Thermal Losses of Tertiary Butylhydroquinone (TBHQ) and Its Effect on the Qualities of Palm Oil

Cuifang Liu, Jun Li, Yanlan Bi*, Xuede Wang*, Shangde Sun and Guolong Yang

Lipid Technology and Engineering, School of Food Science and Engineering, Henan University of Technology, Lianhua Road, Zhengzhou 450001, Henan, CHINA

Abstract: The rules and patterns of thermal losses of tertiary butylhydroquinone (TBHQ) in palm oil (PO) and its effect on the qualities of PO were investigated by oven heating method. Volatilization and transformation products of TBHQ in PO were also studied in detail under heating treatment. Results showed that at low temperature (< 135°C), TBHQ had better antioxidative properties, while its antioxidative potency to PO was significantly weakened at high temperature (≥ 135°C). In addition, as heating temperatures increased and heating time prolonged, losses of TBHQ significantly increased in PO. Volatilization was the major pathway for losses of TBHQ in PO under heating treatment. Meanwhile, a small portion of TBHQ was transformed and the major transformation product was 2-tertbutyl-1,4-benzoquinone (TQ). Moreover, TQ and several decomposition products of PO were also observed in the volatilization products of TBHQ.

Key words: TBHQ, TQ, PO, thermal losses, volatilization, transformation

1 Introduction

Edible oils, especially high unsaturated oils, were prone to oxidative rancidity because of their own natures and effects of external factors such as oxygen, heat, light and metal ions etc.1-3. At present, it was commonly acknowledged that using synthetic antioxidants was the most effective method to delay oxidative rancidity of oils4-6. Due to its low price, high efficacy and wide availability, tertiary butylhydroquinone (TBHQ) was commonly applied to edible oil and food industry in many countries, such as America, Malaysia, Australia, Peru and China etc.7,8. Currently, the maximum legal level established by U.S. Food and Drug Administration for TBHQ in oils and food products was 0.02% of the oil content of the food, based on tests of acute and chronic toxicity9, and Japan, Canada and many European countries, such as England, Denmark, Norway, Switzerland and Sweden etc., banned the use of TBHQ for foods.

In past decades, many studies7,8,10,11 concerning the antioxidant activity of TBHQ, the comparison of antioxidant properties of TBHQ and other synthetic antioxidants(butylated hydroxytoluene[BHT], 3-tert-butyl-4-hydroxyanisole[BHA] and propyl gallate[PG],etc.) had been reported. Results7,8,10,11 showed that the antioxidant property of TBHQ was better than that of other synthetic antioxidants regardless of low temperatures and high temperatures. However, antioxidant activity of TBHQ under heating condition was much lower than that at room temperature, which might be related to thermal losses of TBHQ under heating condition12-14. Hamama et al.12 studied the thermal decomposition of pure TBHQ under nitrogen condition, and several thermal oxidation products were separated and identified by gas chromatography/mass spectra (GC/MS) and relative mechanisms for their formation were proposed. Buck13 reported that losses of TBHQ were observed in frying systems and that steam distillation significantly increased the rate of TBHQ losses. Asap et al.14 studied the effect of TBHQ on quality characteristics of refined, bleached and deodorized palm olein during the frying and found that losses of TBHQ increased with prolonged frying time. Lin et al.15 studied the alteration of TBHQ using isotope labeling method in heated vegetable oils and found several products of lower polarity, but their structures remained unidentified. Pho et al.16 studied the fate of TBHQ coated on the inner surface of polyethylene bags used to package ramyon and found an unknown dark band above the band of TBHQ by the method of thin layer chromatography(TLC), but not qualitatively analyzed the unknown band.

The primary objective of current study was to investigate...
the rules of TBHQ losses in palm oil (PO) under heating condition. In addition, volatilization and transformation products of TBHQ in PO were analyzed and identified in detail.

2 Materials and Methods

2.1 Chemicals

Standards of TBHQ (purity > 99.0%) and 2-tert-butyl-1,4-benzoquinone (TQ) (purity > 98.0%) were purchased from Sigma-Aldrich (St Louis, USA) and used without any purification. 24C fractionated PO (acid value of 0.20 mg KOH/g, peroxide value of 0.50 mmol/kg, 1031.40 mg/kg of α-V_E, 403.80 mg/kg of γ-V_E and 126.80 mg/kg of δ-V_E) without any synthetic antioxidants was obtained from Zhenjiang Sinograin oils and Fats Co., Ltd. (Zhenjiang, China). Methanol (purity > 99.9%, HPLC grade) was purchased from VBS Biologic Inc. (New York, USA). Glacial acetic acid (purity > 99.8%, HPLC grade) was purchased from Kerch Chemical Reagent Co., Ltd. (Tianjin, China). All other reagents were of analytical grade and used without further purification.

