 °C and filtered. The residue was again extracted with 5000 mL of n-hexane again. The supernatant from both steps was collected, combined and concentrated by using Rotary evaporator under vacuum at 40 °C to remove n-hexane.

Rosemary extract antioxidant (carnosic acid≥15.00%, salvio≥8.00%, rosemano≥3.50%) was purchased from Xi'an Chenyi Biological Technology Co., LTD (Xi'an, China). All other reagents were of analytical grade.

#### *Experimental Design*

All samples, which were distinguished by additions and density, contained 250ml of radish seed oil and were stored in iodine flask of 500ml respectively. Samples of radish seed oil (control) and radish seed oil with different density of TBHQ, RE

---

\*To whom all correspondence should be addressed.

and their compound were heated in incubator to a temperature of  $(60\pm 1)$  °C continuously for whole day and for 28 days, which was used to determine acid value, peroxide value, sulfuraphene content and tocopherol composition of samples and evaluate oil stability.

#### *Determination of Acid Value and Peroxide Value*

The acid value and peroxide value were measured by the IUPAC Methods [19].

#### *Determination of Fatty Acid Composition*

According to previous report [20], the fatty acid composition of samples was measured by using an Agilent 6890N gas chromatograph equipped with a flame ionization detector and a DB-FFAP capillary column (30 m  $\times$  0.32 mm, 0.25  $\mu$ m of film thickness). Temperature and analytical conditions of chromatographic evaluation were as follows: FID 230 °C, injector 230 °C; oven 140 °C for 3 min, 140–190 °C at 4 °C/min, 190 °C for 15 min, 190–250 °C at 2 °C/min for 2 min, 250–280 °C at 15 °C/min for 2 min; carrier gas helium 1 mL/min; ionization energy 70 eV; mass range 40–500 m/z; 1  $\mu$ L of the sample was injected with a split ratio of 1:20. The fatty acids were identified according to the retention times of standard fatty acid methyl ester performed at the same conditions. For all fatty acids the mean value of five replicates was expressed as the percentage of the total fatty acids pool.

#### *Determination of Sulfuraphene Content*

Measurement of the sulfuraphene content in the oil was performed on an Agilent GC-MS according to the previous studies [21-22]. The sample was separated on a HP-5MS capillary column (30 m  $\times$  0.32 mm, 0.25  $\mu$ m of film thickness). Column temperature program was 80 °C (1 min) isotherm, then increased to 150 °C at the rate of 20 °C/min and then to 280 °C at the rate of 40 °C/min, finally held at 280 °C for 3 min; Injector temperature was set at 250 °C; Chose helium as carrier gas at flow rate of 1 mL/min and ionization energy 70 eV; Mass range 40–500 m/z and scan mode on electron impact. 1  $\mu$ L of the sample was injected with a split ratio of 1:20. The identification of sulfuraphene was based on comparison of relative retention time with authentic sample and a linear calibration curve was prepared at concentrations of 2.5–50  $\mu$ g/mL to quantify the levels of sulfuraphene.

The data were recorded and processed by Xcalibur software.

#### *Determination of Tocopherol Composition*

According to the previous report [23], the tocopherol ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -isomers) contents of the oil were determined by using a LC-10Avp HPLC (Shimadzu, Japan) with a RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan). The oil was dissolved in n-hexane and eluted on a silica column (250 $\times$ 4.6 mm, 5 $\mu$ m) (Dalian Yilite, Dalian, China) with n-hexane/isopropyl ether (99/1, v/v) at 0.8 mL/min. And the column temperature was set at 40 °C. The excitation and emission wavelengths were set at 298 and 325 nm, respectively. The absolute contents of tocopherols were determined according to the calibrated standard curves.

