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Determination of tertiary butylhydroquinone in edible vegetable oil by liquid chromatography/ion trap mass spectrometry

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Abstract

A simple, sensitive and accurate analytical method for quantification of tertiary butylhydroquinone (TBHQ) in edible vegetable oil was established by liquid chromatography/ion trap mass spectrometry (LC/ITMS). After extraction, 5 μl of the extracts was directly injected into LC/ITMS for TBHQ determination. Ethanol was selected as the extraction solvent. The optimized fragmentation amplitude was 1.70 V and electrospray ionization (ESI) was more suitable than atmospheric pressure chemical ionization (APCI) for TBHQ detection. The calibration curve showed good linearity ($R^2 = 0.9990$) and recoveries from spiked samples ranged from 81.9% to 110.5%. Relative standard deviations of intra-day and inter-day were in the ranges 2.5–5.7% and 3.9–13.8%, respectively. The procedure allows the detection of 0.3 mg/kg TBHQ in edible vegetable oil. Typical edible vegetable oils in the market were detected for TBHQ by the proposed method. As results, TBHQ was detected in blend oil, soybean salad oil and camellia oil samples.

Keywords: Tertiary butylhydroquinone (TBHQ); Liquid chromatography/ion trap mass spectrometry (LC/ITMS); Quantitative determination

1. Introduction

Edible vegetable oils tend to encounter problems of autoxidation, which would affect oil quality and may endanger human health. The addition of antioxidants, including natural antioxidants and synthetic antioxidants, is preferentially considered to prevent the oxidative deterioration of the lipid fraction during storage and processing. The antioxidants to be used are determined by various factors including legislation, cost, stability and effectiveness. However, when permitted, synthetic phenolic antioxidants (SPAs), such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ), are commonly used because of their chemical stability, low cost and availability. But the safety of these SPAs was questioned due to their potential risk (Okubo, Nagai, Ushiyama, & Kano, 1997; Okubo, Yokoyama, Kano, & Kano, 2003; Williams, Iatropoulos, & Whysner, 1999). For example, BHA was banned in Japan in 1982 and BHT was deleted from the "GRAS" (generally recognized as safe) list of FDA (Food and Drug Administration) in the United States in 1997. As for TBHQ, although it has not been approved as a food antioxidant yet in some countries, such as the European Union and Japan (Pinho, Ferreira, Oliveira, & Ferreira, 2000), it is permitted to be used in foods up to a maximum limit of 200 mg/kg in some other countries, such as China, the United States, Australia, Brazil, New Zealand and Philippines (Christian & Liliane, 2002). Therefore, the regulations for controlling the use of TBHQ in food applications are quite different among individual countries and the content of TBHQ in foods should be carefully monitored.

Experimental studies have been made to quantify TBHQ in various food matrixes, mainly by gas and liquid chromatographic methods (Karovicova & Simko, 2000).

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Oil samples dissolved in diethyl ether could be directly injected into the gas chromatography (GC) with glass wool inserted in the glass liner of the injector port (Yang, Lin, & Choong, 2002). Gas chromatography/hydrogen flame ionization detector (GC/FID) could provide a lower limit of detection for TBHQ (0.20 mg/l) than gas chromatography/mass spectrometry (GC/MS) (1.0 mg/l) with the injection volume of 1 μl (González, Gallego, & Valcárcel, 1999). Liquid chromatography (LC) was used for the determination of TBHQ in bakery products (Rafecas, Guardiola, Illera, Codony, & Boatella, 1998), dry foods (Christian & Liliane, 2002) and liver pâtés (Pinho et al., 2000). Due to unsatisfied sensitivity and selectivity, most of these methods using GC and LC require complicated preconcentration procedures after sample extraction, such as the procedure of evaporating the sample extracts to dryness by a vacuum rotary evaporator and then dissolving the residue, which cannot meet the simple, sensitive and accurate demand of detection.

Although TBHQ has an exceptional stabilizing effect in unsaturated fats, particularly in polyunsaturated vegetable oils and in edible animal fats, it is still controversy of its applications due to its toxicological effects. However, methods are still needed for some purposes, e.g. detecting the presence of illegal TBHQ in oil. The objective of this work is to establish a rapid, sensitive and accurate method to quantify TBHQ in edible vegetable oils by LC/ITMS. Moreover, TBHQ is detected in 10 typical edible vegetable oils in the market, including blend oil, soybean salad oil, peanut oil, grape seed oil, camellia oil, sesame oil, olive oil, corn oil and sunflower seed oil.

