Influence of industrial refining on some characteristics of olive pomace oil

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The scope of this study was to determine the changes in fatty acid composition, sterol distribution; as well as volatiles and 3-MCPD content of olive pomace oils throughout refining process. For this purpose, olive pomace oils were provided from a chemical refining plant at each refining step and evaluated for their fatty acid composition, individual sterol concentrations, volatiles and 3-MCPD ester content. The results have shown that the main fatty acid in crude oil was oleic acid (70.71-71.45%) followed by linoleic (11.6-11.72%) and palmitic acid (11.55-11.88%). During refining fatty acid composition remained mainly unchanged, however, small but statistically significant changes have been observed for stearic, linoleic and arachidic acids. β-sitosterol was dominant sterol in the crude oil covering 83.58-86.55% of the total sterols (5170 mg/kg) and Δ-5-avenasterol (270 mg/kg) and campesterol (160 mg/kg) were determined in considerable amounts. Total sterol content decreased throughout the process, for more than 50%. The content of campestanol lowered by 95%, the content of stigmasterol, sitosterol and Δ-5-avenasterol by more than 60%, and campesterol, clerosterol and β-sitosterol by more than 50%. Total volatile contents decreased gradually, with a sharp reduction at the deodorisation step up to 97%. 2.84 mg/kg 3-MCPD ester was determined in crude oil. Their content decreased for about 40% up to the end of bleaching, however a moderate increase was observed at the end of the deodorisation step.

Keywords: 3-MCPD ester, fatty acid, olive pomace oil, refining, sterol, volatile compounds.

1. INTRODUCTION

Olive pomace oil is an important olive oil processing technology by-product and has economic importance for olive oil producing countries. Olive pomace oil is obtained by subjecting olive pomace with organic solvents and needs to be refined for further use due to its intense colour, wax and free fatty acid content. Crude vegetable oils are generally refined before consumption to remove impurities (e.g. free fatty acids, phosphatides, oxidation products, colour pigments, odorants, and waxes), to provide oxidative stability and to attain an end-product with desirable organoleptic properties. The typical refining method consists of several steps: degumming, neutralisation, bleaching and deodorisation. Several changes take place in each step and have prominent effects on the composition and content of the minor compounds because of both high processing temperature and chemicals used. Phospholipids are largely removed during degumming; free fatty acids are reduced during neutralisation; peroxides, chlorophyll and carotenoids are separated during the bleaching step; and, lastly, volatiles are removed during deodorisation [1]. It has been reported that the amount of tocopherols decrease at each refining step and significant reductions were observed during deodorisation [2].

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Fatty acid composition is known to remain unchanged during the refining treatment [3], however *cis* isomers are transformed into *trans* forms at the deodorisation stage [4, 5]. Phytosterols, known as major components of the unsaponifiable fraction of oils, are used as authenticity indicators due to their uniqueness for each oil type. The amounts of sterols mainly reduce during deodorisation due to the high distillation temperature and high vacuum pressure. Slight reductions were also reported for the amount of sterols attained at the neutralisation and bleaching steps [6].

Considerable numbers of reports have been published on the influence of refining on minor components of various types of oils. However, there is little information available on the influence of refining steps on the minor constituents of olive pomace oil, namely triglyceride oligopolymers and polar compounds [7]. 3-monochloropropane-1,2-diol (3-MCPD) is a process contaminant found in various foods either in the free or bound form. It is formed at elevated temperatures with the existence of chloride ions. However, the toxicity of the compound is still under evaluation, based on the current knowledge. The International Agency for Research on Cancer (IARC) classified 3-MCPD as a probable human carcinogen (group 2B) [8]. Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EC Scientific Committee on Food established a tolerable daily intake (TDI) of 2 µg/kg body weight [9]. Refined oils are possible sources of these contaminants because of the high temperatures used in the process especially in the deodorisation step. The current study investigates the 3-MCPD (3-monochloropropane-1, 2-diol) ester content of olive pomace oils at different refining steps. Likewise, to the best of the author's knowledge, there has been no study that deals with the changes in individual volatiles during the refining process. Hence, the current study examines the effects of refining steps on volatiles, fatty acid and sterol distribution as well as the 3-MCPD (3-monochloropropane-1, 2-diol) ester content of olive pomace oils.

