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Improvement of Canola Oil Frying Stability by Bene Kernel Oil's Unsaponifiable Matter

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Abstract The anti-rancidity effect of the unsaponifiable matter fraction of bene kernel (UFB) oil on canola oil (CAO) during frying was compared to that of *tert*-butyl hydroquinone (TBHQ). The UFB was separated into hydrocarbons (12.9%), carotenes (9.6%), tocopherols and tocotrienols (65.8%, mainly γ-tocopherol), linear and triterpenic alcohols (3.8%), methyl sterols (2.8%), sterols (3.0%, mainly β-sitosterol, stigmasterol, Δ^5-avenasterol, and Δ^7-avenasterol, respectively), and triterpenic dialcohols (2.2%). The results obtained from the measurements of the total polar compounds, the conjugated diene value, the carbonyl value, and total tocopherols showed that the stability of CAO improves similarly in the presence of UFB or TBHQ, and even more in the presence of UFB in some cases (especially inhibition of oxidized triglyceride monomers and triglyceride dimers). The analysis of polar components showed that the antioxidative additives were more effective to resist the formation of thermo-oxidative than hydrolytic products during the frying of CAO.

Keywords Bene kernel oil · Canola oil · Deep-frying · Rancidity · Unsaponifiable matter

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Introduction

During frying, the combination of prolonged heating at high temperature in the presence of moisture and oxygen causes an interrelated series of chemical reactions in the frying oils. As a result, a number of harmful compounds are produced that make drastic changes in the quality of oils and fried foods. Canola oil (CAO), because of its high content of polyunsaturated fatty acids (PUFA), is considered superior to many vegetable oils, but it is thermally unstable [1]. One of the most common methods to stabilize and increase the shelf life of frying oils is to add antioxidative compounds. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroanisol (BHA), and tertiary butyl hydroquinone (TBHQ) retard oxidation at ambient temperature. However, they become less effective at frying temperatures due to losses through volatilization or decomposition [2]. Moreover, the commercial use of synthetic antioxidants is strictly controlled, and increasing consumer awareness of the safety and toxicity of such synthetic antioxidants related to their metabolism and possible absorption and accumulation in the body organs and tissues have prompted increased interest in the use of natural antioxidants as alternatives to synthetic compounds [3].

Pistacia atlantica widely grows in the Zagrossian region of Iran at 600–3,000 m above the sea level. Its fruits, which are called bene in Iran, are round to oval, somewhat flat, 0.5–0.7 cm in diameter, and covered with a rather dry hull that can be easily removed by pressing between the fingers. A previous study on *P. atlantica* dealt with the chemical composition and oxidative stability of the kernel oils from its current subspecies in Iran. Considering the fatty acid composition, total tocopherols and phenolic compounds of the oil extracted from the kernel of *P. atlantica* subsp. *mutica* (bene kernel oil, BKO), this oil can be considered as

one of the most oxidatively stable vegetable oils in the world [4]. Our previous work indicated that the oxidative stability of CAO was significantly improved by the BKO [5]. In addition, the unsaponifiable matter (USM) fraction of vegetable oils, including hydrocarbons, terpenic alcohols, sterols, tocopherols and other phenolic compounds, is of great importance for oil characterization and stability. The USM fraction typically constitutes 0.5–2.5% of the vegetable oils, although some vegetable oils have exceptional amounts (5–6%). The effectiveness of the USM fraction of sesame and rice bran oils in retarding oil deterioration has been studied previously [6, 7].

The aim of this study was to investigate the effect of the USM fraction of the BKO (UFB) on the rancidity of CAO during frying at 180 °C, to fractionate the UFB, and to compare its anti-rancidity activity with that of TBHQ as a very strong synthetic antioxidant.

Materials and Methods

Materials

The ripe fruits of bene were collected from the fields of Islamabad in the Ilam province. Refined, bleached, and deodorized CAO with no added antioxidants was supplied by Segol (Nishaboor, Iran) and was stored at −18 °C until analysis. Peroxide and acid values of the CAO were 0.51 mequiv O_2/kg oil and 0.2 mg KOH/g oil, respectively, indicating that it was unoxidized and of high initial quality. Its fatty acid composition mainly consisted of palmitic (16:0, 10%), stearic (18:0, 3.7%), oleic (18:1, 50.5%), linoleic (18:2, 24%), linolenic (18:3, 8%) and erucic (22:1, 0.44%) acids. The same concentrations (100 ppm) of the UFB and TBHQ were individually added to the CAO before frying. All chemicals and solvents used in this study were of analytical reagent grade and supplied by Merck and Sigma Chemical Companies.

UFB Extraction

After drying in the shade, the pericarps of the *Pistacia* fruits were removed and the kernels were ground to powder in a grinder. The oil was extracted from the powders with *n*-hexane (1:4 w/v) by agitation in the dark at ambient temperature for 48 h. The solvent was evaporated in vacuo at 40 °C to dryness.