## **Results and Discussion**

#### *Effect of RE and TBHQ on Stability of Radish Seed Oil Respectively*

##### *Acid Value and Peroxide Value*

As shown in Table-1, With the concentration of RE increased from 0.01% to 0.07%, the value of oil acid reduced from 7.124 mgKOH/kg to 4.015 mgKOH/kg, the value of peroxide reduced from 8.067 meq/kg to 3.844 meq/kg, the change of acid value reduced from 3.856 mgKOH/kg 0.747 mgKOH/kg, and the change of peroxide value reduced from 6.386 meq/kg to 2.163 meq/kg. With the concentration of TBHQ increased from 0.005% to 0.02%, the value of oil acid reduced from 6.961 mgKOH/kg 4.178 mgKOH/kg, the value of peroxide reduced from 7.725 meq/kg to 4.030 meq/kg, the change of the acid value reduced from 3.693 mgKOH/kg 0.91 mgKOH/kg, and the change of peroxide value reduced from 6.044 meq/kg to 2.349 meq/kg. Acid value and peroxide value in oil of added RE and TBHQ were lower than that of the control group (9.897 mgKOH/kg and 14.622 meq/kg).

Compared with control group, RE and TBHQ had a significant effect on keeping acid value and peroxide value of oil during storage. According to the change of these two parameters, 0.07% of RE was most effective, followed by 0.02% of TBHQ, 0.05% of RE, 0.01% of THBQ, 0.03% of RE, 0.005% of THBQ and 0.01% of RE orderly. Thus, in maintaining acid value and peroxide value, TBHQ performed better than RE.

Table-1: Effect of single antioxidant on acid value and peroxide value.

	Original	Control	RE				TBHQ		
			0.01%	0.03%	0.05%	0.07%	0.005%	0.01%	0.02%
Acid value /(mgKOH/kg)	3.268± 0.001	9.897± 0.001	7.124± 0.003	6.564± 0.004	5.395± 0.003	4.015± 0.002	6.961± 0.003	6.064± 0.003	4.178± 0.002
Peroxide value /(meq/kg)	1.681± 0.001	14.622±0.005	8.067± 0.004	6.645± 0.001	5.212± 0.003	3.844± 0.002	7.725±0.003	5.846± 0.004	4.030± 0.003
Change of acid value/(mgKOH/kg)	0	6.629	3.856	3.296	2.127	0.747	3.693	2.796	0.91
Change of peroxide value/(meq/kg)	0	12.941	6.386	4.964	3.531	2.163	6.044	4.165	2.349

\*Change of acid value=Average of acid value-3.268, Change of peroxide value=Average of peroxide value-1.681

Table-2: Fatty acids composition of original radish seed oil (%).

Fatty acids	C16: 0	C16: 1	C18: 0	C18: 1	C18: 2	C20: 0	C18: 3	C22: 0	C22: 1	C24: 0	UFA
Content	5.964	0.183	2.282	19.873	12.351	1.509	20.232	1.165	35.779	0.666	88.415

UFA stand for unsaturated fatty acids.

Table-3: Effect of single antioxidant on fatty acids content of radish seed oil (%).

	Original	Control	0.07%RE	0.02%TBHQ	0.05%RE	0.01%TBHQ	0.03%RE	0.005%TBHQ	0.01%RE
SFA	11.586	33.637	12.764	13.8	14.532	21.131	23.631	26.775	28.541
MUFA	55.832	48.692	55.973	55.446	56.853	56.107	56.301	54.382	53.455
PUFA	32.583	17.66	31.263	30.057	28.605	22.762	20.067	18.843	18.003
UFA	88.415	66.352	87.236	86.203	85.458	78.869	77.368	73.225	71.458
Preserving rate of UFA/%	100	75.05	98.67	97.50	96.66	89.206	87.51	82.82	80.82

\*SFA, MUFA, PUFA and UFA stand for saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and unsaturated fatty acids, respectively.

\*Preserving rate of UFA/%=Content of UFA/88.415×100

### Sulforaphene Content

Table-4: Effect of single antioxidant on sulforaphene content of radish seed oil (mg/kg).