2. EXPERIMENTAL

MATERIAL

Olive pomace oil samples were obtained from the chemical refining processing lines of Orpir Olive Cake and Oil Industry Factories (Balikesir, Turkey). Oil samples were taken from each refining step: neutralisation, bleaching and deodorisation.

REAGENTS AND STANDARDS

Toluene, tert-butyl methyl ether, methanol, ethyl acetate, diethyl ether, isooctane, sodium methoxide, sodium chloride, sodium bromide, sodium sulphate, phenyl boronic acid and d5-3-MCPD-1,2-bis-palmitoyl ester, silica gel, 5α-cholestan-3β-ol were obtained from Sigma (St-Louis, USA); hexane, β-sitosterol, pyridine, chloroform were purchased from Merck (Darmstadt, Germany), N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMSC) and 37 fatty acid methyl ester mix were bought from Supelco (Bellefonte, USA).

REFINING PROCEDURE

The crude olive pomace oil obtained using solvent extraction was subjected to chemical refining in industrial scale as follows: Acid degumming was applied at 8-12°C, phosphoric acid (85%) was added at 0.05% level as degumming agent and the mixture was stirred for 5-10 minutes. Afterwards, the oil was mixed with 20°Be' NaOH for 20 minutes. In bleaching step, neutralised oil was heated to 90-100°C and 2% of bleaching earth was added. The mixture was heated for 30-45 min and filtered. Finally, the oil was deodorised at deodorizer at 180-200°C under 1-3 mbar vacuum. All samples were kept in 250 mL bottles in dark ambiences at -18°C in nitrogen atmosphere until analysis.

ANALYSES

Fatty acid composition

The methyl esters of fatty acids were prepared according to International Union of Pure and Applied Chemistry [10] and analysed using a gas chromatograph GC 2010 (Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector (T = 240°C) and split/splitless injector (T = 290°C). DB-23 fused silica capillary column (60 m × 0.25mm i.d. and 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used to separate individual fatty acids. Oven temperature was set to 195°C. Carrier gas was nitrogen with a flow rate of 1 ml/min and split ratio was 80:1. Peaks were identified by comparing their retention times with Supelco (Bellefonte, USA) 37 fatty acid methyl ester mix. Analyses were repeated twice.

Sterol composition

Sterol composition was determined according to AOCS Official Method Ch 6-91 [11]. Sterol derivatives were analysed by a gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split/splitless injector (T = 280°C) and flame ionisation detector (T = 290°C). HP-5 fused silica capillary column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) (Chrom Tech., Apple Walley, MN, USA) was employed for chromatographic elution. 5α-cholestan-3β-ol was used as internal standard for quantification. Carrier gas was nitrogen

with a flow rate of 0.8 ml/min and the split ratio was 50:1. Each sample was analysed at least twice.

Volatile compound analysis

The volatile compound profile of oils was determined using static headspace gas chromatography-mass spectrometry (SHSGC-MS). The headspace of samples was analysed by Agilent 7697A model (Agilent Technologies, Santa Clara, CA, USA) headspace sampler. The headspace was associated with 7820A model (Agilent Technologies, Santa Clara, CA, USA) gas chromatograph and coupled to a mass selective detector of Agilent 5975 model (Agilent Technologies, Santa Clara, CA, USA). A 10 ml of sample was introduced in a 20-ml vial and sealed with poly (tetrafluoroethylene) (PTFE)/silicone septum. The extraction settings were as follows: Oven temperature, 120°C; loop temperature, 130°C; transfer line temperature, 140°C; vial equilibration time, 120 min. The injection loop (volume = 1 ml) was filled by depressurising the headspace and loop was swept with Helium (loop equilibration, 0.05 min; sample injection, 0.5 min). The chromatographic separation was achieved by HP-5MS column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) (Chrom Tech., Apple Walley, MN, USA) with the following temperature program: 40°C, held for 5 min; 2°C/min up to 110°C, held for 1 min; 5°C/min up to 150°C, held for 1 min; 10°C/min up to 250°C, held for 20 min. Total running time was 80 min and the carrier Helium flow rate was 1 ml/min. The injection port, MS source and MS quadrupole temperatures were set to 160°C, 230°C and 150°C respectively. The volatile compounds were identified by comparing their mass spectra to those of the Wiley library. Analyses were repeated at least twice.

Analysis of 3-MCPD esters

The concentrations of 3-MCPD esters were determined according to the DGF Standard Method C-VI 18 [12]. Each sample was analysed at least twice.