In a volume flask, 5 g of the BKO was saponified with 50 mL 1 N ethanolic KOH. Potassium hydroxide in a capped flask was heated in an oven for 1 h at 95 °C. After cooling, 100 mL of distilled water was added and mixed. The resulting solution was extracted two times with 100 mL diethyl ether. The upper organic layers were combined and washed twice with 75 mL distilled water, once with 100 mL 0.5 N ethanolic KOH, and then 100 mL distilled water until neutrality. The organic layer was then separated and dried over Na_2SO_4. After filtration of this solution, the solvent was evaporated to dryness under a vacuum at 45 °C. To purify more effectively, the dry UFB was dissolved with chloroform and, after filtration, was evaporated to dryness under a vacuum at 45 °C [8]. The yield of UFB extraction was about 5.7 wt%.

Thin-layer Chromatography Fractionation of the UFB

A chloroform solution (5%) of the UFB (50 mg/plate) was streaked using a thin-layer chromatography (TLC) applicator (CAMAG, Muttenz, Switzerland) along a line at 1 cm from the edges of a 20 cm × 20 cm plate coated with a 0.5 mm layer of silica gel (G), which had been activated for 15 min at 110 °C. The plate was developed in the ascending direction for 15 cm with the solvent system *n*-hexane/diethyl ether (7:3, v/v). The developed plate was then dried with a hair dryer, and visualization of the chromatogram was carried out by spraying a saturated solution of $K_2Cr_2O_7$ in H_2SO_4 (80%) and then carbonating at 130 °C for 25 min. Fractions with the same R_f were carefully scraped from the plate and thoroughly extracted with chloroform; then the extract was filtered through a 0.45-mm membrane filter (Millipore, HVLP) and evaporated to near dryness in vacuo below 40 °C. The residue was weighed to determine the yield of each fraction [9].

Frying Process

Potatoes were peeled and cut into pieces (7.0 cm × 0.5 cm × 0.3 cm) and submerged in water until needed. Potato pieces were fried in the frying oil. The oil (2.5 L) was placed in a 2.5-L capacity bench-top deep-fryer (Tefal model 1250, Paris, France) and heated to 180 °C. Potato pieces were fried in 20 g batches at constant frying temperature. The batches were fried at 7-min intervals for 8 h per day for six consecutive days. At the end of each 4 h, about 10 g of the frying oil was filtered into a screw-cap vial and promptly stored in the dark at 4 °C until analyzed. The volume of oil was not replenished during the frying process. Frying experiments were conducted in duplicate on each frying medium [10].

Total Tocopherols, Conjugated Diene Value, and Carbonyl Value

Total tocopherols (TT) content was determined according to the colorimetric method described by Wong, Timms, and Goh [11]. For the conjugated diene value (CDV) determination the oil samples were diluted to 1:600 with

hexane and measured spectrophotometrically at 234 nm and read against HPLC-grade hexane as a blank. An extinction coefficient of 29,000 mol/L was used to quantify the concentration of conjugated dienes formed during oxidation [12]. The carbonyl value (CV) of the oils was measured according to the method developed by Endo et al. [13], using 2-propanol and 2,4-decadienal as the solvent and standard, respectively [14].

HPSEC Analysis for Polar Compounds

The total polar compounds (TPC) content was determined according to the economical micro-method developed by Schulte [15]. The altered compounds that constitute the polar fraction were separated into free fatty acids (FFA), diglycerides (DG), oxidized triglyceride monomers (oxTGM), triglyceride dimers (TGD), and triglyceride polymers (TGP) by high-performance size-exclusion chromatography (HPSEC), according to Dobarganes et al. [16]. Isolated polar fractions were analysed in a GPC-SEC chromatograph (Knauer, Berlin, Germany) with a 20 µl sample loop. A 2300 refractive index detector and two Nucleogel GPC columns (Macherey–Nagel, Duren, Germany) with 100- and 500 Å pore size connected in series were operated at 40 °C. The columns were 300 × 7.7 mm I.D., packed with a macro-porous, highly cross-linked and spherical polystyrene/divinylbenzene copolymer (5 lm particle size). HPLC-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min. Sample concentration was 10 mg/mL in tetrahydrofuran.

GC Analysis for Sterols

The composition of the sterol fraction was determined by gas chromatography (GC) using betulin as the internal standard [17]. The compounds were separated on a SE 54 CB (Macherey–Nagel, Duren, Germany; 50 m long, 0.25 mm ID., 0.25 µm film thickness). Further parameters were as follows: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program, 240–255 °C at 4 °C/min.

HPLC Analysis for Tocopherols

Tocopherols were analyzed directly in the oils by HPLC with fluorescence detection [18]. A unit fitted with a "Nucleosil" Si 50—5 mm column and a 250 × 4 mm "Merck-Hitachi" F 1000 fluorescent detector was used. The operating conditions were as follows: excitation wavelength set at 295 nm, emission wavelength at 330 nm, mobile phase *n*-hexane:propan-2-ol, 95:5 (v/v), and rate of mobile phase 1 ml/min. The peaks were identified by using authentic individual tocopherol standards.

Statistical Analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA) and regression analyses were performed according to the MStatC and Excel software. Significant differences between means were determined by Duncan's multiple range tests. *P* values less than 0.05 were considered statistically significant.

