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Analytical Methods

Polar compounds distribution of sunflower oil as affected by unsaponifiable matters of Bene hull oil (BHO) and tertiary-butylhydroquinone (TBHQ) during deep-frying

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ABSTRACT

Total polar compounds (TPC) contents of the sunflower oil (SFO) increased linearly ($R^2 > 0.99$) with frying time. At the same concentration (100 ppm), the increase rate of the TPC content for the SFO containing the unsaponifiable matters (USM) of the Bene hull oil (BHO) was lower than that for the SFO containing the tertiary-butylhydroquinone (TBHQ). The TPC analysis by high-performance size-exclusion chromatography allowed the separation and quantification of triglyceride polymers (TGP), triglyceride dimers (TGD), oxidised triglyceride monomers (oxTGM), diglycerides (DG), and free fatty acids (FFA) during frying. The ability of the USM to resist the TGP formation was higher than that of the TBHQ. The USM and TBHQ showed lower influences on the changes in TGD and oxTGM contents, as well as there was an effectiveness better for the USM than for the TBHQ. The increase rate of DG and FFA contents more effectively decreased by the USM rather than by the TBHQ.

1. Introduction

Determination of the contents of polar compounds is considered to be the most reliable method for the evaluation of stability and quality of fats and oils during frying of foods. Polar compounds are principally due to the action of atmospheric oxygen, water content of the foodstuff, and the high temperature at which frying takes place. They consist of dimeric and higher polymeric triglycerides formed through thermal polymerisation of triglycerides, monomeric oxidised products, as well as mono- and diglycerides and free fatty acids formed through hydrolytic cleavage of triglycerides. The analysis of polar fraction by high-performance size-exclusion chromatography (HPSEC) allows the separation and quantification of triglyceride polymers (TGP), triglyceride dimers (TGD), oxidised triglyceride monomers (oxTGM), diglycerides (DG), and free fatty acids (FFA) (Houhoula, Oreopoulou, & Tzia, 2003). These components differ not only in polarity or molecular weight but also in nutritional significance, and so, it would be interesting to know the contribution of each of them to the total alteration (Dobarganes, Perez-Camino, & Marquez-Ruiz, 1988).

Addition of synthetic antioxidants is one of the major treatments used to maintain the quality of fats and oils during frying. However, the use of chemical additives has raised questions regarding food safety and toxicity (Linderschmidt, Trylka, Goad, & Witschi, 1986; Tappel, 1995). Consumers are becoming increasingly aware of the nutritional value and safety of their food and its ingredients. Natural antioxidants are believed to be safer than synthetic ones (Tian & White, 1994). Unsaponifiable mater (USM) fraction of vegetable oils naturally contains hydrocarbons, terpene alcohols, sterols, tocopherols and other phenolic compounds which may act as oxidation inhibitors under a range of conditions (Bosku & Morton, 1976). Vegetable oils typically contain 0.5–2.5% USM, although some others have exceptional amounts, 5–6% (Maleka, 1994). The effectiveness of the USM fraction of vegetable oils in retarding oil deterioration has been studied by many investigators (Abdel-Aziz, 1985; Awatif, Khalil, & Badawy, 1996; Gopala Krishna, Prashanth, Pragasam, Raghavendra, & Matoon, 2003; Mohamed & Awatif, 1998).

Pistacia atlantica subsp. *mutica* widely grows in the Zagrossian region of Iran at 600–3000 m above sea level (Sabeti, 1994). Its fruits, which are called "Bene" in Iran, are round to oval, somewhat flat, and 0.5–0.7 cm in diameter. Bene have a wooden hard shell covered with a rather dry hull which could be easily removed by pressing between fingers. This soft hull is dark green in colour, comprises ~24% of the whole fruit (~25% kernel and ~51% hard shell), and yields up to ~30% oil (Daneshrad & Aynehchi, 1980). Previous studies on *P. atlantica* deal with the chemical composition and oxidative stability of the kernel oil from its current subspecies in Iran (Farhoosh & Tavakoli, 2008), oxidative stability and antioxidant activity of the hull oil from *P. atlantica* subsp. *mutica* (Farhoosh, Haddad Khodaparast, & Sharif, 2009), and anti-rancidity effect

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of Bene hull oil (BHO) compared with sesame and rice bran oils during the frying process of sunflower oil (Sharif, Farhoosh, Haddad Khodaparast, & Tavassoli Kafrani, 2009). The BHO contains about 6.5% USM (Farhoosh et al., 2009), which is considered to be the highest content among all common vegetable oils. In a recent research, the unsaponifiable constituents of the BHO were separated into hydrocarbons, carotenes, tocopherols and tocotrienols, linear and triterpenic alcohols (4,4′-dimethylsterols), methylsterols (4-methylsterols), sterols (desmethylsterols), triterpenic dialcohols, and triterpenic dialcohols methylesters by means of silica gel thin-layer chromatography. Tocopherols and tocotrienols were the major constituents of the USM of the BHO, followed by the steroidal phytochemicals, hydrocarbons, and carotenes (Farhoosh & Tavassoli Kafrani, 2010). As can be seen, most of them are particularly important functional compounds which have potentials to retard the degradation of unsaturated fatty acids in lipid systems.

