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GC ANALYSIS OF CHANGES IN THE FATTY ACID COMPOSITION OF SUNFLOWER AND OLIVE OILS HEATED WITH QUERCETIN, CAFFEIC ACID, PROTOCATECHUIC ACID, AND BUTYLATED HYDROXYANISOLE ◈

R. Kowalski

Subdepartment of Food Quality Assessment and Central Apparatus Laboratory, Agricultural University, 20-950 Lublin, Akademicka 13, Poland

SUMMARY

The objective of this study was to determine the effect on fatty acid composition of heating olive and sunflower oils with selected natural and synthetic antioxidants. The antioxidants investigated were quercetin, caffeic acid, protocatechuic acid, and butylated hydroxyanisole (BHA), each at concentrations of 0.02, 0.04, and 0.06%. Oils with no added antioxidants were also heated. After heating of the samples at 90°C for 72 h and 120 h, then preliminary saponification of the fat and esterification of the acids, the fatty acids were determined as the methyl esters by gas chromatography; heptadecanoic acid was used as internal standard. The high temperatures had a negative effect on fatty acid composition. Olive oil was more resistant than sunflower oil to changes during heating. In general, the effectiveness of natural antioxidants (quercetin, caffeic acid, and protocatechuic acid) was no less than that of the synthetic antioxidant (BHA).

INTRODUCTION

Although the high level of unsaturation of fatty acids contained in oils of plant origin is appreciated by nutritionists, it causes severe technological problems, because of their greater susceptibility to oxidation. Oxidation of the fats degrades the organoleptic quality of food, reduces its nutritional value, and products of the oxidation processes can participate in the

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aging of an organism and in the aetiology of cardiovascular diseases and cancer [1,2]. Lipids can be protected against uncontrolled oxidation by addition of antioxidants with the ability to remove the free radicals and reactive oxygen species (ROS) that damage cellular and tissue structures [3].

There is currently much interest in natural antioxidants, including polyphenols, isolated from plants [4]. Because of their natural occurrence and consumption with plants, they are fully accepted by consumers, in contrast with synthetic antioxidants which may initiate disease [5,6]. Much research is therefore being conducted to find completely safe, naturally active substances that strongly inhibit degradation of fats.

Evaluation of the protection afforded to fats by antioxidants consists mainly in measurement of antioxidant activity under different experimental conditions, e.g. type of fat, temperature, pH, light, presence of enzymes or microorganisms, antioxidant concentration, aeration, presence of heavy metals, presence of ionizing radiation, etc. The course of lipid oxidation is monitored by measurement of the concentration of conjugated dienes, hexanal, or compounds reacting with thiobarbituric acid, mainly malonyl dialdehyde. The analytical methods most often used for evaluation of the efficiency of antioxidants are iodometry [7,8], the thiocyanate method [9], the thiobarbituric acid (TBA) method [10], or use of the automated Rancimat device [11]. None of these methods can be used to characterize in detail the quantitative changes occurring within the fatty acid glyceride mixture.

Gas chromatography is more convenient and precise method for qualitative and quantitative analysis of fatty acid methyl esters, and comparative chromatographic analysis of changes in the concentrations of fatty acid methyl esters released by saponification of fat previously subjected to oxidation with and without the presence of antioxidant can be used for indirect characterization of the efficiency of the antioxidants at inhibiting unfavourable processes within the fat. It is, therefore, plausible to use gas chromatography to evaluate the efficiency of antioxidants used in studies of inhibition of quantitative changes in the glyceride fraction of fatty acids.

The objective of this study was to use GC analysis of fatty acids to monitor changes in the composition of sunflower and olive oils heated with selected natural (quercetin, caffeic acid, and protocatechuic acid) and synthetic (butylated hydroxyanisole BHA) antioxidants.

EXPERIMENTAL

Chemicals and Reagents

Sunflower oil and olive pomace were purchased from a Lublin supermarket. Quercetin, caffeic acid, protocatechuic acid, butylated hydroxyanisole (BHA), and heptadecanoic acid were from Sigma.