Results and Discussion

The unsaponifiable constituents of the BKO were separated into hydrocarbons, carotenes, tocopherols and tocotrienols, linear and triterpenic alcohols (4,4′-dimethyl sterols), methyl sterols (4-methyl sterols), sterols (4-desmethyl sterols), and triterpenic dialcohols by means of silica gel TLC. Tocopherols and tocotrienols were the major constituents (65.8 ± 0.6%) of the UFB. These compounds have nutritional importance because they are known to have an antioxidative activity, which protects the oil against oxidative deterioration and protect cells against oxidative stresses [19]. Gamma-tocopherol was the predominant tocopherol component in the BKO, followed by β-, α-, and δ-tocopherols (Fig. 1). Weiser and Vecchi [20] showed that α-tocopherol has the highest biologic activity and it is generally considered as the most important antioxidant in living systems. However, recent studies have demonstrated that γ-tocopherol is a more effective free radical scavenger than α-tocopherol [21].

The UFB contained 12.9 ± 1.1% hydrocarbons. The hydrocarbons consist of a homologous series of linear compounds that are mainly saturated chains of 15–33 carbon atoms in food matrices. Most of the hydrocarbons have an odd number of carbon atoms and are mainly squalene [22]. There is evidence which indicates the antioxidant activity of squalene, especially on heating [23].

Carotenes constituted 9.8 ± 0.0% of the UFB. These compounds are thermolabile substances regardless of the molecular structure. These compounds have been shown to protect lipids from free-radical autoxidation by reacting with peroxyl radicals, thereby inhibiting propagation and promoting termination of the oxidation chain reaction [24], and also to be effective quenchers of singlet oxygen during inhibition of photooxidation [25].

The sterols (3.0 ± 0.6%), methyl sterols (2.8 ± 0.4%), triterpenic alcohols (3.8 ± 0.1%), and triterpenic dialcohols (2.2 ± 0.6%) constituted about 12% of the UFB. These steroidal phytochemicals contained in vegetable oils are hypocholesterolemic and may also be potent antioxidants [26]. Figure 1 shows the sterol composition of the BKO. The composition of sterols in the BKO was

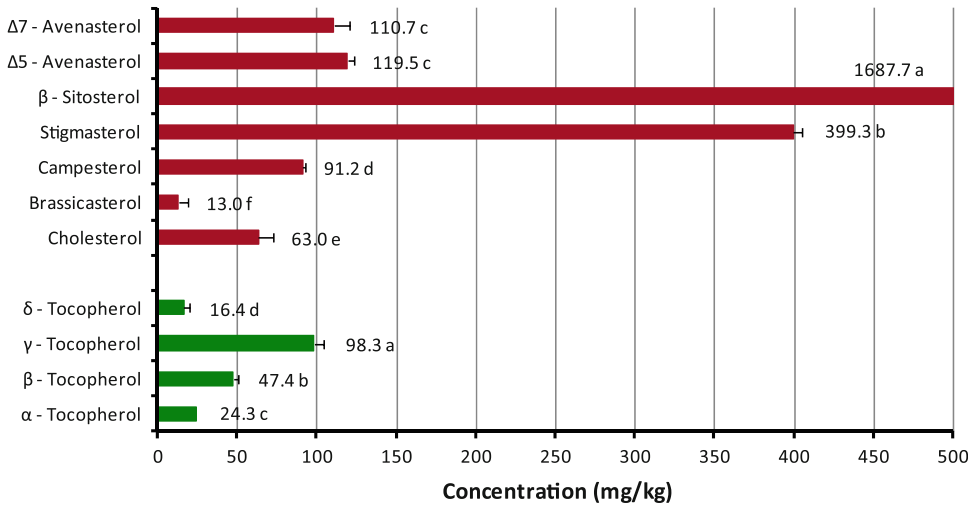

Fig. 1 The contents of tocopherol compounds and sterols of the bene kernel oil (BKO). The columns showing the similar components with the same lowercase letters are not significantly different at $P < 0.05$. Error bars indicate standard deviations

dominated by β-sitosterol, which accounted for about 80% of the total sterols (1,687.7 mg/kg) in the oil. Beta-sitosterol is the predominant and most widely distributed phytosterol [19]. Other sterols with some importance were stigmasterol, Δ^5-avenostrol, Δ^7-avenasterol and campesterol, respectively. Phytosterols with an ethylidene group in position 24 (24′) of the side chain, such as (24*Z*)-24-ethylidencholest-5-en-3β-ol (Δ^5-avenasterol) and (24*Z*)-24-ethylidencholest-7-en-3β-ol (Δ^7-avenasterol), have been reported to have antioxidant and anti-polymerization activity in oils subjected to high temperatures and to frying, whereas (24*R*)-ethylcholest-5-en-3β-ol (β-sitosterol), (24*R*)-24-methylcholest-5-en-3β-ol (campesterol) and (24*S*)-24-ethylcholest-5, 22-dien-3β-ol (stigmasterol), the most representative phytosterols in nature, do not exhibit this trend [27, 28]. The contents of cholesterol and brassicasterol were 62.9 and 13.0 mg/kg, respectively. It is conspicuous that the BKO contained brassicasterol. This sterol is characteristic for rapeseed oil and oils from other members of the family *Brassicaceae*. Matthaus and Ozcan [19] observed similar results and reported that some samples of *Pistacia terebinthus L. (P. terebinthus-3, P. terebinthus-7, and P. terebinthus*-8) contained brassicasterol in amounts from 8.6 to 23.9 mg/kg. An explanation of the finding is not possible from this data. Cholest-5-en-3β-ol (cholesterol) did not exhibit any antioxidant activity [27].