The purpose of this study was to investigate the effect of USM of the BHO on the thermo-oxidative and hydrolytic reactions occurring during deep-frying of sunflower oil (SFO), and to compare its anti-rancidity activity with that of tertiary-butylhydroquinone (TBHQ) as a very strong synthetic antioxidant.

2. Materials and methods

2.1. Materials

Refined, bleached, and deodorized SFO with no added antioxidants was supplied by Segol (Nishaboor, Iran) and was stored at −18 °C until analysis. Peroxide value (measured according to Shantha and Decker (1994)) and acid value (measured according to the AOCS Official Method Cd 3d-63) of the sunflower oil were 0.3 meq O_2 per kg oil and 0.2 mg KOH per gram oil, respectively, indicating that it was unoxidized and of high initial quality. The fatty acid composition (measured according to Farhoosh, Niazmand, Rezaei, and Sarabi (2008)) mainly consisted of palmitic (16:0, 8.5%), stearic (18:0, 4.8%), oleic (18:1, 28.0%), linoleic (18:2, 54.2%), and linolenic (18:3, 2.8%) acids. The same concentrations (100 ppm) of USM and TBHQ were individually added to the SFO. Fatty acid methyl ester standards, and all chemicals and solvents used in this study were of analytical reagent grade and supplied by Merck and Sigma Chemical Companies.

2.2. USM extraction

In a volume flask, 5 g of the BHO 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 distiled 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 distiled water, once with 100 mL 0.5 N ethanolic KOH, and then 100 mL distiled water until neutrality. The organic layer was then separated and dried over Na_2SO4. After filtration of this solution, the solvent was evaporated to dryness under vacuum at 45 °C. To purify more effectively, the dry USM was dissolved with chloroform and, after filtration, was evaporated to dryness under vacuum at 45 °C (Lozano, Dhuique Mayer, Bannon, & Gaydou, 1993). The yield of USM extraction was about 6.5 wt.%.

2.3. Total polar compounds (TPC) content

The TPC content was determined according to the economical micro method developed by Schulte (2004). Silica gel 60 (63–100 μm) was dried overnight at 160 °C, was filled still warm into a glass bottle, was added five parts of water to 95 parts of it, and was shaken vigorously for about 1 min to disperse lumps; after standing overnight the material was ready for use. One gram of the silica gel 60 was compressed and filled between two cotton wool balls into a 5 mL pipette tip (15 cm long). Oil sample (500 mg) was pipetted into a 5 mL volumetric flask. It was dissolved in 4 mL toluene, and then filled with the toluene. Under a well ventilated fume hood, 1 mL of the solution was pipetted on top of the pipette tip fixed over a tared aluminium tray, so that it ended 1 mm above the bottom of the tray. After the solution was soaked in, the pipette tip was washed with 1 mL eluent and after soaking in, were added 7 mL (2 mL × 3.5 mL) of eluent. After elution (∼15 min), the end of the tip was washed with 500 μL of toluene. The solvent was removed from the eluate with dry compressed air; the evaporation was speeded up by placing the tray on a temperature-controlled hot plate of 50 °C. After weighing, TPC, in per cent (w/w), was calculated by the formula of $100(w - w1)/w$, in which w and $w1$ are the sample weight and the weight of nonpolar components in milligram, respectively.

2.4. Polar compounds distribution (HPSEC analysis)

The altered compounds that constitute the polar fraction were separated into FFA, DG, oxTGM, TGD, and TGP by HPSEC, according to Dobarganes et al. (1988). 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 μm 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.

2.5. 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 oil. The oil (2.5 L) was placed in a 2.5-L capacity bench-top deep-fryer (Tefal model 1250, 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 4 consecutive days. At the end of each 4 h, about 20 g of the frying oil was filtered into a screw-cap vial and promptly stored in the dark at 4 °C until use. The volume of oil was not replenished during the frying process. Frying experiments were conducted in duplicate (Farhoosh & Moosavi, 2008).

2.6. Statistical analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). 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.

3. Results and discussion

The TPC contents of the SFO as affected by the TBHQ and USM of the BHO during frying are given in Table 1. The initial amount of TPC in vegetable oils has a pronounced contribution to create the off-flavour compounds as well as a marked effect on the primary oxidation (Farhoosh & Pazhouhanmehr, 2009). The fresh sunflower