2. Materials and methods

2.1. Equipment

An Agilent HP 1100 series LC/MSD trap SL system, equipped with a binary pump with a degasser, an automatic sample injector, a LC column ZORBAX Eclipse XDB-C18 (Narrow-Bore, 2.1 mm × 150 mm, 5 μm), a guard column ZORBAX Eclipse XDB-C8 (Narrow-Bore, 2.1 mm × 12.5 mm, 5 μm), a diode array detector (DAD), an electrospray ionization (ESI) interface and an atmospheric pressure chemical ionization (APCI) interface, was used for TBHQ analysis.

Centrifuge LD5-2A (Beijing, China) with the maximum rotate speed of 5000 r/min and microvibrant mixer WH-90A (Shanghai, China) with the vibrant frequency of 1250/min were also used in the experiment.

2.2. Materials and reagents

Ten typical edible vegetable oils including blend oil, soybean salad oil, peanut oil, grape seed oil, camellia oil, sesame oil, olive oil, corn oil and sunflower seed oil were purchased from local supermarkets.

The purity of TBHQ (J&Kchemica, Germany) was higher than 98.0%. Methanol and acetonitrile (Fisher Scientific, America) were of HPLC grade. Ethanol, aqua ammonia and acetic acid (Beijing, China) were of analytic grade. Water was purified using a Milli-Q gradient system (Millipore Corporation, France).

2.3. Preparation of solutions

The stock standard solution of TBHQ (1000 mg/l) was prepared in methanol. Working standard solutions at concentration ranges of 0.048–4.543 mg/l for LC/ITMS analysis and 0.50–200.00 mg/l for LC/DAD analysis were obtained by dilution with methanol. The standard solutions were stored at −20 °C and were used for one month.

2.4. Extraction procedures

A 0.2000 g edible vegetable oil sample was weighed into a centrifuge tube with plug. Six hundred microliters of hexane was added and the mixture was vigorously shaken for 2 min by using the microvibrant mixer. Then 2 ml extracting solvent was added and the mixture was vigorously shaken for 5 min. After the mixture was centrifuged at 4000 r/min for 5 min, the supernatant was transferred to another empty centrifuge tube with plug. The extracting procedure was repeated twice and the combined extracts were then centrifuged at 4000 r/min for another 5 min. At last, 1 ml of the final supernatant was transferred to a sample bottle for LC/ITMS analysis. The concentration of TBHQ in edible vegetable oil was calculated as the mean of four replicates.

2.5. LC/ITMS analysis

TBHQ was analyzed in standard and sample solutions using the mobile phase of water and methanol (40:60, v/v). The column was at 35 °C, with a flow rate of 0.40 ml/min. Injection volume was 5 μl. Stop time was 6 min. TBHQ was detected at 290 nm.

ITMS system was in negative ionization mode, using helium as collision gas, and using nitrogen as nebulizer gas and drying gas. Multiple reaction monitoring (MRM) mode was used to acquire MS^1 and MS^2 spectra of TBHQ standard solutions and oil extracts, with a scan range of m/z 50–300. Product ion spectra were obtained using the optimized fragmentation amplitude of 1.70 V when the target mass was set to m/z 165 and the Cutoff value was set to m/z 80. When ESI was adopted, nebulizer gas was set at 35 psi, drying gas was set at 8 l/min and 330 °C, capillary voltage was set at 3000 V, and compound stability was set to 100%. When APCI was adopted, nebulizer gas was set at 60 psi, drying gas was set at 4 l/min and 350 °C, vaporizer was set at 350 °C, capillary voltage was set at 3000 V, corona current was set to 20,000 nA, and compound stability was set to 100%.

3. Results and discussion

3.1. Optimization of ITMS parameters

TBHQ was well ionized to parent ion m/z 165 with the retention time of 3.7 min in negative ionization mode, but no signal was detected for TBHQ in positive ionization mode. In the MS^2 spectrum (Fig. 1), the product ions of m/z 165 were m/z 149 and m/z 108. The main product ion m/z 149 was due to the methyl cleavage from tertiary butyl and the other product ion m/z 108 came from the cleavage of tertiary butyl. In order to avoid the matrix interference, extracted ion chromatogram (EIC) of the main product ion m/z 149 was used to detect TBHQ in edible vegetable oil.