Methods

Samples (1 g, in triplicate) of the fats were placed in tubes with addition of sufficient volumes of solutions of the antioxidants in ethanol (2 mg mL^{-1}) to achieve antioxidant concentrations of 0.02, 0.04, and 0.06% (Table I); these concentrations were selected on the basis of literature data [12]. Samples were stirred to produce an emulsion and then left for 24 h at ambient temperature for evaporation of the alcohol. Another sample of the oil (1 g) was then added to each tube, giving a total mass of 2 g and the mixtures were stirred again then thermostatted at 90°C protected from light. Samples (approx. 50 mg) were taken for chromatographic analysis after 72 and 120 h.

Table I

Designation of the samples

No.	Sample	Designation
1	Raw oil	0
2	Oil with added quercetin (0.02%)	Q 0.02
3	Oil with added quercetin (0.04%)	Q 0.04
4	Oil with added quercetin (0.06%)	Q 0.06
5	Oil with added caffeic acid (0.02%)	C 0.02
6	Oil with added caffeic acid (0.04%)	C 0.04
7	Oil with added caffeic acid (0.06%)	C 0.06
8	Oil with added protocatechuic acid (0.02%)	P 0.02
9	Oil with added protocatechuic acid (0.04%)	P 0.04
10	Oil with added protocatechuic acid (0.06%)	P 0.06
11	Oil with added BHA (0.02%)	B 0.02
12	Oil with added BHA (0.04%)	B 0.04
13	Oil with added BHA (0.06%)	B 0.06
14	Oil with no added antioxidant (control)	K

A solution of heptadecanoic acid in hexane (10 mg mL^{-1}, 300 μL) was added to weighed oil samples (approx. 50 mg) and the fats were sapo-

nified and the fatty acids esterified (with a 14% solution of BF_3 in methanol) in accordance with AOAC methods [13,14].

Gas Chromatography

Chromatography was performed with Unicam 610 Series gas chromatograph equipped with a flame-ionization detector and a 60 m × 0.25 mm i.d. column coated with a 0.25 μm film of HP-23. Split injection (split ratio 1:50) was performed, with hydrogen as carrier gas at a flow rate of 43 m s^{-1}. The column temperature was maintained at 160°C for 1 min after injection then programmed at 2.75° min^{-1} to 215°C, which was held for 2 min, and then at 40° min^{-1} to 230°C, which was held for 2 min. The injection port and detector temperatures were 270°C. Calculations were based on previous analysis of standard mixtures and calculation of individual correction coefficients.

The activity of the antioxidants in the experimental systems was determined on the basis of calculated inhibition $I_{h\Sigma}$ of quantitative changes of total fatty acids:

$$I_{h\Sigma} = \left(\frac{C_{aox\Sigma}}{C_{k\Sigma}} \times 100\% \right) - 100\%$$

where $I_{h\Sigma}$ is the inhibition of quantitative changes relative to the total amount of fatty acids, $C_{aox\Sigma}$ is the total concentration of fatty acids (%) in the sample tested with added antioxidant, and $C_{k\Sigma}$ is the total concentration of fatty acids (%) in the control with no added antioxidant.

RESULTS AND DISCUSSION

Experiments revealed the oils tested contained large amounts of unsaturated fatty acids. The most abundant was the mono-unsaturated oleic acid (C18:1), approximately 55% in olive oil and the bi-unsaturated linoleic acid (C18:2), approximately 47% in sunflower oil (Tables II and III). This means the fats tested are significant sources of unsaturated fatty acids.

