J Food Sci Technol (May–June 2011) 48(3):382–385
DOI 10.1007/s13197-010-0190-2

SHORT COMMUNICATION

The effect of consecutive steps of refining on squalene content of vegetable oils

Cevdet Nergiz · Deniz Çelikkale

Revised: 13 November 2010 / Accepted: 22 November 2010 / Published online: 21 December 2010

Abstract The aim of this study is to evaluate the effect of refining steps on the squalene content of some vegetable oils. A comparison has been made between the crude oils and consecutive steps of refining process (neutralization, bleaching, deodorization, winterization) in the amounts of squalene of the oil samples. Among the oils, virgin and refined olive oils contained higher amounts of squalene. A mean of 491.0±15.55 mg/100 g squalene was found in virgin olive oil samples. While appreciable quantities of squalene has been reduced during refining, considerable level of squalene were still present in refined olive oils (290.0±9.89 mg/100 g). The squalene content of crude seed oils varied from 13.8±0.39 mg/100 g to 26.2±0.08 mg/100 g as average. It has been determined that refining process reduced the level of squalene in examined oils. The highest reduction in squalene content of the oils was detected during deodorization. The effect of refining steps on the amount of squalene in vegetable oils was found to be significant ($p<0.05$). Olive oil has been considered an important source of squalene, even after it has been refined, compared to seed oils.

Keywords Vegetable oils · Refining · Squalene

C. Nergiz (✉)
Department of Chemistry, Faculty of Arts and Sciences,
Fatih University,
34500 Büyükçekmece, Istanbul, Turkey
e-mail: cnergiz@fatih.edu.tr

D. Çelikkale
Department of Food Engineering, Engineering Faculty,
Celal Bayar University,
45140 Muradiye, Manisa, Turkey

Introduction

In recent years, understanding of the positive effect of some minor components of foods on human health has encouraged the scientific research on this topic. A great interest has been on the Mediterranean populations who have longer life and more healthy living. This was due to the their diets as extra virgin olive oil which is more consumed by this populations as compared to other countries (Strandberg et al. 1990; Owen et al. 2000a).

Squalene is one of the minor constituents of vegetable oils and has a role on human health. Epidemiological studies have shown that it can effectively inhibit chemically induced colon, lung and skin tumourigenesis in rodents (Smith et al. 2000). It has been also used in several cosmetic applications as a solute component in fats because it is absorbed easily by skin (Üstündağ and Temelli 2004). It was reported that the decreasing risk for various cancers and reducing serum cholesterol levels has been ascribed to the squalene in vegetable oils (He et al. 2003). Several studies have been carried out to obtain squalene hydrocarbon from vegetable sources or marine animals by using different methodologies (Vazquez et al. 2007). Squalene has been sold in the markets as capsules for the beneficial effect on human health recently.

Crude vegetable oils contain squalene in the minor components which are generally constitute 1–3% of oil. One of the most important differences between the olive oil and other vegetable oils is the amount of squalene present in the oil. It's concentration in olive oil varies between 0.2 and 0.7%, whereas in other edible vegetable oils it constitutes only 0.002–0.03% (Rao et al. 1998). On the other hand, the techniques of olive growing (Psomiadou and Tsimidou 1999), oil extraction methods (Nergiz and Ünal 1990), Olive fruit variety (Draman and Hışıl 2005),

refining process (Owen et al. 2000a) and adulteration of virgin olive oil with seed oils affect squalene content of the oils. The amount of squalene in virgin olive oil has also been considered as an indicator for adulteration.

Despite variable the squalene content found in several vegetable oils, there is no detailed investigation on the factors affecting the amount of squalene in vegetable oils. Among the factors, refining process is important, since all the crude vegetable oils cannot be consumed without refining, except good quality virgin olive oil. The purpose of this study is to investigate the changes in the amount of squalene in different vegetable oils during refining process.

Materials and methods

Materials

Oil samples were collected from four different commercial refineries representing for five different vegetable oils as two replicates. For the analysis, about 150 mL of oil samples were taken from each refining steps from selected refineries. The collected oil samples of olive (10), sunflower seed (10) and rapeseed (10) were refined by conventional method. Soybean (8) and corn oil (8) samples were refined by physical method. Their processing conditions were usually the same as encountered in industry.

Reagents Squalene standard (purity≥97%) was purchased from Fluka (Switzerland), Chloroform, diethylether and hexane (A.C.S. grade) were obtained from Merck (Darmstadt, Germany). TLC plates (20×20 cm), pre coated (0.2 mm) with silica gel 60 F_{254} were obtained from Fluka (Switzerland), The other reagents were analytical grade.

Equipment A gas chromatograph apparatus (Agilent 6890N Series Network GC System) was used with a flame - ionization detector and a capillary column (HP-5.30 m long × 0.32 i.d.) coated with a 0.25 μm film thickness of liquid phase (5% phenyl) methylpolysilioxane (Agilent Technology, USA).