As for ITMS, the parameter of fragmentation amplitude is very important in order to obtain the best MS^2 signal. The optimization of fragmentation amplitude is shown in Fig. 2. When the amplitude of fragmentation was lower than 1.50 V, most of the compound existed in the form of the parent ion m/z 165 because the fragmentation energy was not high enough. With the amplitude of fragmentation increasing, the signal of the parent ion m/z 165 decreased and the signal of the product ion m/z 149 increased, because more and more parent ions were fragmentated to product ions. However, when the amplitude of fragmentation was higher than 1.70 V, the signals of the product ion m/z 149 and the parent ion m/z 165 both kept decreasing, maybe because the ions became unsteady in the trap when the fragmentation energy was too high. So, the best MS^2 signal could be obtained at the fragmentation amplitude of 1.70 V.

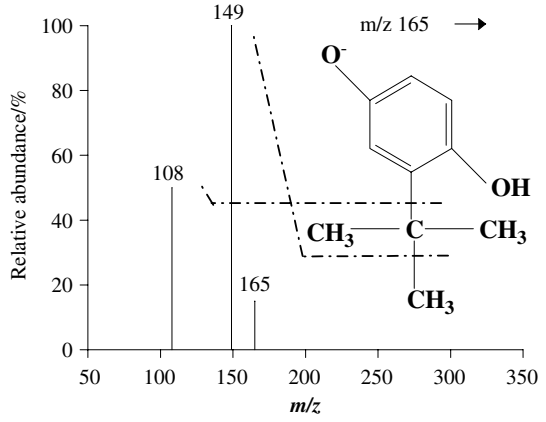

Fig. 1. MS^2 spectrum of TBHQ.

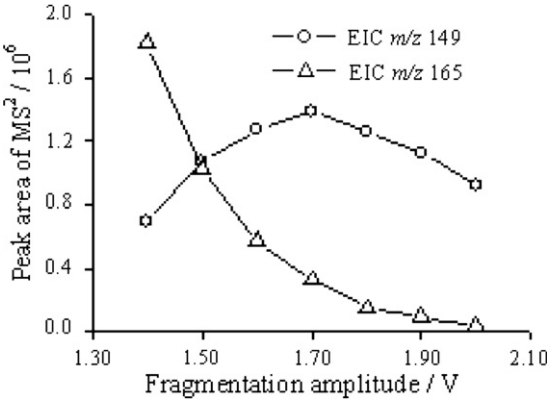

Fig. 2. Plots of peak area of EIC m/z 149 or EIC m/z 165 (MS^2) vs fragmentation amplitude.

The selection of ionization interface has an important effect on compound analysis. For TBHQ, two kinds of ionization interfaces, ESI and APCI, were compared. Analyzing standard solutions, linear calibration curves were obtained, by plotting the peak area of EIC m/z 149 against the concentration of TBHQ, in the range of 0.048–4.543 mg/l. The regression equations using ESI and APCI were $y = (4.14 \pm 0.07) \times 10^6 x + (3.78 \pm 0.14) \times 10^3$ and $y = (1.05 \pm 0.14) \times 10^5 x + (3.38 \pm 2.35) \times 10^2$, respectively. The correlation coefficient of the regression equation using ESI ($R^2 = 0.9990$) was much higher than that using APCI ($R^2 = 0.9427$). So the linearity of the response obtained by ESI was much better than that obtained by APCI for TBHQ quantification. The sensitivity (slope of the calibration equation) using ESI was higher (almost 40 times) than that using APCI for TBHQ analysis. The limit of detection was defined as the minimum concentration providing a chromatographic signal three times higher than background noise (González et al., 1999). When the injection volume was 5 μl, the detection limit for TBHQ using ESI was 0.010 mg/l, lower than that using APCI (0.075 mg/l), probably as the result of the higher sensitivity of ESI. In a word, comparing the linearity, sensitivity and limit of detection, ESI was more suitable to TBHQ detection rather than APCI.