	Original	Control	0.07%RE	0.02%TBHQ	0.05%RE	0.01%TBHQ	0.03%RE	0.005%TBHQ	0.01%RE
Sulforaphene	62.268	8.872	56.522	56.235	49.374	45.758	40.364	36.246	34.557
Preserving rate/%	100	14.25	90.78	90.31	79.29	73.49	64.82	57.88	55.50

\*Preserving rate/%=Content of Sulforaphene/62.268×100

The reason why antioxidant effect of RE was worse than that of TBHQ may be that the content of antioxidants in RE, such as salvia, salviol and rosemary phenol were low.

### Fatty Acid Composition

According to Table-2, radish seed oil contained various fatty acids, such as C18:1 (19.873%), C18:2 (12.381%), C18:3 (20.232%), C22:1 (35.779%) and C16:1 (0.183%), of which 88.415% were unsaturated fatty acids with low oxidation resistance.

Data in Table-3 showed that samples with single antioxidant had larger proportion of polyunsaturated fatty acids and monounsaturated fatty acids and smaller proportion of saturated fatty acids than control group, which illustrated that single antioxidant protected unsaturated fatty acids from being oxidized effectively.

With the concentration of RE and TBHQ increasing, the content of SFA in oil reduced from 28.541% of 0.01% RE to 12.764% of 0.07% RE, the content of UFA increasing from 71.458% of 0.01% RE to 87.236% of 0.07% RE, the content of PUFA increased from 18.003% of 0.01% RE to 31.263% of

0.07% RE, and the survival rate of UFA E increased from 80.828% of 0.01% RE to 98.67% of 0.07% RE.

Similar with effect on acid value and peroxide value, 0.07% of RE performed best, followed by 0.02% of TBHQ, 0.05% of RE, 0.01% of THBQ, 0.03% of RE, 0.005% of THBQ and 0.01% of RE orderly. Oil of added single antioxidant, MUFA changed irregularly. Except samples added 0.01% of RE and 0.005% of TBHQ, the monounsaturated fatty acids content of other samples with single antioxidant were almost the same with or even higher than the original oil. One possible reason for this phenomenon could be oxidation of polyunsaturated fatty acids produced monounsaturated fatty acids.

According to Table-4, content of sulforaphene in original radish seed oil was 62.268 mg/kg. However, this index dropped to 8.872 mg/kg rapidly after storage in the control group with a preserving rate merely of 14.25% which was relevant to the low oxidation resistance of sulforaphene [24-25] and high storage temperature.

With the concentration of RE and TBHQ increasing, the content of sulforaphene in oil increased from 34.557 mg/kg of 0.01% RE to 56.235 mg/kg of 0.02% TBHQ and 56.522 mg/kg of 0.07% RE. The

survival rate of sulforaphen increased from 55.50% of 0.01% RE to 90.31% of 0.02% TBHQ and 90.78% of 0.07% RE.

Sulforaphene content and preserving rate of samples with single antioxidant were much higher than of control group, among which 0.07% of RE and 0.02% of TBHQ were most effective with highest sulforaphene content and preserving rate of their samples followed by 0.05% of RE, 0.01% of THBQ, 0.03% of RE, 0.005% of THBQ and 0.01% of RE orderly.

As shown in Table-5, content of tocopherol in original oil was 601.596 mg/kg, among which content of  $\gamma$ -VE was highest with a value of 536.914 mg/kg accounting for 89.25% of the total content of tocopherol and the content of  $\beta$ -VE was lowest with a value merely of 9.218 mg/kg. Every factor shown in Table-5 of samples with single antioxidant was higher than of control group, which indicated that single antioxidant protected tocopherol.