Methods

Extraction of the unsaponifiable fraction of the oil samples were conducted IUPAC Method 2.401 (1987). Five gram of the oil sample was weighed into a flask and 50 mL 1 N ethanolic potassium hydroxide solution was added. The mixture was saponified for 1 h under reflux condenser. After the saponification, 100 mL distilled water was added. The solution was poured into the a 500 mL separating funnel and extracted with 50 mL portions of diethyl ether. The ethereal extracts were combined into the another decanting funnel and were washed several times with 100 mL portions of distilled water until the wash-water gave neutral pH. The ether solution was dried over anhydrous sodium sulfate and evaporated in a rotary evaporator under vacuum. The residue was dissolved in 1 mL chloroform and applied to the TLC plates, it was developed with hexane -diethyl ether mixture (80:20, *v*/*v*) in a developing tank. After drying bands were marked by viewing under UV light at 254 nm. The spot of squalene with same Rf value of the authentic squalene standard was scraped off and dissolved with diethyl ether and filtered. The ethereal solution was evaporated on a water bath by passing through the nitrogen gas and dissolved in a certain mL of hexane. The amount of squalene was determined gas chromatographically and calculated using a calibration curve of peak heights versus amount of injected squalene standards. The chromatographic conditions were: initial oven temperature 180 °C/min then programmed at 8 °C/min to 270 °C. Injector temperature: 290 °C, detector temperature: 300 °C. Split ratio was 1:50 using hydrogen as carrier gas with a flow of 1.0 mL/min. One μL sample was injected by automatic injector (Agilent 7683 ALS series automatic liquid sampler). Variance analysis (ANOVA) was used statistical evaluation of the results by using a package programme SAS

Table 1 Changes in squalene content (mg/100 g) of vegetable oils during refining steps[a]

Refining Steps	Olive oil (n=10)	Sunflowerseed oil (n=10)	Rapeseed oil (n=10)	Corn oil (n=8)	Soybean oil (n=8)
Crude oil	491.0a±15.55	13.8a±0.39	26.2a±0.08	24.7a±0.40	18.1a±0.11
Neutralization/Physic. Refining	427.0a±9.89 (13.0)	12.8b±0.36 (6.9)	25.7b±0.11 (1.7)	23.0b±0.31 (7.3)	15.6b±0.11 (13.5)
Bleaching	392.5b±7.77 (7.0)	12.1b±0.25 (5.3)	24.2c±0.10 (5.5)	20.4c±0.29 (11.9)	13.3c±0.06 (13.0)
Deodorization	315.5c±6.36 (15.6)	9.9c±0.32 (16.9)	22.0d±0.08 (3.2)	–	–
Winterization	290.0d±9.89 (5.2)	9.2d±0.30 (4.0)	21.1e±0.06 (8.7)	25.9b±0.27 (6.8)	12.5d±0.08 (4.4)

The values given in parenthesis are the % reduction of squalene during each of refining steps

a–d Values with same letters within each column are not significantly different (p>0.05)

[a] Data are mean values of duplicate analysis ± standard deviation

(Statistical Analysis System) and Duncan's multiple range test (Anon 2001).

Results and discussion

The amounts of squalene in five different crude oil samples and after each refining steps were given Table 1. Squalene content of crude oil samples ranged from 13.8±0.39 mg/100 g to 491.0±15.55 mg/100 g as average. These results are in agreement with the values reported in the literature related with squalene content of olive and seed oils (Gutfinger and Letan 1974; Kiritsakis et al. 1998; Owen et al. 2000b).

Table 1 shows that the average squalene content of the crude olive oil samples found to be 491.0±15.55 mg/100 g and decreased for all the refining steps and the largest reduction has occurred during the deodorization. This was followed by the neutralization step. The least reduction in squalene was determined during the vinterization. Squalene reductions occurred during refining in olive oil samples were very close. Total reductions during all the stages of refining was found to be 40.94% as compared to crude olive oil samples. A significant difference was found among the refining steps in terms of squalene reductions statistically ($p \leq 0.05$), which is in agreement with values reported earlier by Vazquez et al. (2007). They also reported that deodorization distillate is quite rich in squalene content and can be used as source of 'vegetal squalene'. It was recovered from deodorization distillate in the grade of 93% and 91% purity by supercritical fluid extraction method (Vazquez et al. 2007).

The amount of squalene in sunflower seed oil was found to be quite low level as compared to olive oil (Table 1). Total average reduction for sunflower seed oil samples during refining process was 32.9%. Table 1 shows that the most reduction has occurred during deodorization. Statistical evaluation has been made between the average values pertaining to sunflower seed oil samples and a significant difference was found among the refining steps in squalene reductions ($P<0.05$).

Rapeseed oil samples showed similar results as shown by olive and sunflower seed oil samples in squalene reductions during refining process. Total decrease in the amount of squalene belonging to rapeseed oil samples was found to be 19.10% averagely (Table 1). The largest reduction has also been found to occur during the deodorization step. A significant difference was found among the refining steps in terms of squalene reductions statistically ($P<0.05$). In the physical refining process the amount of squalene content of corn oil was reduced marginally (Table 1). Total lowering of squalene was 25.90% in corn oil during refining. In contrast to other oils, the largest decrease was detected during bleaching step in corn oil. Differences among the refining steps of corn oil in terms of squalene content reduction were also found to be significant ($P<0.05$).