These results indicate that long-term high-temperature treatment had an adverse effect on the quantitative fatty acid composition of the oils; this has a direct implication on use of these fats for cooking. A decrease in fatty acid concentrations was observed when the fats were heated at 90°C (Tables II and III), with the fatty acid content decreasing in proportion to heating time. The decreases for olive oil and sunflower oil, respectively,

Table II

Changes of the fatty acid (FA) content of olive oil during heating at 90°C

Fatty acid	Fatty acid content ± *SD* (g per 100g)		
	After heating for 0 h	After heating for 72 h	After heating for 120 h
C16:0[a]	12.14 ± 0.35	11.77 ± 0.30	11.01 ± 0.30
C16:1	1.42 ± 0.04	1.21 ± 0.05	1.03 ± 0.05
C18:0	2.17 ± 0.05	1.75 ± 0.06	1.80 ± 0.04
C18:1	55.13 ± 2.50	50.14 ± 2.25	44.83 ± 2.00
C18:2	13.85 ± 0.60	8.71 ± 0.40	7.37 ± 0.35
α-C18:3	0.64 ± 0.03	–	–
C20:0	0.34 ± 0.02	0.23 ± 0.01	0.20 ± 0.01
C20:2	0.70 ± 0.04	1.10 ± 0.05	2.13 ± 0.06
Σ	86.39	74.91	68.37

[a] C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; α-C18:3, α-linolenic acid; C20:0, arachidonic acid; C20:2, eicosadienoic acid

Table III

Changes of the fatty acid (FA) content of sunflower oil during heating at 90°C

Fatty acid	Fatty acid content ± *SD* (g per 100g)		
	After heating for 0 h	After heating for 72 h	After heating for 120 h
C16:0[a]	5.35 ± 0.11	5.84 ± 0.12	4.99 ± 0.10
C18:0	3.41 ± 0.08	2.94 ± 0.03	3.39 ± 0.03
C18:1	19.58 ± 0.39	17.32 ± 0.35	16.12 ± 0.32
C18:2	46.87 ± 1.17	34.80 ± 0.87	31.08 ± 0.78
C20:0	0.70 ± 0.01	0.30 ± 0.01	0.26 ± 0.00
C20:2	0.06 ± 0.00	0.55 ± 0.01	0.17 ± 0.00
Σ	75.97	61.75	56.01

[a] C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C20:0, arachidonic acid; C20:2, eicosadienoic acid

were approximately 11% and 15% after heating for 72 h and approximately 18% and 20% after 120 h (relative to the initial concentrations). It seems the lower loss of total fatty acids from olive oil compared with sunflower oil was because of its lower content of linoleic acid, which is readily oxidized, and its higher amount of oleic acid, which is less readily oxidized [15,16]. It thus follows that olive oil, with its large mono-unsaturated fatty

acid content is slightly better for cooking than sunflower oil, which is abundant in bi-unsaturated fatty acids.

Comparison of results for samples with and without added antioxidants revealed the antioxidants had a protective effect on the fatty acid content of the oils tested (Figs 1 and 2). Natural antioxidants were, furthermore, no less effective, in general, than the synthetic antioxidant BHA and, under favourable conditions (type of antioxidant, antioxidant concentration, type of fat), sometimes resulted in better inhibition of fatty acid degradation (Table IV). Zia-ur-Rehman et al. [6] and Jarosławska et al. [17,18] have also reported the comparable performance of natural and synthetic antioxidants in experiments with sunflower oil.