Changes in the CDV can be used as a relative measure of oxidation. Oxidation of PUFA is accompanied by increased ultraviolet absorption [29]. As can be seen in Table 1, the amount of conjugated dienes in all treatments increased with frying time. The CDV of CAO showed an increase of 359% after 48 h of frying (40 mmol/L). The CAOs stabilized with 100 ppm TBHQ or 100 ppm UFB showed lower levels of the CDV increase (237.1%, 28.4 mmol/L; and 263.3%, 29.5 mmol/L, respectively), indicating their antioxidant potential for resistance to the production of conjugated diene hydroperoxides under the frying process conditions. It was interesting to find that the UFB and TBHQ statistically revealed the same behavior.

The CV is considered to be a good index of oxidative changes in lipid systems. It does not measure primary products of oxidation (hydroperoxides), but secondary decomposition products such as aldehydes and ketones. These often contribute to rancid and unpleasant flavors and reduce the nutritional value of fried foods [13]. During the frying process of CAO, the CV increased and reached a maximum value of 39.9 μmol/g after 24 h and then decreased as a result of further heat treatment (Table 1). This phenomenon has been attributed to the decomposition of carbonyl compounds during prolonged frying period and formation of new compounds which are not detectable by the CV assay [30]. However, the CV of CAOs containing 100 ppm TBHQ or 100 ppm UFB increased steadily with lower rates till the end of the frying process, so that maximum values of 31.0, and 30.8 μmol/g, respectively, were observed.

Prior to performing the frying process, the CAO had a TT content of 817.9 mg/kg, and the addition of 100 ppm of TBHQ or UFB caused no statistically significant changes in the initial TT content of the CAO (Table 1). The TT content of the CAO decreased by 62% to 309.7 mg/kg at the end of the frying process. The decrease for the CAO containing TBHQ or UFB was about 37.2 and 34.1%, respectively, indicating the protective effect of the UFB and TBHQ on the CAO's tocopherols. Since tocopherols act as antioxidants, oils in which tocopherols degrade rapidly would be expected to exhibit lower stability.

Determination of polar compounds in used edible oils and fats is a well-accepted method due to its accuracy and reproducibility. It provides the most reliable measure of the extent of deterioration in frying oils and fats in most situations. There was no statistically significant difference

Table 1 Conjugated diene value (CDV, mmol/L), carbonyl value (CV, μmol/g), and the content of total tocopherols (TT, mg/kg) of the canola oil (CAO) as affected by the tertiary butyl hydroquinone (TBHQ, 100 ppm) and unsaponifiable matter (USM, 100 ppm) of the bene kernel oil (UFB) during frying at 180 °C