With the concentration of RE and TBHQ increasing, the content of Total VE in oil increased from 455.156 mg/kg of 0.01% RE and 458.889 mg/kg of 0.05% TBHQ to 573.787 mg/kg of 0.02% TBHQ and 580.905 mg/kg of 0.02% RE. The survival rate of total VE increased from 75.66% of 0.01% RE and 76.28% of 0.005% TBHQ to 95.38% of 0.02% TBHQ and 96.56% of 0.07% RE. The varying rule of Alpha, Beta, Gamma, and Delta – VE was the same with Total VE.

After storage, sample with 0.07% of RE had the highest tocopherol ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -isomers) content and preserving rate, followed by samples with 0.02% of TBHQ, 0.05% of RE, 0.01% of THBQ,

0.03% of RE, 0.005% of THBQ and 0.01% of RE orderly. From the results in Table-1 to Table-5, the effects of single antioxidant on acid value, peroxide value, fatty acids composition, sulforaphene content and tocopherol composition had the same pattern. Hence, TBHQ was a better antioxidant of radish seed oil than RE.

Comparing the data in Table-4 and Table-5, under single antioxidant of the same concentration, the survival rate of VE in oil was higher than that of sulforaphene. It was speculated that sulforaphene was more easily oxidized than VE in line with the outcome reported in the literature [26-27]. Or the antioxidants used in this experiment were more conducive to protect the VE.

The test results of effect of composite antioxidant on acid value and peroxide value are given in Table-6.

The value of acid in Composite antioxidant 0.01% TBHQ + 0.03% RE was 3.305 mgKOH/kg, the value of peroxide was 2.648 meq/kg, and the changes of the acid value and peroxide value were 0.237 mgKOH/kg and 0.967 meq/kg. Compared with other composite antioxidant, the change of peroxide value, acid value and peroxide value was the lowest, followed by oil of added 0.01% TBHQ + 0.01% RE. The value of acid was 3.778 mgKOH/kg, the value of peroxide was 3.834 meq/kg, and the changes of the acid value and peroxide value were 0.51 mgKOH/kg and 2.153 meq/kg. The worst was the oil of 0.005% TBHQ + 0.01% RE. The value of acid was 6.197 mgKOH/kg, the value of peroxide was 5.945 meq/kg, and the changes of the acid value and peroxide value were 2.929 mgKOH/kg and 4.264 meq/kg.

#### Tocopherol Composition

Table-5: Effect of single antioxidant on tocopherol composition of radish seed oil (mg/kg).

	Original	Control	0.07%RE	0.02%TBHQ	0.05%RE	0.01%TBHQ	0.03%RE	0.005%TBHQ	0.01%RE
$\alpha$ -VE	27.178	9.851	25.512	22.867	21.291	20.751	18.774	16.768	16.153
$\beta$ -VE	9.218	4.135	8.364	7.719	6.674	5.404	4.651	4.242	4.011
$\gamma$ -VE	536.914	95.761	521.355	519.336	461.244	450	421.326	419.315	417.236
$\delta$ -VE	28.286	8.167	25.674	23.865	21.483	20.632	19.127	18.564	17.756
Total	601.596	117.914	580.905	573.787	510.692	496.787	463.878	458.889	455.156
Preserving rate/%	100	19.60	96.56	95.38	84.89	82.58	77.11	76.28	75.66

\*Total= $\alpha$ -VE+ $\beta$ -VE+ $\gamma$ -VE+ $\delta$ -VE; Preserving rate/%=Total/601.596 $\times$ 100.

#### Effect of Compound of TBHQ and RE on Stability of Radish Seed Oil

##### Acid Value and Peroxide Value

Table-6: Effect of composite antioxidant on acid value and peroxide value.