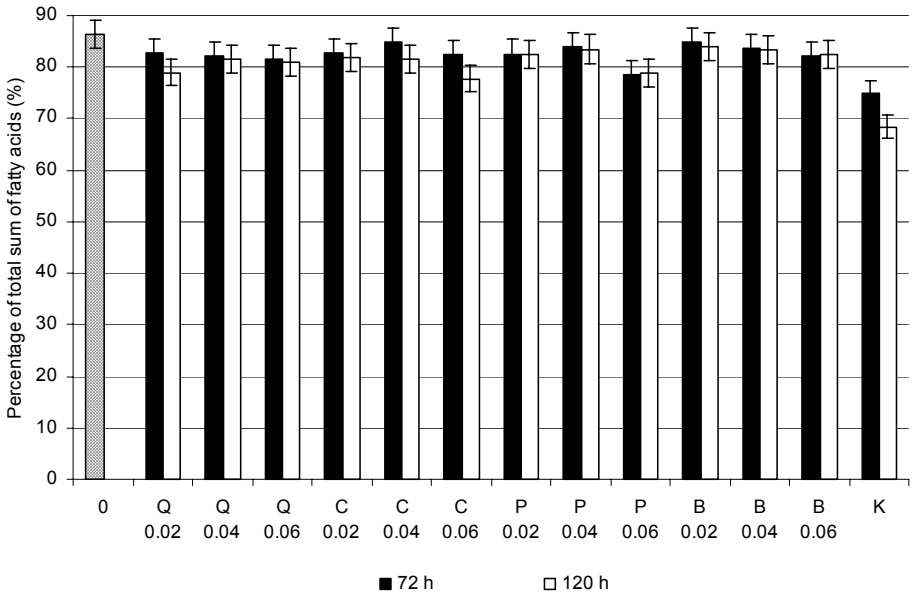

Fig. 1

Fatty acid content of olive oil heated at 90°C (designations as in Table I)

It is, however, impossible to judge unequivocally which natural antioxidant has the best protective properties and at which concentration it should be used. It is possible they interact synergistically with other active substances naturally present in the oils and may thus both act as inhibitors of oxidation and as pro-oxidants catalyzing oxidation in the bulk oil [19, 20]. This effect was observed in this work for 0.02% and 0.04% quercetin

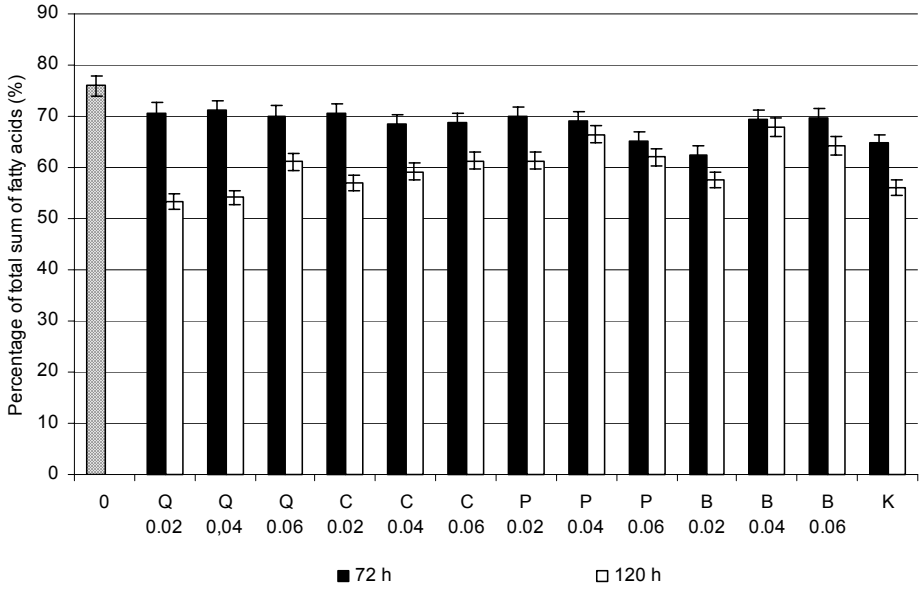

Fig. 2

Fatty acid content of sunflower oil heated at 90°C (designations as in Table I)

Table IV

Effectiveness of quercetin, caffeic acid, protocatechuic acid, and BHA at inhibition of quantitative changes of the total fatty acid content of olive and sunflower oils