Time (h)	CDV			CV			TT		
	CAO	CAO + TBHQ	CAO + UFB	CAO	CAO + TBHQ	CAO + UFB	CAO	CAO + TBHQ	CAO + UFB
0	8.7 ± 0.2 Kab	8.4 ± 0.2 Jab	8.1 ± 0.8 Jab	8.4 ± 0.1 Ga	8.9 ± 0.6 Ha	8.5 ± 1.1 Ha	817.9 ± 3.9 Aa	820.4 ± 12.6 Aa	814.9 ± 7.6 Aa
4	12.4 ± 0.3 Ja	11.0 ± 0.7 Ib	10.5 ± 1.3 Ib	8.8 ± 1.8 Ga	10.0 ± 1.5 GHa	9.2 ± 1.5 Ha	758.7 ± 6.5 Ba	768.2 ± 12.4 Ba	753.5 ± 13.6 Ba
8	15.5 ± 0.3 Ia	13.0 ± 0.5 Ib	12.8 ± 2.1 Hb	17.4 ± 3.0 Fa	13.0 ± 1.4 FGb	12.7 ± 1.5 Gb	717.7 ± 6.7 Cb	754.6 ± 10.6 Ba	716.4 ± 10.3 Cb
12	19.7 ± 0.6 Ha	16.3 ± 1 Hb	14.1 ± 0.4 Hc	25.2 ± 1.9 Ea	15.3 ± 2.4 Fb	15.4 ± 1.4 Fb	578.7 ± 15.4 Dc	708.2 ± 10.7 Ca	660.2 ± 14.8 Db
16	22.3 ± 1.1 Ga	17.8 ± 1.1 GHb	16.0 ± 0.7 Gc	28.0 ± 2.4 Ea	19.4 ± 4.0 Eb	18.0 ± 2.1 EFb	538.6 ± 17.6 DEb	656.9 ± 8.6 Da	653.5 ± 19.7 Da
20	25.2 ± 0.5 Fa	19.0 ± 0.6 FGb	18.3 ± 0.8 Fc	28.2 ± 3.6 Ea	20.5 ± 2.6 DEb	19.5 ± 0.8 Eb	507.3 ± 8.7 Ec	630.6 ± 10.2 DEa	600.4 ± 7.2 Eb
24	28.0 ± 0.8 Ea	20.1 ± 1.0 EFc	20.1 ± 1.1 Ec	39.9 ± 2.0 Ca	21.3 ± 1.9 DEb	20.2 ± 2.0 Eb	477.3 ± 9.9 Fc	607.2 ± 11.8 Ea	579.7 ± 4.5 Fb
28	28.8 ± 0.2 Eba	21.8 ± 1.6 DEc	22.8 ± 0.2 Dc	33.8 ± 2.7 Da	24.2 ± 3.7 CDb	24.4 ± 1.3 Db	359.4 ± 6.0 Gb	550.3 ± 10.3 Fa	573.7 ± 12.2 FGa
32	31.4 ± 1.3 Da	23.8 ± 1.7 CDc	23.4 ± 0.4 Dc	35.8 ± 1.5 Da	24.3 ± 2.3 CDb	25.3 ± 1.5 CDb	355.8 ± 3.8 Gc	543.3 ± 3.5 Fb	560.9 ± 3.4 Ga
36	36.3 ± 0.7 Ca	25.8 ± 1.1 BCc	24.8 ± 0.5 Cc	55.7 ± 2.8 Ba	26.5 ± 2.8 BCb	27.1 ± 3.2 BCDb	340.8 ± 5.7 Hb	531.6 ± 7.5 FGHa	552.4 ± 9.4 GHa
40	37.2 ± 0.2 Ca	26.2 ± 1.6 ABc	26.6 ± 1.0 Bc	64.0 ± 1.8 Aa	28.5 ± 1.8 ABb	27.9 ± 3.3 BCbc	336.2 ± 6.2 Hc	525.9 ± 13.4 FGHb	559.4 ± 2.3 Ga
44	38.8 ± 1.2 Ba	27.3 ± 3.2 ABc	29.0 ± 0.5 Ac	58.0 ± 1.4 Ba	29.4 ± 2.5 ABc	29.9 ± 1.4 ABc	312.9 ± 7.2 Ib	520.9 ± 8.3 GHa	544.8 ± 9.6 GHa
48	40.0 ± 0.6 Aa	28.4 ± 2.5 Ac	29.5 ± 0.2 Ac	47.5 ± 3.4 Ba	31.0 ± 2.5 Ac	30.8 ± 1.2 Ac	309.7 ± 13.8 Ic	515.3 ± 6.1 Hb	536.7 ± 5.5 Ha

Means ± SD (standard deviation) within a column with the same uppercase letters are not significantly different at $P < 0.05$. Means ± SD within a row with the same lowercase letters are not significantly different at $P < 0.05$

between the initial TPC content of the CAO and those containing the UFB or TBHQ (3.79–3.94%). The TPC contents during the frying process increased linearly with high correlation coefficients ($R^2 > 0.98$). Research has shown that the fraction of polar compounds isolated from oxidized oils is toxic to laboratory animals [31]. Therefore, it has been suggested that a level of 24% total polar materials be the limit beyond which frying oil should be discarded. This is an indication of high stability of the oil to changes in triacylglycerols that occur during the period of frying. The CAO reached the discarding range of TPC content during the frying process, but the CAO containing the UFB or TBHQ did not. Assuming that the limit of acceptance for the TPC content is 24%, the time required to reach this limit was considered as a measure of frying stability. Regarding the calculated measures (Fig. 2), the CAO showed a frying stability significantly lower (32.8 h) than that of the CAO containing the TBHQ (52.13 h) or UFB (52.17 h). As can be seen, there was statistically similar stabilizing effects on the CAO in the presence of equal concentrations of the UFB and TBHQ.

The contents of thermo-oxidative (oxTGM, TGD, and TGP) and hydrolytic (FFA and DG) components of the CAO as affected by the TBHQ or UFB during frying are shown in Tables 2 and 3, respectively. Changes in the polar components during frying were significantly fitted to power equations with high determination coefficients ($R^2 > 0.96$). The parameters of the power equations, the percentage of increase in each polar component after 48 h of frying (PI_{48}), and the ratio between the PI_{48} of the CAO and that of the CAO containing TBHQ or UFB, as an effectiveness index (EI) of the corresponding additive, are given in Table 4.

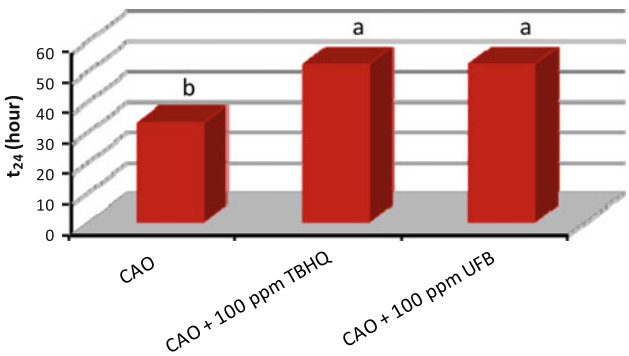

Fig. 2 The time required to reach total polar compounds (TPC) content of 24% (t_{24}) for the canola oil (CAO) as affected by the tertiary butyl hydroquinone (TBHQ, 100 ppm) and unsaponifiable matter (USM, 100 ppm) of the bene kernel oil (UFB) during frying at 180 °C. The corresponding linear equations: CAO, $y = 0.61x + 3.97$, $R^2 = 0.997$; CAO + 100 ppm TBHQ, $y = 0.39x + 3.67$, $R^2 = 0.993$; CAO + 100 ppm UFB, $y = 0.42x + 2.09$, $R^2 = 0.985$. Means with the same lowercase letters are not significantly different at $P < 0.05$