	0.01%TBHQ +0.03%RE	0.01%TBHQ +0.01%RE	0.005%TBHQ +0.05%RE	0.005%TBHQ +0.03%RE	0.005% TBHQ +0.01%RE
Acid value/(mgKOH/kg)	3.505 $\pm$ 0.003	3.778 $\pm$ 0.001	5.518 $\pm$ 0.003	5.804 $\pm$ 0.001	6.197 $\pm$ 0.001
Peroxide value/ (meq/kg)	2.648 $\pm$ 0.001	3.834 $\pm$ 0.002	5.035 $\pm$ 0.001	5.464 $\pm$ 0.002	5.945 $\pm$ 0.002
Change of acid value/(mgKOH/kg)	0.237	0.51	2.25	2.536	2.929
Change of peroxide value/ (meq/kg)	0.967	2.153	3.354	3.783	4.264

\*Change of acid value=Average acid value-3.268; Change of peroxide value=Average peroxide value-1.681.

With fixed concentration of TBHQ (RE), the effect of composite antioxidant on maintaining acid value and peroxide value grew with the increase of RE (TBHQ) addition. Comparing the data in Table-1 and Table-6, the oxidation resistance ability of samples were arranged from high to low as following: 0.01%TBHQ+0.03%RE, 0.01%TBHQ+0.01%RE, 0.07%RE, 0.02%TBHQ, 0.005%TBHQ+0.05%RE, 0.05TBHQ+0.03RE, 0.05%RE, 0.005%TBHQ+0.01%RE, 0.01%TBHQ, 0.03%RE, 0.005%TBHQ, 0.01%RE which indicated that RE and TBHQ had a synergistic effect on holding acid value and peroxide value. The effect of 0.01% of TBHQ+0.01% of RE were even better than 0.02%TBHQ which was the highest addition amount allowed [28]. Thus, 0.01% of TBHQ and 0.01% of RE compound has both better antioxidation and economic effect.

Table-7 displays the test result of effect of composite antioxidant on fatty acid composition of radish seed oil.

Among components of composite antioxidant, with the concentration of RE and TBHQ decreasing, the oil content of SFA in oil rose from 12.535% of 0.01% TBHQ + 0.03% RE to 12.626% of 0.01% TBHQ + 0.01% RE, 17.852% of 0.005% + 0.01% RE. The content of UFA reduced from 87.465% of 0.01%TBHQ + 0.03% RE to 87.375% of

0.01% TBHQ + 0.01% RE, 82.147% of 0.005% + 0.01% of RE. The content of PUFA decreased from 31.594% of 0.01% TBHQ + 0.03% RE to 31.442% of 0.01% TBHQ + 0.01% RE, 25.737% of 0.005% + 0.01% RE. The survival rate of UFA decreased from 98.93% of 0.01% TBHQ + 0.03% RE to 98.82% of 0.01% TBHQ + 0.01% RE, 92.91% of 0.005% + 0.01% RE.

According to the data, with the same concentration of TBHQ (RE), the saturated fatty acids content decreased as the concentration of RE (TBHQ) increased and the unsaturated fatty acids content, polyunsaturated fatty acids content and preserving rate of unsaturated fatty acids increased as the concentration of RE (TBHQ) increased. This phenomenon indicated that the antioxidation effect of composite antioxidant was positively correlated with the concentration of TBHQ and RE. However, when the combination came to 0.01% of TBHQ + 0.01% of RE, the increasing effect almost diminished as density increasing. Comparing the data in Table-3 and Table-7, the pattern of effect of composite antioxidant on oxidation resistance was same with on acid value and peroxide value, which also indicated that there was a synergistic effect between TBHQ and RE and the compound of 0.01% of TBHQ and 0.01% of RE performed better than 0.02% of TBHQ.

#### Fatty Acid Composition

Table-7: Effect of composite antioxidant on fatty acid content of radish seed oil (%).