3.2. Optimization of the extraction procedures

Preliminary tests were carried out to determine the most appropriate extraction solvent. Recoveries obtained for one edible vegetable oil sample spiked with TBHQ at two concentration levels of 10.0 mg/kg and 20.0 mg/kg are shown in Table 1. Acetonitrile, methanol and ethanol were all able to extract TBHQ from edible vegetable oil with

Table 1
Comparison of extraction solvents for analysis of TBHQ in edible vegetable oil ($n = 4$)

Extraction solvent	Added (10.0 mg/kg)		Added (20.0 mg/kg)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acetonitrile	96.3	9.8	95.4	8.3
Methanol	90.8	8.6	96.1	7.6
Ethanol	95.8	7.0	93.3	8.1
Ethanol–ammonia (95:5, v/v)	0.0	0.0	0.0	0.0
Ethanol–acetic acid (95:5, v/v)	11.9	12.8	8.7	11.4

good recoveries of 90.8–96.3%. Ethanol was selected in this study, since acetonitrile and methanol were unsuitable for routine analysis of TBHQ considering their toxicity and high price. In order to investigate the effect of acidity on the extraction efficiency, ethanol–acetic acid (95:5, v/v) and ethanol–ammonia (95:5, v/v) were also used for extraction, but the recoveries of less than 15% were unsatisfied. Acetic acid in ethanol could inhibit the extraction of TBHQ, for TBHQ was also a weak acid and acetic acid may compete with TBHQ for dissolution in ethanol. As for ethanol/ammonia, the result was opposite to our expectation, possibly because TBHQ in edible vegetable oils reacted and was lost in the existence of alkalinity.

Besides, when the sample extracts were filtered through a 0.45 μm nylon membrane filter before LC/ITMS analysis, about 10% TBHQ was lost. So, direct injection without filtering was taken for TBHQ analysis.

3.3. Method performance

For TBHQ detection, the linearity, sensitivity and limit of detection using LC/ITMS were compared with those using LC/DAD. Analyzing standard solutions, the linear calibration curves using LC/ITMS and LC/DAD were $y = (4.14 \pm 0.07) \times 10^6 x + (3.78 \pm 0.14) \times 10^3$ ($R^2 = 0.9990$) in the range of 0.048–4.543 mg/l and $y = (29 \pm 0)x - (15 \pm 2)$ ($R^2 = 0.9999$) in the range of 0.50–200.00 mg/l, respectively. Both of the correlation coefficients exceeded 0.999 for TBHQ, which indicated that the linearity of the MS^2 m/z 149 response was as good as that of the UV response. As indicated by slope of the calibration graph, it was obvious that LC/ITMS provided a higher sensitivity (5 magnitudes) for TBHQ than did LC/DAD. For example, the UV response of one blend oil sample containing TBHQ after extraction was almost out of detection, but the peak was very high and easy to be quantified in EIC m/z 149 (Fig. 3). The more sensitive ITMS response of TBHQ could allow simpler sample preparation procedures without preconcentration. Moreover, the detection limit for TBHQ obtained by LC/ITMS was 0.010 mg/l, much lower than that obtained by LC/DAD (0.20 mg/l).

Repeatability was checked by carrying out four replicate analyses on four edible vegetable oil samples containing TBHQ. The relative standard deviation (RSD) of repeatability ranged from 2.5% to 5.7%. Intermediate reproducibility was checked by carrying out replicate analyses on