2.2 Apparatus

The HPLC spectra were measured with a 2695 separation module and an ultraviolet (UV)/Visible-2489 detector (Agilent, Santa Clara, USA). The GC spectra were measured with a 6890N separation module and a flame ionization detector (FID) (Agilent, Santa Clara, USA). The MS spectra were measured with a 5973N quadrupole spectrometer equipping an electron impact (EI) ion source detector (Agilent, Santa Clara, USA). The UV/Visible spectra were measured with a TU-1860 spectrophotometer (Beijing, China). Vortex mixer (Staufen, German), SCQ-250B ultrasonic apparatus (Shanghai, China) and 800 low speed centrifuge (Jintan, China) were also used in the experiment. The oven was purchased from Taisite Instrument Co., Ltd. (Tianjin, China).

2.3 Measurement of acid value (AV), peroxide value (PV), p-anisidine value (p-AnV) and total oxidation value (TV)

The measurement of AV and PV was respectively based on AOCS Cd 3d-6322 and AOCS Cd 8-5322. The measurement of p-AnV and TV was based on ISO 6885:2006 (E)22.

TV = 2 × PV + p-AnV

2.4 Preparation of oil samples

Refined PO was preheated in 40°C until completely melted. 0.20 g of TBHQ (accurately weighted to 0.1 mg, similarly hereinafter) was added to 999.80 g of melted PO and then shaken until homogeneous. Finally, finished oil samples contained 200.00 mg/kg TBHQ.

2.5 Heating experiment

6 copies of 500.00 g of oil samples were respectively held at different heating temperatures (80, 105, 120, 135, 150 and 180°C). Samples were taken at various intervals (0, 1, 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h).

2.6 Extraction procedure

The extraction procedure was as followings22: 2.00 g of each of melted oil samples was weighed into a 10 mL test tube, and 4 mL of methanol was added. The mixture was agitated for 2 min using a vortex mixer and then centrifuged at 2,000 rpm for 2 min at room temperature. The supernatant was quantitatively transferred to a 10 mL volumetric flask. The extraction procedure was repeated twice, adding 3 mL of methanol each time. Finally, sufficient methanol was added to achieve a 10 mL solution. The solution was filtered through a 0.45 µm membrane before HPLC analysis.

2.7 HPLC analysis

HPLC analysis was performed under the following conditions22: the injection volume was 20 µL; the column was Symmetry C18 (4.6 x 250 mm, 5 µm, Waters); the solvent system was methanol-H_2O containing 0.5% AcOH (65 : 35, v/v); isocratic elution; the flow rate was 0.8 mL/min; the detection wavelength was 280 nm; the column temperature was 35°C. Methanol and H_2O containing 0.5% AcOH were both degassed for 15 min by an ultrasonic apparatus.

2.8 GC analysis

1 µL of each of samples was injected in split mode. The injector was maintained at 250°C. The capillary chromatographic column was an HP-5 MS (Agilent Technologies), 30 m x 0.32 mm i.d., film thickness 0.25 µm, with the following temperature program: initial temperature was 180°C and was held for 5 min; then this was increased to 220°C at a rate of 15°C/min and held for 6 min. UHP helium was used as carrier gas at 1.0 mL/min.

2.9 GC/MS analysis

2.9.1 GC/MS analysis of extracted samples

1 µL of each of samples was injected in splitless mode. The injectors of GC and MS were respectively maintained at 200 and 250°C. The temperature program was as followings: initial temperature was 60°C and was held for 2 min; then this was increased to 250°C at a rate of 10°C/min and held for 5 min. The range of mass-charge ratio was 50 ~ 650. The MS detector was operated at 70 eV in EI mode with full scan or in selected ion monitoring (SIM) mode. The software used was D.01.02 with the NIST02 mass database. The rest conditions were the same as GC analysis.
2.9.2 GC/MS analysis of headspace samples

The injection volume was 100 μL. The temperature program was as follows: initial temperature was 40°C and was held for 2 min; then it was increased to 80°C at a rate of 10°C/min and held for 2 min, finally increased to 230°C at a rate of 5°C/min. The range of mass-charge ratio was 40–450. The rest conditions were the same as GC/MS analysis of extracted samples.