	0.01%TBHQ +0.03% RE	0.01%TBHQ +0.01% RE	0.005%TBHQ +0.05% RE	0.005%TBHQ +0.03% RE	0.005%TBHQ +0.01% RE
SFA	12.535	12.626	14.135	14.364	17.853
MUFA	55.871	55.933	56.24	56.522	56.41
PUFA	31.594	31.442	29.625	29.114	25.737
UFA	87.465	87.375	85.865	85.636	82.147
Preserving rate of UFA/%	98.93	98.82	97.12	96.86	92.91

\*SFA, MUFA, PUFA and UFA stand for saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and unsaturated fatty acids, respectively.  
\*Preserving rate of UFA/%=Content of UFA/88.415×100

#### Sulforaphene Content

Table-8: Effect of composite antioxidant on sulforaphene content of radish seed oil (mg/kg).

	0.01%TBHQ +0.03%RE	0.01%TBHQ +0.01%RE	0.005%TBHQ +0.05%RE	0.005%TBHQ +0.03%RE	0.005%TBHQ +0.01%RE
Sulforaphene	59.342	58.122	53.615	52.546	48.658
Preserving rate/%	95.30	93.34	86.10	84.39	78.14

\*Preserving rate/%=Content of Sulforaphene/62.268×100.

#### Tocopherol Composition

Table-9: Effect of composite antioxidant on tocopherol composition of radish seed oil (mg/kg).

	0.01%TBHQ +0.03%RE	0.01%TBHQ +0.01%RE	0.005%TBHQ +0.05%RE	0.005%TBHQ +0.03%RE	0.005%TBHQ +0.01%RE
α-VE	26.214	25.974	22.336	21.874	20.751
β-VE	8.526	8.463	7.367	7.012	5.404
γ-VE	529.365	527.414	483.652	476.454	450.000
δ-VE	27.003	26.847	22.221	21.866	20.632
Total	591.108	588.698	535.576	527.206	496.787
Preserving rate/%	98.26	97.86	89.03	87.633	82.58

\*Total=α-VE+β-VE+γ-VE+δ-VE; Preserving rate/%=Total/601.596×100.

From Table-8, among the composition of composite antioxidants, with the concentration of RE and TBHQ decreasing, the content of sulforaphene and survival rate in oil decreased from 59.342 mg/kg and 95.30% of 0.01% TBHQ + 0.03% RE to 58.122 mg/kg and 93.34% of 0.01% TBHQ + 0.01% RE, as well as 48.658 mg/kg and 78.14% of 0.005% + 0.01% RE.

Data in Table -4 and Table-8 performed a same effect arrangement of composite antioxidant on sulforaphene with on previously discussed indexes, acid value, peroxide value and fatty acid content, which thirdly illustrated a synergistic effect between TBHQ and RE and suggested the compound of 0.01% of TBHQ and 0.01% of RE as suitable antioxidant.

Among the components of composite antioxidant, with the concentration of RE and TBHQ decreasing, the content of Total VE and the survival rate in oil decreased from 591.108 mg/kg and 98.29% to 588.698 mg/kg and 97.86% of 0.01% TBHQ + 0.01% RE, 496.787 mg/kg and 82.58% of 0.005% + 0.01% RE. The varying rule of Alpha, Beta, Gamma, and the Delta - VE was in line with Total VE.

The same pattern discussed above again appeared in effect on tocopherol composition according to the data in Table-5 and Table-9. Besides, the preserving rate of 98.26% and 97.86% arose in samples with compound of 0.01% of TBHQ + 0.03% of RE and compound of 0.01% of TBHQ + 0.01% of RE needed more attention. These preserving rates were both higher than of 0.07% of RE with a preserving rate of 96.56% and of 0.02% of TBHQ with a preserving rate of 95.38%, which indicated a synergistic effect between TBHQ and RE. However, despite the concentration of RE tripled, the outcome of preserving rate merely increased by 0.4%, which suggested the compound of 0.01% of TBHQ and 0.01% of RE as suitable antioxidant when considering economic effect.

Comparing the data in Table-8 and Table-9, under composite antioxidants of the same concentration, the survival rate of VE in oil was higher than that of sulforaphene. Once again, it showed that sulforaphene was more easily oxidized than VE.