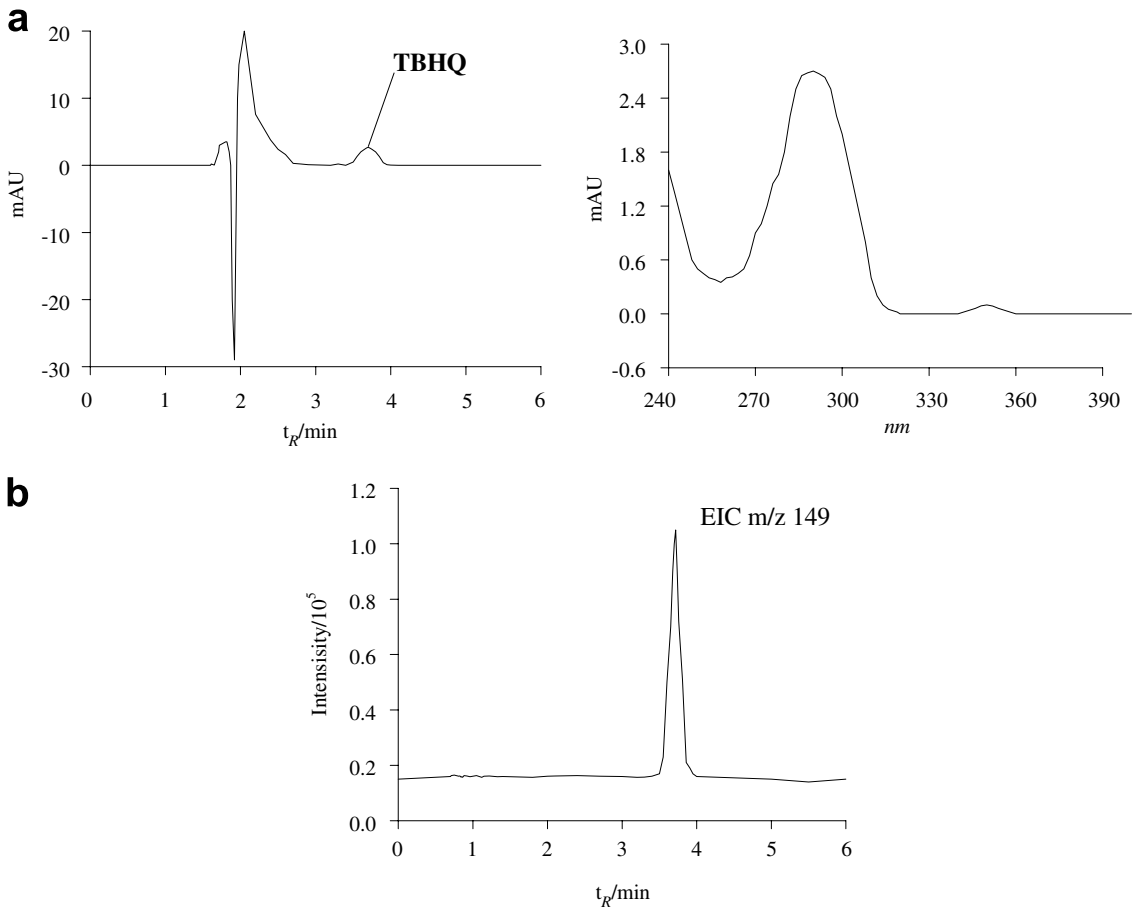

Fig. 3. Analysis of one blend oil sample containing TBHQ after extraction with ethanol (a) LC chromatogram and DAD spectrum taken across the peak of TBHQ. (b) LC/MS^2 chromatogram.

Table 2
Determination results of TBHQ in edible vegetable oils ($n = 4$)

Sample	Concentration (mg/kg, mean ± SD)	Recovery (%)	RSD (%)
Blend oil 1	87.7 ± 2.4	96.9	5.5
Blend oil 2	43.8 ± 1.1	110.5	4.5
Soybean salad oil	38.5 ± 2.2	94.6	11.4
Peanut oil	n.d.[a]	96.2	3.6
Grape seed oil	n.d.	88.4	5.7
Camellia oil	41.3 ± 1.8	94.4	14.2
Sesame oil	n.d.	99.4	4.2
Olive oil	n.d.	81.9	1.3
Corn oil	n.d.	96.2	3.6
Sunflower oil	n.d.	109.5	4.2

[a] n.d., not detected.

the same edible vegetable oil samples containing TBHQ, on four different days. The RSD ranged from 3.9% to 13.8%.

Recoveries were determined by spiking 10 kinds of edible vegetable oil samples with 10.0 mg/kg TBHQ. The results indicated that the recoveries were between 81.9% and 110.5% (Table 2), which met the demand of quantitative determination, although the matrix of each edible vegetable oil was different.