Statistical analysis

A statistical analysis was performed using SPSS 15 statistical software (SPSS Inc., Chicago, USA). The data were evaluated by one-way ANOVA procedure using the Duncan's multiple range test to determine if there were any significant differences between refining steps. A *p* value of less than 0.05 was considered as significant.

3. RESULTS AND DISCUSSION

Fatty acid composition of olive pomace oils was given in Table I. The main fatty acid was oleic acid ranging in 70.71-71.45% of total acids. The linoleic and palmitic acids were the second predominant fatty acids with-about 11.60-11.72% and 11.55-11.88% respectively in total acids, followed by stearic acid (2.74-2.81%). Additionally, myristic (C 14:0), pentadecanoic acid (C 15:0), pentadecenoic acid (C 15:1), palmitoleic (C16:1), linolenic (C18:3), arachidic (C20:0), myristic (C14:0), heptadecanoic (C17:0), heptadecenoic (C17:1) and gadoleic (C20:1) acids were determined in smaller ratios, lower than 1%. The fatty acid composition of the oil samples covered the legal limits established by national and international regulations determined for olive pomace oils. Table II shows the changes in the fatty acid composition during refining in comparison with crude olive pomace oils. The results have shown that some of the acids were unchanged, while palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic and gadoleic acid ratios underwent little changes throughout the process. However, the changes in stearic, linolenic and arachidic acids, although small, were statistically significant. These findings are like those obtained for other types of oils [3, 13].

The sterol contents of oils at each step of refining are given in Table I. The total sterol contents differed among 5169.67 to 2148.07 mg/kg and reduction ratio was 55.70% between the first and last steps of refining. The major sterol was β-sitosterol ranging in 1795.53-4474.70 mg/kg comprising 83.58-86.55% of total sterols. Δ-5-avenasterol was the second important phytosterol (102.61-270.03 mg/kg) for olive pomace oil covering 4.48-5.22% of all sterols. Campesterol was the third outstanding phytosterol (72.79-160.01 mg/kg) forming 3.09-3.38% of the total sterols. Remaining sterols; brassicasterol, 24-methylene-cholesterol, campestanol, stigmasterol, Δ-7-campesterol, clerosterol, sitostanol, Δ-5-24-stigmastadienol, Δ-7-stigmastenol and Δ-7-avenasterol covered less than 8% of the sum of the sterols. Individual sterol content and composition were mainly comparable to previous works determined for olive pomace [14] oils. Total sterol content and individual sterol ratios were generally within the limits established for vegetable oils, set by the Turkish Food Codex [15]. The changes in sterol contents throughout refining are given in Table II. The refining process mainly caused significant losses nearly for all individual sterols; except for campesterol, Δ-7-campesterol and Δ-7-stigmastadienol that exhibited statistically insignificant changes. The highest losses (> 60%) were observed at campestanol, sitostanol, stigmasterol, clerosterol and Δ-5-avenasterol levels. The main reductions were determined at the neutralisation step for 24-methylene-cholesterol, Δ-5-24-stigmastadienol and Δ-7-avenasterol; at bleaching step for brassicasterol, campesterol, stigmasterol, clerosterol and β-sitosterol; deodorisation

Table I - Fatty acid, sterol, volatile and 3-MCPD ester content of olive pomace oils at each step of refining