2.10 Statistical analysis

All experiments were performed at least in duplicate. All data were presented as the mean plus or minus the standard deviation (SD). The significance of the differences was assessed using analysis of variance (ANOVA). Data evaluation was performed using SPSS software for Windows (version rel. 16.0, SPSS Inc., Chicago, IL, USA). Differences were considered significant when p value was < 0.05.

3 Results and discussion

3.1 Effect of TBHQ on the qualities of PO under heating treatment

3.1.1 Effect of TBHQ on AV of PO

AVs of PO with or without TBHQ were almost same under the same heating temperatures \( p > 0.05 \) (Fig. 1), suggesting that TBHQ almost had no effect on AVs of PO. In addition, AVs of PO with or without TBHQ both had a slightly increasing trend with prolonged heating time. Similar results were reported by Asap et al.\(^{14}\). This was mainly because hydrolysis of oils & fats and decomposition of hydroperoxides produced a small amount of free fatty acids\(^{21}\).

3.1.2 Effect of TBHQ on PV of PO

As heating time prolonged, PVs of PO with or without TBHQ both increased \( p < 0.05 \) (Fig. 2). In addition, under the same sets of conditions, PVs of PO with TBHQ were less than that without TBHQ \( p < 0.05 \). For example, when PO with or without TBHQ was both held at 105°C for 48 h, PVs were respectively 9.22 and 35.27 meq/kg. This indicated that TBHQ can inhibit the increasing of PV of PO.

As heating temperatures \( < 135°C \) increased, PVs of PO with or without TBHQ both increased \( p < 0.05 \). For example, when oil samples were held at 80, 105 and 120°C for 48 h, PVs of oil samples without TBHQ were respectively 18.68, 35.27 and 45.23 meq/kg and PVs of oil samples with TBHQ were respectively 8.28, 9.22 and 27.56 meq/kg. This indicated that the contents of hydroperoxides in PO increased with the increasing of heating temperatures \( < 135°C \). However, As heating temperatures \( ≥ 135°C \) increased, PVs of PO with or without TBHQ both decreased \( p < 0.05 \). For example, when oil samples were held at...
135, 150 and 180°C for 4 h, PVs of oil samples without TBHQ were respectively 11.37, 9.51 and 6.15 meq/kg and PVs of oil samples with TBHQ were respectively 10.85, 5.96 and 4.50 meq/kg. This was mainly due to the lower level of oxygen dissolved in oils & fats and rapider decomposition of hydroperoxides with higher heating temperatures\(^{24, 25}\).

As heating temperatures increased, the difference of PVs of PO between with TBHQ and without TBHQ was smaller and smaller. This further indicated that the inhibition of TBHQ on the increasing of PVs of PO was also affected by heating temperatures. This was mainly because TBHQ itself easily sublimated, resulting in a large amount of losses of TBHQ itself (specific results and analysis were presented in sections 3.2 and 3.4) and only a small amount of TBHQ involving in the lipid radical chain reaction. Therefore, antioxidant activity of TBHQ under heating conditions was less than that at room temperature.

3.1.3 Effect of TBHQ on p-AnV of PO

As heating temperatures increased and heating time prolonged, p-AnVs of PO with or without TBHQ both increased\((p<0.05)\) (Fig. 3). In addition, under the same sets of conditions, p-AnVs of PO with TBHQ were less than that without TBHQ\((p<0.05)\). For example, when oil samples were held at 80, 105 and 120°C for 24 h, p-AnVs of oil samples with TBHQ were respectively 1.99, 2.05 and 18.96, and p-AnVs of oil samples without TBHQ were respectively 3.23, 4.33 and 31.87. This indicated that TBHQ can significantly inhibit the increasing of p-AnVs of PO. However, as heating temperatures increased, this inhibition was weaken.

3.1.4 Effect of TBHQ on TV of PO

Based on the analysis of sections 3.1.2 and 3.1.3, it was concluded that at higher heating temperatures, changes of PVs of PO were unobvious; at lower heating temperatures, changes of p-AnVs of PO were unobvious. Thus, TV from the combination of PV and p-AnV can be used to characterize the oxidative degree of PO (Fig. 4).