As the use of TBHQ is not permitted in foods in some countries, it is of interest to determine the detection limit of TBHQ in edible vegetable oils by this method. The detection limit of MS^2 m/z 149 analysis was 0.010 mg/l when the injection volume was 5 μl corresponding to 0.3 mg/kg in 0.2000 g oil sample, well below the maximum limit of 200 mg/kg. So, the detection limit of TBHQ in edible vegetable oils by LC/ITMS method (0.3 mg/kg) is much lower than that in dry foods by LC method (2 mg/kg) (Christian & Liliane, 2002) and that in edible oils and fats by GC method (3.3 mg/kg) (Yang et al., 2002).

3.4. TBHQ detection in commercial samples

There are various edible vegetable oils, such as salad oil, blend oil, peanut oil, corn oil and olive oil, in China. In this paper, 10 typical edible vegetable oils in the market were detected for TBHQ. The results are shown in Table 2. Two blend oil, one soybean salad oil and one camellia oil samples were found to contain 38.5–87.7 mg/kg TBHQ, and the other six edible vegetable oils including one peanut oil, one grape seed oil, one sesame oil, one olive oil, one corn oil and one sunflower oil samples contained no detectable TBHQ. According to the packaging instructions, no other antioxidants were added to these six oil samples. So we wondered why some kinds of edible vegetable oils were added antioxidants and other kinds need not add antioxidants.

Whether to add antioxidants or not and how much to add antioxidants are mainly related to the oxidizability of edible vegetable oil. On one hand, the oxidizability is positive correlated with the unsaturation degree of fatty acid. The content of unsaturated fatty acid is higher than 90% both in blend oils (Huang, Chen, & Chen, 1999) and in camellia oils (Li & Lu, 2003). The fatty acid of soybean salad oils is mainly composed of linolic acid, a kind of polyunsaturated fatty acid with high unsaturation degree (Jiang, Liu, & Liu, 2000). So these three kinds of edible vegetable oils are very easy to be oxidized because of their high unsaturation degree. However, sunflower seed oils have excellent oxidative stability because the unsaturated fatty acid in sunflower seed oils is mainly oleic acid, a kind of monounsaturated fatty acid, much more stable than linolic acid, although the content of unsaturated fatty acid in sunflower seed oils is also high (Jiang, Guan, Fan, & Sun, 2004). On the other hand, the oxidizability is negative correlated with the antioxidant capacity and the content of natural antioxidant components in edible vegetable oils. The abundance of various natural antioxidants may contribute to the oxidative stability of the other five edible vegetable oils. Peanuts (Talcott, Passeretti, Duncan, & Gorbet, 2005) contain numerous polyphenols, such as *p*-coumaric acid, tocopherol and ferulic acid. Internal polyphenols could protect grape seed oils from oxidation (Zhang, Ji, & Qi, 2001). A combination of a number of minor constituents, including sesamol, tocopherol, sesamin, sesamolin, sterol, squalene and some active browning substances, could have a synergistic role in increasing the oxidative stability of sesame oils (Mohamed & Awatif, 1998). There are abundant natural antioxidants, such as hydroxytyrosol, hydroxytyrosol acetate, oleuropein, 3,4-dihydroxyphenylelenolic acid (3,4-DHPEA-EA) and 3,4-dihydroxyphenylelenolic acid dialdehyde (3,4-DHPEA-EDA) in olive oils (Paiva-Martins, Gordon, & Gameiro, 2003). The content of tocopherol in corn oils is more than 900 mg/kg (You, 2004), which may be helpful to retard oil autoxidation.

4. Conclusions

A simple, fast and reliable routine LC/ITMS method to determine TBHQ in edible vegetable oil is established. The optimized method has satisfactory validation characteristics with respect to recovery, sensitivity, selectivity and repeatability. In addition, this method is much less time-consuming. Considering the complexity of the analysis, which includes extraction and LC/ITMS determination, it is fully satisfactory that each analysis takes only 50 min. And also, the extraction solvent is ethanol, nontoxic and economical. Therefore, the method proposed is suitable for routine monitoring of TBHQ in edible vegetable oil.

Among 10 typical edible vegetable oils in the market, two blend oil, one soybean salad oil and one camellia oil samples were found to contain 38.5–87.7 mg/kg TBHQ, and the other six oil samples contained no detectable TBHQ. It is summarized from the TBHQ detection results that whether to add antioxidants or not and how much to add antioxidants are decided by the oil oxidizability, correlating with the unsaturation degree of fatty acid and the antioxidant capacity and the total amount of its containing natural antioxidants.

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