Crude soybean oil contained 18.1±0.11 mg/100 g squalene as average (Table 1). Similar values in soybean oil has been reported by Kiritsakis et al. (1998). Squalene reductions showed differences during physical refining of soybean oil. In contrast to corn oil, the highest decrease in amount of squalene has been found to occur during deacidification process. This differences may be due to both differences in nature of oils and refining conditions. Whereas it was the deodorization step that gave largest drop in squalene content for olive, sunflower seed and rapeseed oils during chemical refining. Shahidi and Wanasundara (1999), reported that the most losses of squalene (63%) occurred during the deodorization step for sea blubber oil refining. It was thought that high temperature applied during deodorization caused evaporation and degradation of squalene.

Conclusion

During the refining of five different types of vegetable oils the decrease in the squalene level varied from 19,1 to 40,9%. In olive oil which had a higher level of squalene, losses are relatively higher whereas in the seed oils that contain lower level of squalene, it was found that the losses are relatively lower. It has been determined that there is a considerable difference between natural and refined olive oil and seed oils in terms of squalene level; furthermore, olive oil, even it is refined, contains 25 to 30 times more squalene compared to seed oils. The oils, like olive, sunflower and rapeseed, processed by chemical refining, exhibited largest drop in squalene content during deodorization step. In consideration of the fact that only 60% of squalene that has been taken through a diet, could be absorbed in human body, it is believed that other types of vegetable oils could not be considered as a source of squalene but olive oil.

Acknowledgement This study was supported financially by the Scientific Research Fund of Celal Bayar University under project number Müh.2007/037.

References

Anon (2001) Statistical Analysis Systems (SAS/STAT) User Guide 8.02, SAS Institute Inc. Cary, NC, USA

Draman H, Hışıl Y (2005) Bazı önemli yerli ve yabancı zeytin çeşitlerinin cis-trans yağ asitleri kompozisyonu ve skualen düzeylerinin kapiler kolon gaz kromatografisi ile incelenmesi

üzerine bir çalışma. IV. GAP Tarım Kongresi, 21-23-Eylül 2005, Şanlıurfa. Bildiriler Kitabı, sayfa 538–546

Gutfinger T, Letan A (1974) Studies of unsaponifiables in several vegetable oils. Lipids 9:658–663

He HP, Cai JG, Corke H (2003) Supercritical carbon dioxide extraction of oil and squalene from Amaranthus grain. J Agr Food Chem 51:7921–7925

IUPAC (1987) Standard methods for the analysis of oils, fats and derivatives, method number 2.401 determination of the unsaponifiable matter-methods using diethyl ether extraction. Blackwell Scientific, Boston

Kiritsakis AP, Lenart EB, Willet VC, Hernandez MD (1998) Olive oil from the tree to the table, 2nd edn. Food and Nutrition Press, p. 121

Nergiz C, Ünal K (1990) The effect of extraction systems on triterpene alcohols and squalene content of virgin olive oil. Grasas Aceites 41:117–121

Owen RV, Giacosa A, Hull EW, Haubner R, Wurtele G, Spiegelhalder B, Bartsch H (2000a) Olive oil consumption and health: the possible role of antioxidants. Lancet Oncol 1:107–112

Owen RV, Mier W, Giacosa A, Hull EW, Spiegelhalder B, Bartsch H (2000b) Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem Toxicol 38:647–659

Psomiadou E, Tsimidou M (1999) On the role of squalene in olive oil stability. J Agr Food Chem 47:4025–4032

Rao CV, Newmark HL, Reddy BS (1998) Chemo-preventive effect of squalene on colon cancer. Carcinogenesis 19:287–290

Shahidi F, Wanasundara UN (1999) Effect of processing and squalene on composition and oxidative stability of seal blubber oil. J Food Lipids 6:159–172

Smith D, Espino-Montoro A, Perez-Jimenez F, Pedro-Botet J, Smith D, Espino-Montoro A, Perez-Jimenez F, Pedro-Botet J, Jimenez Pereperez J, Ordovas JM (2000) Effect of a high saturated fat and cholesterol diet supplemented with squalene or β-sitosterol on lipoprotein in fib hamsters. Nutr Res 20:1309–1318

Strandberg TE, Silvis RS, Miettinen TA (1990) Metabolic variables of cholesterol during squalene feeding in humans: comparision with cholestyramine treatment. J Lipid Res 31: 1637–1643

Üstündağ Ö, Temelli F (2004) Correlating the solubility behavior of minor lipid components in supercritical carbon dioxide. J Supercrit Fluids 31:235–253

Vazquez L, Torres CF, Fornari T, Senorans FJ, Reglero G (2007) Recovery of squalene from vegetable oil sources using counter-current supercritical carbon dioxide extraction. J Supercrit Fluids 40:59–66