	Crude	Neutralized	Bleached	Deodorized
Fatty acids (% in total acids)				
C 14:0	0.02[a]	0.02[a]	0.02[a]	0.02[a]
C 15:0	0.01[a]	0.01[a]	0.01[a]	0.01[a]
C 15:1	0.01[a]	0.01[a]	-	-
C 16:0	11.55[a]	11.72[a]	11.74[a]	11.88[a]
C 16:1	0.64[a]	0.66[a]	0.69[a]	0.61[a]
C 17:0	0.12[a]	0.12[a]	0.12[a]	0.13[a]
C 17:1	0.19[a]	0.19[a]	0.19[a]	0.19[a]
C 18:0	2.81[a]	2.74[b]	2.74[b]	2.77[ab]
C 18:1	70.71[a]	71.29[a]	71.21[a]	71.45[a]
C18:2	11.60[a]	11.72[a]	11.67[a]	11.69[a]
C 18:3	0.03[ab]	0.05[ab]	0.06[a]	0.01[b]
C 20:0	0.62[a]	0.61[a]	0.62[a]	0.55[b]
C 20:1	0.50[a]	0.47[a]	0.44[a]	0.47[a]
Sterols (mg/kg oil)				
Brassicasterol	5.51[a]	3.62[ab]	2.36[b]	4.30[ab]
24-methylene-cholesterol	24.06[a]	15.95[b]	20.81[ab]	17.37[b]
Campesterol	160.01[a]	97.99[b]	72.79[c]	74.07[bc]
Campestanol	6.90[a]	3.58[a]	1.85[a]	0.38[a]
Stigmasterol	78.49[a]	30.61[b]	22.09[c]	26.24[bc]
Δ-7-campesterol	5.73[a]	5.07[a]	8.17[a]	4.85[a]
Clerosterol	30.64[a]	16.44[b]	11.60[b]	14.40[b]
β-sitosterol	4474.70[a]	2511.89[b]	1795.53[c]	1980.28[c]
Sitostanol	55.16[a]	43.02[b]	38.73[bc]	17.41[c]
Δ-5-avenasterol	270.03[a]	156.44[b]	123.38[c]	102.61[c]
Δ-5,24-stigmastadienol	27.89[a]	19.50[d]	21.27[c]	23.32[b]
Δ-7-stigmastenol	13.52[a]	10.41[a]	11.87[a]	10.06[a]
Δ-7-avenasterol	16.98[a]	12.89[b]	17.57[a]	14.85[ab]
Total Sterols	5169.67[a]	2927.47[b]	2148.07[c]	2290.22[c]
Volatiles (GC-MS-HS peak area $\times 10^{-6}$)				
Benzaldehyde	-	-	295.00	-
Bicyclo[2.2.1]heptane,2-methyl-,exo-	302.55[a]	191.28[ab]	106.65[ab]	34.86[b]
Dicyclopropylacetylene	5.35[a]	2.99[a]	-	-
3-heptyne	728.44[a]	486.70[a]	294.67[a]	-
7-oxabicyclo[4.1.0]heptane,2-methylene	308.68[a]	117.81[b]	112.51[b]	25.13[b]
Cycloheptene	2.54	-	-	-
1 H-Inden-1-one,2,3-dihydro	-	8.70[a]	5.83[a]	-
2-octyne	-	-	-	31.00
Acenaphytelene	1.65[a]	1.70[a]	1.08[a]	-
7,13-C-methylene-cyclohexane	1.82[a]	1.88[a]	1.57[a]	-
Trans- 5-octadecene	3.03[c]	5.24[a]	3.68[b]	-
Total volatiles	1349.17[a]	813.67[ab]	525.20[ab]	42.55[b]
3-MCPD ester (mg/kg oil)	2.84[a]	1.70[b]	1.51[b]	1.77[b]

Different superscript letters in the same row indicate significant differences ($p < 0.05$) between different steps of refining

step for campestanol, sitostanol, Δ-5-avenasterol and Δ-7-stigmastenol.

Static headspace extraction technique analysed oil pomace oils and volatile compounds were characterised by gas chromatography coupled with mass spectrometry. Peak areas of volatiles and the relative changes in peak areas were given in Table I and II, respectively. Total volatile compound areas decreased gradually, at bleaching 39.69%, neutralisation 61.07% and deodorisation 96.85%. Major volatile compounds determined in olive pomace oils were 3-heptyne; bicyclo [2.2.1] heptane,2-methyl-,exo-; and 7-oxabicyclo[4.1.0]heptane,2-methylene. 3-heptyne was predominant in crude oil and decreased by 33.19% at neutralisation, by 59.55% at bleaching and couldn't be detected at deodorised oil. Bicyclo[2.2.1]heptanes, 2-methyl-,exo-, underwent significant losses by 36.78, 64.75 and 88.48% throughout the process. 7-oxabicyclo [4.1.0] heptane,2-methylene were also found in significant amounts in oil samples and decreased about 61.83% at neutralisation, 63.55% at bleaching and 91.86 at deodorisation phases. Benzaldehyde was only detected in bleached oil; while 1 H-Inden-1-one,2,3-dihydro was determined at the neutralised phase and bleached oils, which may probably come up as a re-

Table II - Changes (%) in fatty acids, sterols, volatiles and 3-MCPD content of olive pomace oils throughout refining in comparison with crude olive pomace oil