Table 2 The contents (mg/g oil) of thermo-oxidative components (oxidised triglyceride monomers, oxTGM; triglyceride dimers, TGD; and triglyceride polymers, TGP) of the canola oil (CAO) as affected by the tertiary butyl hydroquinone (TBHQ, 100 ppm) and unsaponifiable matter (USM, 100 ppm) of the bene kernel oil (UFB) during frying at 180 °C

Time (h)	TGP			TGD			oxTGM		
	CAO	CAO + TBHQ	CAO + UFB	CAO	CAO + TBHQ	CAO + UFB	CAO	CAO + TBHQ	CAO + UFB
0	0.2 ± 1.0 Ka	0.3 ± 0.2 Ja	0.2 ± 0.4 Ja	4.0 ± 0.0 La	4.2 ± 0.4 Ja	4.3 ± 0.5 Ia	17.4 ± 0.6 Ja	16.6 ± 0.0 Ja	16.9 ± 0.5 Ka
4	0.9 ± 0.2 Ja	0.9 ± 0.2 Ia	1.0 ± 0.6 Ia	10.9 ± 2.0 Ka	8.9 ± 0.1 Ib	6.9 ± 0.6 Ic	28.7 ± 1.8 Ia	20.7 ± 0.4 Ic	19.2 ± 0.7 Jd
8	2.75 ± 0.3 Ia	1.7 ± 0.1 Ib	1.9 ± 0.3 Ha	22.9 ± 0.6 Ja	13.9 ± 0.1 Hc	12.9 ± 1.5 Hc	33.8 ± 1.0 Ha	22.9 ± 0.4 Ic	22.1 ± 0.3 Ic
12	6.6 ± 0.5 Ha	2.1 ± 0.7 Hc	2.3 ± 0.3 Hbc	32.7 ± 0.3 Ia	21.4 ± 1.8 Gb	18.2 ± 1.8 Jc	40.0 ± 2.2 Ga	32.3 ± 0.8 Hb	25.7 ± 2.1 Hc
16	10.6 ± 1.4 Ga	3. 9 ± 0.9 Gc	3.1 ± 0.5 Gc	45.3 ± 1.5 Ha	32.6 ± 2.2 Fc	24.1 ± 2.0 Fd	57.0 ± 0.5 Fa	36. 4 ± 0.5 Gc	28.8 ± 1.5 Gd
20	15.4 ± 0.7 Fa	4.0 ± 0.6 FGc	3.9 ± 0.8 Fc	53.6 ± 1.3 Ga	34.9 ± 2.9 Eb	33.9 ± 1.8 Ec	59.7 ± 1.7 EFa	40.5 ± 1.6 EFc	35.9 ± 4.7 Fd
24	19.1 ± 1.6 Ea	5.6 ± 0.7 EFc	4.5 ± 0.6 Ed	60.4 ± 0.3 Fa	41.5 ± 2.8 Db	36.7 ± 2.6 Ec	61.7 ± 1.8 Ea	42.9 ± 1.0 Ec	38.8 ± 2.9 Ed
28	22.1 ± 1.9 Ea	8. 9 ± 0.6 DEc	6.2 ± 0.1 Dd	74.0 ± 0.2 Ea	45.6 ± 0.3 Cc	43.7 ± 0.6 Dd	67.4 ± 3.1 Da	46.8 ± 0.8 Dc	40.9 ± 0.6 Dd
32	29.0 ± 1.1 Da	11.8 ± 1.2 CDc	14.0 ± 0.6 Db	79.4 ± 1.5 Da	50.2 ± 0.4 Dc	46.6 ± 0.5 Dd	70.9 ± 2.7 Ca	52.6 ± 2.5 Cb	46.6 ± 1.3 Cc
36	36.2 ± 0.5 Ca	14.4 ± 1.1 BCc	16.7 ± 1.6 Cb	91.3 ± 2.9 Ca	54.7 ± 0.4 Bc	54.0 ± 0.5 Cc	74.2 ± 0.5 Ba	53.2 ± 3.0 Cb	50.0 ± 0.5 Bc
40	42.3 ± 1.8 Ca	15.3 ± 2.0 ABc	19.6 ± 1.7 Bbd	91.0 ± 0.9 Ca	56.1 ± 0.6 Bd	59.5 ± 0.5 Bc	79.1 ± 1.9 Aa	55.5 ± 2.8 ABb	51.4 ± 0.4 Bc
44	51.7 ± 0.4 Ba	16.8 ± 1.9 ABc	21.5 ± 1.4 Ab	103.0 ± 2.2 Ba	60.7 ± 0.7 Bd	62.1 ± 1.7 Ac	80.1 ± 3.2 Aa	56.3 ± 2.1 ABc	54.5 ± 1.2 Ac
48	55.4 ± 0.9 Aa	19.4 ± 0.3 Ab	22.6 ± 1.3 Ad	108.2 ± 2.9 Aa	65.1 ± 2.6 Ad	65.8 ± 1.1 Ac	84.2 ± 1.0 Aa	57.7 ± 0.7 Ac	56.0 ± 0.3 Ac