Changes of TVs of PO were very obvious regardless of low temperatures and high temperatures\((p<0.05)\). This indicated that the increasing of heating temperatures would accelerate the oxidation of oils & fats. In addition, under the same heating temperatures, TVs of PO with TBHQ were less than that without TBHQ. For example, when oil samples was held at 80, 105 and 120°C for 48 h, TVs of oil samples without TBHQ were respectively 40.59, 76.66 and 149.45, and TVs of oil samples with TBHQ were respectively 18.39, 22.28 and 87.07. This indicated that TBHQ can inhibit the increasing of TVs of PO. Moreover, as heating temperatures increased, the difference of TVs of PO between with TBHQ and without TBHQ was also smaller and smaller. This further indicated that the increasing of heating temperatures would weaken the antioxidant activity of TBHQ.
Thermal Losses of Tertiary Butylhydroquinone (TBHQ) and Its Effect on the Qualities of Palm Oil

Fig. 3  Effect of TBHQ on $p$-AnV of PO under different heating conditions.

Fig. 4  Effect of TBHQ on TV of PO under different heating conditions.

J. Oleo Sci.
3.2 Effect of heating temperature and time on TBHQ losses in PO

As heating temperatures increased and heating time prolonged, losses of TBHQ in PO increased significantly ($p < 0.05$) (Fig. 5). For example, when oil samples were heated at 150°C for 4, 8, 12, 16 and 24 h, losses of TBHQ were respectively 27.93, 54.66, 73.40, 86.48 and 95.61%. When oil samples were heated for 12 h, losses of TBHQ at 80, 120, 150, 180°C were respectively 9.07, 46.75, 73.40 and 80.83%. This was due to the increasing volatility of TBHQ as heating temperatures increased.

3.3 Identification of volatilization and transformation products of TBHQ in PO

According to previous reports, losses of TBHQ in frying systems included its volatilization, transformation and absorption of the fried food. In the current study, PO was considered as heating medium to investigate the volatilization and transformation products of TBHQ in oils & fats.

3.4 Identification of volatilization products of TBHQ in PO

To investigate the volatilization products of TBHQ in PO under heating conditions, 150.00 g of PO with 200 mg/kg TBHQ were weighed into a 100-mL round-bottom flask and then heated for 1.5 h at 150°C under reflux condensation mode. Considering that some volatile gases were uneasily condensed, 100 mL of methanol solution was used to absorb these volatile gases escaping from the end of condenser. Upon completion of the reaction, the condenser and connectors were eluted with methanol, then all the methanol solution was collected and concentrated to 20 mL using nitrogen-blowing method at low temperature.

3.5 Identification of transformation products of TBHQ in PO

In order to identify the transformation products of TBHQ in PO under heating conditions, 25.00 g of PO with 200 mg/kg TBHQ were weighed into a 150-mL round-bottom flask and then heated for 2 h at 150°C under a sealed state. Meanwhile, the control experiment was carried out without TBHQ. After finishing the reaction, extracting solutions of oil samples were analyzed with GC/MS (Fig. 8). Two new peaks were clearly observed at 9.72 and 15.09 min in TBHQ-containing experiment. Then through matching standard mass spectral library (Figs. 9 and 10), it was concluded that the two peaks were respectively TQ at 9.72 min and TBHQ at 15.09 min. This indicated that partial TBHQ in PO was transformed to TQ during heating treatment.

Finally, the concentrated samples was analyzed by GC (Fig. 6). Results showed that besides the peak of TBHQ (relative content: 52.5%) at 5.44 min retention time, two new peaks (relative contents: 1.13 and 46.4% respectively) were observed at 3.42 and 5.22 min retention time, respectively. This indicated that TBHQ itself was the main volatilization product of TBHQ in PO under the heating condition. Similar results were reported by Li et al. Several transformation and decomposition products were also observed in volatilization products. In addition, UV absorption spectra of concentrated samples were also qualitatively analyzed (Fig. 7). Results showed that besides four similar peaks of TBHQ and the concentrated samples, a new and relative weak peak between 250 and 270 nm was also observed in the concentrated samples. This further confirmed the above conclusion. Similar results were reported by Hamama et al., but types of volatilization products of TBHQ in the current study were much less than that (more than 30) in the previous study. This was mainly because Hamama et al. studied volatilization products of pure TBHQ under nitrogen condition, which was not affected by oil samples as heating medium and air etc.
Thermal Losses of Tertiary Butylhydroquinone (TBHQ) and Its Effect on the Qualities of Palm Oil