### Conclusion

TBHQ and RE have a significant positive effect on the stability of radish seed oil. Single TBHQ is more effective than single RE. However, when combined, there is a synergistic effect on antioxidation between TBHQ and RE. The effect of each concentration of antioxidant on the stability of radish

seed oil can be arranged as follows: 0.01%TBHQ+0.03%RE > 0.01%TBHQ+0.01%RE > 0.07%RE > 0.02%TBHQ > 0.005%TBHQ+0.05%RE > 0.005%TBHQ+0.03%RE > 0.05%RE > 0.005%TBHQ+0.01%RE > 0.01%TBHQ > 0.03%RE > 0.005%TBHQ > 0.01%RE. Taking both efficiency and economy into account, compound of 0.01% of TBHQ and 0.01% of RE is a suitable antioxidant.

### Acknowledgements

The financial support provided by the Program of Science and Technology Department in Henan (132300410359, 152300410096) was greatly appreciated.

### References

1. N. Prasad, B. Siddaramaiah, M. Banu, Effect of Antioxidant Tertiary Butyl Hydroquinone on the Thermal Oxidative Stability of Sesame Oil (*sesamum indicum*) by ultrasonic studies, *J. Food Sci Technol*, **52**, 2238 (2015).
2. A. S. Bhatnagar, A. G. Gopala Krishna, Stability of Cold-Pressed Oil from Commercial Indian Niger (*Guizotia abyssinica (L.f.) Cass.*) Seed as Affected by Blending and Interesterification, *J. Am Oil Chem Soc*, **10**, 1 (2015).
3. N. M. Ricardo, J. Páscoa, S. Machado, Value Adding to Red Grape Pomace Exploiting Eco-friendly FT-NIR Spectroscopy Technique, *J. Food Bioprocess Technol*, **8**, 865 (2015).
4. B. Matthäus, Utilization of High-Oleic Rapeseed Oil for Deep-Fat Frying of French Fries Compared to other Commonly Used Edible Oils, *Eur J. Lipid Sci Technol*, **108**, 200 (2006).
5. K. Petersen, G. Jahreis, M. Busch-Stockfisch, Chemical and Sensory Assessment of Deep-Frying Oil Alternatives for the Processing of French Fries. *Eur J. Lipid Sci Technol*, **115**, 935 (2013).
6. F. Nor, S. Mohamed, N. Idris, Antioxidative Properties of Pandanus amaryllifolius Leaf Extracts in Accelerated Oxidation and Deep Frying Studies, *J. Food Chem*, **110**, 319 (2008).
7. R. Chirinos, M. Huaman, I. Betalleluz-Pallardel, Characterisation of Phenolic Compounds of Inca Muña (*Clinopodium bolivianum*) Leaves and the Feasibility of their Application to Improve the Oxidative Stability of Soybean Oil During Frying, *J. Food Chem*, **128**, 711 (2011).
8. M. Wada, H. Kido, K. Ohshima, Chemiluminescent Screening of Quenching