Means ± SD (standard deviation) within a column with the same uppercase letters are not significantly different at $P < 0.05$. Means ± SD within a row with the same lowercase letters are not significantly different at $P < 0.05$

Table 3 The contents (mg/g oil) of hydrolytic components (free fatty acids, FFA; diglycerides, DG) of the canola oil (CAO) as affected by the tertiary butyl hydroquinone (TBHQ, 100 ppm) and unsaponifiable matter (USM, 100 ppm) of the bene kernel oil (UFB) during frying at 180 °C

Time (h)	DG			FFA		
	CAO	CAO +TBHQ	CAO + UFB	CAO	CAO +TBHQ	CAO + UFB
0	14.1 ± 0.1 Ka	14.6 ± 0.7 Ha	14.4 ± 0.9 Ga	3.1 ± 0.3 Ha	3.0 ± 0.0 Ka	3.1 ± 0.0 Ia
4	17.3 ± 0.4 Ka	17.6 ± 0.8 GHa	15.6 ± 0.2 Ga	3.6 ± 0.7 Ha	3.7 ± 0.0 Ja	3.5 ± 0.0 Ia
8	22.2 ± 0.3 Ja	19.6 ± 1.1 FGab	18.0 ± 0.8 FGb	4.7 ± 0.2 Ga	4.2 ± 0.0 Jbc	3.9 ± 0.0 GIc
12	25.3 ± 0.1 Ia	22.2 ± 1.5 Fab	17.1 ± 2.9 FGb	5.1 ± 0.2 Gb	4.8 ± 0.0 Ib	4.0 ± 0.1 GIc
16	31.5 ± 1.3 Ha	27.8 ± 1.8 Eb	20.7 ± 2.8 EFc	6.5 ± 0.3 Fa	6. 4 ± 0.0 Hc	4.4 ± 0.1 Gd
20	32.5 ± 0.1 Ha	30.1 ± 2.0 DEab	22.7 ± 0.4 Ec	6.7 ± 0.1 Fa	6.8 ± 0.0 Ha	5.2 ± 0.1 Fb
24	36.2 ± 0.4 Ga	34.6 ± 2.0 CDab	29.1 ± 0.5 Dc	7.6 ± 0.0 Fb	7.8 ± 0.1 Gab	6.4 ± 0.1 Ec
28	41.6 ± 1.1 Fa	38.8 ± 2.1 Cab	37.2 ± 1.5 Cbc	9.0 ± 0.0 Ea	9.2 ± 0.1 Fa	8.2 ± 0.1 Db
32	45.5 ± 3.3 Ea	44.0 ± 2.4 Ba	39.1 ± 0.6 Cb	13.3 ± 0.2 Da	11.4 ± 0.0 Eb	9.8 ± 0.1 Cc
36	48.6 ± 2.6 Da	48.8 ± 2.6 Ba	47.1 ± 0.9 Ba	16.9 ± 1.4 Da	13.9 ± 0.1 Db	13.0 ± 0.0 Bc
40	54.8 ± 0.8 Ca	56.0 ± 2.9 Aa	49.6 ± 0.8 Bb	18.5 ± 1.7 Ca	16.6 ± 0.1 Cc	13.3 ± 0.3 Bd
44	62.0 ± 3.2 Ba	57.5 ± 3.4 Ab	52.8 ± 0.9 ABc	21.2 ± 1.9 Ba	18.5 ± 0.0 Bc	16.0 ± 0.2 Ad
48	68.0 ± 3.0 Aa	58.2 ± 3.5 Ab	56.7 ± 0.6 Ab	22.4 ± 1.3 Aa	19.6 ± 0.1 Ac	16.0 ± 0.3 Ad

Means ± SD (standard deviation) within a column with the same uppercase letters are not significantly different at $P < 0.05$. Means ± SD within a row with the same lowercase letters are not significantly different at $P < 0.05$

Table 4 The parameters calculated from the power relationship between the polar components contents (mg/g oil) and frying time at 180 °C for the canola oil (CAO) as affected by the tertiary butyl hydroquinone (TBHQ, 100 ppm) and unsaponifiable matter (USM, 100 ppm) of the bene kernel oil (UFB)