J. Oleo Sci.

Utilization products of TBHQ, 10.00 g of PO with 200.00 mg/kg TBHQ were weighed into a 20-mL headspace vial for the headspace experiment (Fig. 11). Meanwhile, the control experiment was carried out without TBHQ. A new peak was clearly observed at 15.33 min. After referring to the mass spectral library, it was found that the peak was TQ. However, the peak of TBHQ was not observed in the TBHQ-containing oils. This was because heating temperature of headspace experiment was 80°C and TBHQ could not be volatilized under this condition. As TQ easily volatilized, the content of TQ remaining in heated PO was very little. Therefore, the smell and taste of fried foods were not affected. However, based on the toxicologic studies that the toxicity of TQ was much more than that of TBHQ, the safety of fried foods was very noteworthy.

At present, it was commonly acknowledged that there were two pathways for TBHQ transforming to TQ in edible oils (Fig. 12). One pathway was that TBHQ was directly oxidized to TQ by oxygen dissolved in the edible oils. Another pathway was that TBHQ reacted with lipid radicals (R·) or lipid peroxide radicals (ROO·) and finally formed TQ.

![Image 7](https://example.com/image7.png)  
**Fig. 7** The ultraviolet absorption spectra of tertiary butylhydroquinone (TBHQ), 2-tert-butyl-1,4-benzoquinone (TQ) and concentrated samples of volatilization products from heated PO containing TBHQ.

![Image 8](https://example.com/image8.png)  
**Fig. 8** GC/MS analysis of oil samples with and without TBHQ after heating treatment.
Fig. 9  Ion fragment chromatograms of the peak at 9.72 min and standard TQ.

Fig. 10  Ion fragment chromatograms of the peak at 15.09 min and standard TBHQ.

Fig. 11  Headspace GC/MS analysis of oil samples with and without TBHQ under heating treatment.
Thermal Losses of Tertiary Butylhydroquinone (TBHQ) and Its Effect on the Qualities of Palm Oil

3.6 Analysis of the content of TBHQ in frying oils

In order to better understand the changes of the content of TBHQ during the actual frying process, oil samples were taken at various intervals and determined (Fig. 13). Results showed that the content of TBHQ decreased with prolonged frying time. After adding fresh oil containing TBHQ each time, the content of TBHQ would rally. However, throughout the frying experiment, the content of TBHQ was still a decreasing trend. This was mainly because most of TBHQ volatilized from frying oils; partial TBHQ was lost with lipid radical reaction; partial TBHQ was transformed and decomposed. These would reduce the content of TBHQ remained in fried foods, shorten the shelf life of fried foods and result in the content of TBHQ and shelf life of the same batch of foods different. Thus, to ensure the shelf life of fried foods, it was necessary to constantly use fresh oils containing TBHQ to replace the used frying oils or add fresh oils containing TBHQ into the used frying oils.

4 Conclusion

Under different heating conditions, volatilization and transformation of TBHQ and its effect on the qualities of PO were investigated. Results showed that TBHQ had no effect on AV of PO, but can significantly inhibit the increasing of PV, p-AnV and TV of PO. Volatilization was the major pathway for losses of TBHQ in PO under heating treatment. Meanwhile, a small portion of TBHQ was transformed to TQ. Moreover, TQ and several decomposition products of PO were also observed in the volatilization products of TBHQ. Thus, it was proposed that the types, the added level and mode of antioxidants should be considered seriously to extend the shelf life of oils & fats and fried foods.

Acknowledgement

This work was supported by National Natural Science Foundation of China (No. 31271883) and China Agriculture Research System (No. CARS15-1-10).

Reference

1) Eldin, A. K. Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. Eur. J. Lipid...
Sci. Tech. 108, 1051-1061 (2006).

2) Mozuraitytë, R.; Kristinova, V.; Rustad, T.; Storro, I. The role of iron in peroxidation of PUFA: effect of pH and chelators. Eur. J. Lipid Sci. Tech. 16, 57-84 (2015).

3) Giese, J. Antioxidants: tools for preventing lipid oxidation. Food Technol. 50, 73-81 (1996).

4) Ramanathan, L.; Das, N. P. Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. J. Agric. Food Chem. 40, 17-21 (1992).