- Effects of Natural Colorants Against Reactive Oxygen Species: Evaluation of Grapeseed, Monascus, Gardenia and Red Radish Extracts as Multi-Functional Food Additives, *J. Food Chem*, **101**, 980 (2007).
9. P. Kuang, D. Song, Q. Yuan, Separation and Purification of Sulforaphene from Radish Seeds Using Macroporous Resin and Preparative High-Performance Liquid Chromatography, *J. Food Chem*, **136**, 342 (2013).
  10. J. Zhang, X. Zhou, M. Fu, Integrated Utilization of Red Radish Seeds for the Efficient Production of Seed Oil and Sulforaphene, *J. Food Chem*, **192**, 541 (2016).
  11. H. C. Kaymak, Profile of (n-9) and (n-7) Isomers of Monounsaturated Fatty Acids of Radish (*Raphanus sativus* L.) seeds. *J. Am Oil Chem Soc*, **92**, 345 (2015).
  12. P. Kuang, D. Song and Q. Yuan, Preparative Separation and Purification of Sulforaphene from Radish Seeds by High-Speed Countercurrent Chromatography, *J. Food Chem*, **136**, 309 (2013).
  13. T. Songsak, G. B. Lockwood, Glucosinolates of Seven Medicinal Plants from Thailand, *J. Fitoterapia*, **73**, 209 (2002).
  14. A. Shin, M. Ahn, G. O. Kim, Biological Activity of Various Radish Species, *J. Orient Pharm Exp Med*, **15**, 105 (2015).
  15. P. Y. Zhang, Cardioprotection by Phytochemicals via Antiplatelet Effects and Metabolism Modulations, *J. Cell Biochem Biophys*, **73**, 369 (2015).
  16. H. C. Kaymak, Seed Fatty Acid Profiles: Potential Relations between Seed Germination under Temperature Stress in Selected Vegetable Species, *J. Acta Sci Pol-Hortoru*, **13**, 119 (2014).
  17. V. J. Barthet, (n-7) and (n-9) cis-Mono unsaturated Fatty Acid Contents of 12 Brassica species. *J. Phytochemistry*, **69**, 411 (2008).
  18. X. Hu, J. K. Daun, R. Scarth, Proportion of C18:1(n-9) Fatty Acids in Canola Seed Coat Surface and Internal Lipids, *J. Am Oil Chem Soc*, **71**, 221 (1994).
  19. C. Paquot, A. Haunfenne, *IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives*, London Press, Blackwell, p.18 (1987).
  20. M. Khoobchandani, B. K. Ojeswi, N. Ganesh, Antimicrobial Properties and Analytical Profile of traditional Eruca Sativa Seed Oil: Comparison with Various Aerial and Root Plant Extracts, *J. Food Chem.*, **120**, 17 (2010).
  21. S. Lim, J. Lee and J. K. Kim, Analysis of Isothiocyanates in Newly Generated Vegetables, Baemuchae (*Brassicoraphanus*) as Affected by Growth, *Int J Food Sci Tech*, **44**, 1401 (2009).
  22. S. F. Vaughn, M. A. Berhow, Glucosinolate Hydrolysis Products from Various Plant Sources: pH Effects, Isolation, and Purification, *J. Ind Crop Prod*, **21**, 193 (2005).
  23. S. Liang, G. Yang and Y. Ma, Chemical Characteristics and Fatty Acid Profile of Foxtail Millet Bran Oil, *J. Am Oil Chem Soc*, **87**, 63 (2010).
  24. J. W. Fahey, A. T. Zalcmann, P. Talalay, The Chemical Diversity and Distribution of Glucosinolates and Isothiocyanates Among Plants, *J. Phytochemistry*, **56**, 5 (2001).
  25. L. G. West, K. A. Meyer, B. A. Balch, Glucoraphanin and 4-Hydroxyglucobrassicin Contents in Seeds of 59 Cultivars of Broccoli, Raab, Kohlrabi, Radish, Cauliflower, Brussels Sprouts, Kale, and Cabbage, *J. Agric. Food Chem*, **52**, 916 (2004).
  26. Shishu, I. Pal Kaur, Inhibition of Cooked Food-Induced Mutagenesis by Dietary Constituents: Comparison of Two Natural Isothiocyanates, *J. Food Chemistry*, **112**, 977 (2009).
  27. S. Boddupalli, J. R Mein, S. Lakkanna, Induction of Phase 2 Antioxidant Enzymes by Broccoli Sulforaphane Perspectives in Maintaining the Antioxidant Activity of Vitamins A, C, and E, *J. Genetics*, **3**, 8 (2012).
  28. State Standard of the People's Republic of China (GB 2760-2014), CNS no. 04.007, Standards Press of China ,p. 85(2015).