	Neutralized	Bleached	Deodorized
Fatty acids (% in total acids)			
C 14:0	0.00	0.00	0.00
C 15:0	0.00	0.00	0.00
C 15:1	0.00	0.00	0.00
C 16:0	+1.47	+1.65	+2.86
C 16:1	+3.13	+7.81	-4.69
C 17:0	0.00	0.00	+8.33
C 17:1	0.00	0.00	0.00
C 18:0	-2.49	-2.49	-1.42
C 18:1	+0.82	+0.71	+1.05
C18:2	+1.03	+0.60	+0.78
C 18:3	+66.67	+100.00	-66.67
C 20:0	-1.61	0.00	-11.29
C 20:1	-6.00	-12.00	-6.00
Sterols (mg/kg oil)			
Brassicasterol	-34.30	-57.17	-21.96
24-methylene-cholesterol	-33.71	-13.51	-27.81
Campesterol	-38.76	-54.51	-53.71
Campestanol	-48.12	-73.19	-94.49
Stigmasterol	-61.00	-71.86	-66.57
Δ-7-campesterol	-11.52	+42.58	-15.36
Clerosterol	-46.34	-62.14	-53.00
β-sitosterol	-43.86	-59.87	-55.74
Sitostanol	-22.01	-29.79	-68.44
Δ-5-avenasterol	-42.07	-54.31	-62.00
Δ-5,24-stigmastadienol	-30.08	-23.74	-16.39
Δ-7-stigmastenol	-23.00	-12.20	-25.59
Δ-7-avenasterol	-24.09	+3.47	-12.54
Total Sterols	-43.37	-58.45	-55.70
Volatiles (GC-MS-HS peak area $x10^{-6}$)			
Benzaldehyde	-	-	-
Bicyclo[2.2.1]heptane,2-methyl-,exo-	-36.78	-64.75	-88.48
Dicyclopropylacetylene	-44.11	-100.00	-100.00
3-heptyne	-33.19	-59.55	-100.00
7-oxabicyclo[4.1.0]heptane,2-methylene	-61.83	-63.55	-91.86
Cycloheptene	-100.00	-100.00	-100.00
1 H-Inden-1-one,2,3-dihydro	-	-	-
2-octyne	-	-	-
Acenaphytelene	+3.03	-34.55	-100.00
7,13-C-methylene-cyclohexane	+3.30	-13.74	-100.00
Trans- 5-octadecene	+72.94	+21.45	-100.00
Total volatiles	-39.69	-61.07	-96.85
3-MCPD ester (mg/kg oil)	-40.14	-46.83	-37.68

sult of the refining process. Dicyclopropylacetylene, acenaphytelene, 7,13-C-methylene-cyclohexane and *trans*-5-octadecene were completely removed at the deodorisation step.

The 3-MCPD ester content in crude oil and its changes in during refining are given in Table I and II. The crude olive pomace oil contained 2.84 mg/kg 3-MCPD esters and refining led to a decrease in 3-MCPD ester till the end of bleaching, however deodorisation led to a slight increase in their concentration. Reduction ratios were 40.14, 46.83 and 37.68 at neutralisation, bleaching and deodorisation steps, respectively. Zelinková *et al.* [16] reported that the content of ester-bound 3-MCPD was within the range of 0.1 mg/kg and 2.46 mg/kg for various edible oils and refined olive pomace oil was one of the most abundant sources of ester-linked 3-MCPD among the oils analysed. In another study [17], in which 350 edible fat and fat containing products (native or cold pressed vegetable oils; refined vegetable oils; milk fat, lard, tallow, poultry fat; margarine; frying fat; sweet spreads; stuffing and topping of sweet cookies, crackers and bars) were analysed for 3-MCPD ester content; olive pomace oil was found be a rich source of 3-MCPD esters together with grapeseed, walnut and palm oils. Razak *et al.* [18] collected various vegetable oils from

the local markets in Malaysia and analysed for their 3-MCPD ester content. The results showed the highest levels in olive pomace oil, palm olein and blends of olein, peanut and sesame oils (1.6-2.45 mg/kg).

4. CONCLUSION

This work investigates the changes in individual fatty acids, sterols, volatiles and the 3-MCPD ester content of olive pomace oil throughout refining process. Deodorisation was noticed as being the critical step for volatile and sterol reduction, and for the 3-MCPD ester formation. The results presented herein, will form a basis for further works on the refining procedure of olive pomace oil.

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