5) Hawrysh, Z. J.; Shand, P. J.; Lin, C.; Tokarska, B.; Hardin, R. T. Efficacy of tertiary butylhydroquinone on the storage and heat stability of liquid canola shortening. J. Am. Oil Chem. Soc. 67, 585-590 (1990).

6) Man, Y. B. C.; Tan, C. P. Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized palm olein during deep-fat frying of potato chips. J. Am. Oil Chem. Soc. 76, 331-339 (1999).

7) Chung, Y. K.; Yousof, A. E. Inactivation of barotolerant strains of listeria monocytogenes and escherichia coli O157: H7 by ultra high pressure and tert-butylhydroquinone combination. J. Microbiol. 46, 289-294 (2008).

8) Li, J.; Bi, Y. L.; Liu, W.; Sun, S. D. Simultaneous analysis of tertiary butylhydroquinone and 2-tert-butyl-1,4-benzoquinone in edible oils by normal-phase high-performance liquid chromatography. J. Agric. Food Chem. 63, 8584-8591 (2015).

9) U. S. Food and Drug Administration (FDA). Code of Federal Regulations 21, Food and Drugs; Office of the Federal Register (OFR); Washington, D.C., (1980).

10) Ruger, C. W.; Klinker, E. J.; Hammond, E. G. Abilities of some antioxidants to stabilize soybean oil in industrial use conditions. J. Am. Oil Chem. Soc. 79, 733-736 (2002).

11) Merrill, L I; Pike, O. A.; Ogden, L V.; Dunn, M. L. Oxidative stability of conventional and high-oleic vegetable oils with added antioxidants. J. Am. Oil Chem. Soc. 85, 771-776 (2008).

12) Hamana, A. A.; Nawar, W. W. Thermal decomposition of some phenolic antioxidants. J. Agric. Food Chem. 39, 1063-1069 (1991).

13) Buck, D. F. Antioxidants in soya oil. J. Am. Oil Chem. Soc. 58, 275-278 (1981).

14) Asap, T.; Augustin, M. A. Effect of TBHQ on quality characteristics of RBD olein during frying. J. Am. Oil Chem. Soc. 63, 1169-1172 (1986).

15) Lin, F. S.; Warner, C. R.; Fazio, T. Alteration of phenolic antioxidants in heated vegetable oil. J. Am. Oil Chem. Soc. 58, A789-A792 (1981).

16) Rho, K. L.; Seib, P. A.; Chung, O. K.; Chung, D. S. Retardation of rancidity in deep-fried instant noodles (ramyun). J. Am. Oil Chem. Soc. 63, 251-256 (1986).

17) AOCS Official Methods, Cd 3d-63, 5th edn. AOCS Press, Champaign (1999).

18) AOCS Official Methods, Cd 8-53, 5th edn. AOCS Press, Champaign (1997).

19) ISO Official Methods, 6885, 3th edn. ISO press, Switzerland (2006).

20) Li, J.; Bi, Y. L.; Liu, W.; Sun, S. D.; Liu, C. F.; Ma, S. M. Effect of acido value on TBHQ and BHT losses in heating oils: identification of the esterification products of TBHQ and free fatty acids. J. Am. Oil Chem. Soc. 91, 1763-1771 (2014).

21) AOCS Official Methods, Ce 6-86, 5th edn. AOCS Press, Champaign (1997).

22) AOAC Official Methods, 983.15. AOAC Press, Gaithersburg (1994).

23) Bailey, A. E.; Hui, Y. H. Industrial oil and fat products. 5th edn. John Wiley & Sons Inc. Press, Hoboken (1996).

24) Dobarganes, M. C. Formation and analysis of high molecularweight compounds in frying fats and oils. OCL. 5, 41-47 (1998).

25) Kanavouras, A.; Coutelieris, F. A. Shelf-life predictions for packaged olive oil based on simulations. Food Chem. 96, 48-55 (2006).

26) Braeuning, A.; Vetter, S.; Orsetti, S.; Schwarz, M. Paradoxical cytotoxicity of tert-butylhydroquinone in vitro: what kills the untreated cells? Arch. Toxicol. 86, 1481-1487 (2012).

27) Matsunaga, T.; Endo, S.; Takehara, M.; Soda, M.; Yamamura, K.; Tajima, K.; Miura, T.; Terada, T.; El-Kabbani, O.; Hara, A. Reduction of cytotoxic p-quinone metabolites of tert-butylhydroquinone by human aldehyde reductase (AKR1B10). Drug Metab. Pharmacok. 27, 553-558 (2011).