	Polar component = a (time)^b + c				PI_{48}^A	EI^B
	a	b	c	R^2		
TGP						
CAO	0.12	1.60	0.17	0.996	33663.1	
CAO + TBHQ	0.05	1.56	0.16	0.987	12372.8	2.72
CAO + UFB	0.02	1.89	0.20	0.959	12121.6	2.77
TGD						
CAO	3.95	0.86	1.20	0.993	9124.0	
CAO + TBHQ	3.12	0.78	1.88	0.988	3437.5	2.65
CAO + UFB	1.71	0.94	2.19	0.993	3019.7	3.02
oxTGM						
CAO	6.08	0.63	15.65	0.979	449.0	
CAO + TBHQ	2.88	0.72	14.69	0.977	313.1	1.43
CAO + UFB	1.13	0.94	15.49	0.988	275.5	1.63
DG						
CAO	0.47	1.23	15.41	0.992	329.7	
CAO + TBHQ	0.50	1.17	14.22	0.989	332.2	0.99
CAO + UFB	0.20	1.41	12.37	0.981	377.5	0.87
FFA						
CAO	0.01	1.88	3.35	0.980	606.7	
CAO + TBHQ	0.02	1.77	3.30	0.991	517.8	1.17
CAO + UFB	0.01	1.81	2.96	0.978	483.1	1.26

[A] The percentage of increase in each polar component after 48 h of frying at 180 °C

[B] The ratio between the PI_{48} of the CAO and that of the CAO containing TBHQ or UFB

The content of all separate components in the polar fractions increased with frying time. More specifically, the TGP constituted only a minor component of the polar fraction of the fresh oil (0.2 mg/g) (Table 2), but increased consistently with frying time up to 55.4 mg/g (PI_{48} = 33,663.1, Table 4). During frying, the TGP content of the fresh oil in the presence of the TBHQ (0.3 mg/g) or UFB (0.2 mg/g) showed the PI_{48} values of about 12,373 and 12,122 (Table 4) and reached 19.4 and 22.6 mg/g (Table 2), respectively. As shown in Table 4, the EI in the presence of the TBHQ or UFB was 2.72 and 2.77, respectively, indicating the same ability of the UFB and TBHQ to resist the polymer formation.

The TGD content of the fresh oil was more than 20 times of its TGP content (4.0 vs. 0.2 mg/g). The TGD content also consistently increased until the end of the frying process (from 4.0 to 108.2 mg/g) by a PI_{48} of about 9,124. This percentage was considerably lower than that of the TGP content, although the TGD was the major component of the polar fraction throughout the frying process, indicating a higher tendency for formation of the TGP than of the TGD, although the TGD was the major component of the polar fraction throughout the frying process. As can be seen in Tables 2 and 4, the TGD content of the fresh CAO in the presence of the TBHQ or UFB increased from 4.2 and 4.3 mg/g to 65.1 mg/g (PI_{48} = 3,437.5 and EI = 2.65) and 65.8 mg/g (PI_{48} = 3,019.7 and EI = 3.02), respectively.

The oxTGM were the major component of the polar fraction of the fresh CAO (17.4 mg/g) (Table 2), and with a PI_{48} of about 449 (Table 4) reached 84.2 mg/g at the end of the frying process. As can be seen in Tables 2 and 4, the oxTGM content of the fresh CAO in the presence of the TBHQ or UFB increased from 16.6 and 16.9 mg/g to 57.7 mg/g (PI_{48} = 313.1 and EI = 1.43) and 56 mg/g (PI_{48} = 275.5 and EI = 1.63), respectively. The antioxidative additives showed less considerable influences on the changes in TGD and oxTGM contents of the fresh CAO during frying. In addition, there was a better effectiveness for the UFB than for the TBHQ.

The DG was also present in high concentration in the polar fraction of the fresh oil (14.1 mg/g) (Table 3), but increased by a PI_{48} of about 330 (Table 4) as frying proceeded, reaching a final value of 68 mg/g. The FFA content was low (3.1 mg/g) initially and increased by a PI_{48} 607 with frying time to 22.4 mg/g. The FFA content of the fresh CAO in the presence of the TBHQ or UFB increased from 3.0 and 3.1 mg/g to 19.6 (PI_{48} = 517.8 and EI = 1.17) and 16.0 mg/g (PI_{48} = 483.1 and EI = 1.26), respectively, whereas the DG contents as affected by the TBHQ or UFB increased from 14.6 and 14.4 mg/g to 58.2 mg/g (PI_{48} = 332.2 and EI = 0.99) and 56.7 mg/g (PI_{48} = 377.5 and EI = 0.87), respectively. Considering the EI values, it can be observed that the thermo-oxidative reactions were affected by the antioxidative additives totally more than hydrolytic reactions during the frying of the CAO. Also, the UFB showed similar or even better antioxidant effect than TBHQ during the frying process.

Conclusions

Physiochemical reactions such as hydrolysis, thermal degradation, oxidation and polymerization are the most important occurrences in frying processes and lead to the decomposition of frying oil and formation of monomeric and polymeric compounds as well as primary and secondary oxidative compounds, thereby affecting the quality of oil and the fried product. Our results indicated that hydrolytic and oxidative reactions and also the creation of primary and secondary products of lipid oxidation during the frying process of CAO are retarded similarly in the presence of UFB or TBHQ, and even more in the presence

of UFB in some cases. The ability of the UFB to resist the TGP and TGD formation was higher than that of the TBHQ. Also, the UFB had a better protective effect on the indigenous tocopherols of the CAO during the frying process.

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