Oil	Anti-oxidant	Inhibition $I_{h\Sigma}$ (%)					
		After heating for 72 h			After heating for 120 h		
		0.02[a]	0.04	0.06	0.02	0.04	0.06
Olive	Q	10.42 ± 0.34	9.54 ± 0.31	8.93 ± 0.27	15.50 ± 0.53	19.25 ± 0.59	18.57 ± 0.61
	C	10.44 ± 0.21	13.31 ± 0.47	10.06 ± 0.33	19.70 ± 0.65	19.42 ± 0.63	13.66 ± 0.45
	P	10.29 ± 0.31	12.2 ± 0.40	4.92 ± 0.16	20.43 ± 0.71	22.16 ± 0.73	15.36 ± 0.54
	B	13.23 ± 0.45	11.77 ± 0.36	9.74 ± 0.31	22.93 ± 0.74	22.10 ± 0.64	20.81±0.69
Sunflower	Q	9.20 ± 0.17	9.92 ± 0.04	8.32 ± 0.14	−4.77 ± 0.08	−3.36 ± 0.07	9.21 ± 0.16
	C	8.90 ± 0.15	5.56 ± 0.09	6.02 ± 0.11	1.91 ± 0.05	5.68 ± 0.12	9.34 ± 0.11
	P	7.95 ± 0.11	6.64 ± 0.12	0.73 ± 0.01	9.39 ± 0.16	18.75 ± 0.32	10.80 ± 0.16
	B	−3.37 ± 0.05	7.04 ± 0.17	7.46 ± 0.14	2.91 ± 0.06	21.21 ± 0.45	14.77 ± 0.25

[a] Concentration (%) of the antioxidant in the oil

in sunflower oil heated for 120 h and for 0.02% BHA in the same oil heated for 72 h (Table IV). Selection of the optimum antioxidant for a particular

fat is therefore recommended. This will involve performing detailed comparative tests with a wide range of concentrations of chemically different groups of antioxidants [21].

Gas chromatography is, without doubt, a useful analytical technique for studying the effect of different conditions on the fatty acid composition of fats.

REFERENCES

[1] A. Ascherio, Am. J. Med., **113**, 9 (1998)
[2] L.E. Johnson and W.M. Cort, Beverage, **148**, 10 (1985)
[3] G. Bartosz, The second face of oxygen. PWN, Warszawa (1995)
[4] C.A. Rice-Evans, N.J. Miller, and G. Paganga, Trends Plant Sci., **2**, 152 (1997)
[5] S.M. Barlow: Toxicological Aspects of Antioxidants used as Food Additives. In: Food Antioxidants. Elsevier, New York, 171 (1990)
[6] Zia-ur-Rehman, A. Salaria, and F. Habit, J. Sci. Food Agric., **83**, 624 (2003)
[7] N.V. Yanishlieva, A. Popov, and E.M. Marinova, Compt. Rend. Acad. Bulg. Sci., **31**, 869 (1978)
[8] AOCS, Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th edn, Methods CD 8-53 and Cd 1890. American Oil Chemists' Society, Champaign (1990)
[9] T. Osawa and M. Namiki, Agric. Biol. Chem., **45**, 739 (1981)
[10] A.M. Salih, D.M.Smith, J.F. Price, and L.E. Dawson, Poultry Sci., **66**, 1483 (1987)
[11] R. Mateos, M. Trujillo, M.C. Pérez-Camino, W. Moreda, and A. Cert, J. Agric. Food Chem., **53**, 5766 (2005)
[12] N.V. Yanishlieva-Maslarova, Bul. Lias. Groupe Polyphenols, **12**, 470 (1984)
[13] AOAC method 963.22. Methyl Esters of Fatty Acids in Oils and Fats. Official Methods of Analysis of the AOAC, 17th edn, AOAC, Arlington, Virginia USA, (2000)
[14] AOAC method 969.33. Fatty Acids in Oils and Fats. Official Methods of Analysis of the AOAC, 17th edn, AOAC, Arlington, Virginia USA, (2000)
[15] J.P. Cosgrove, D.F. Church, and W.A. Pryor, Lipids, **2**, 299 (1987)

[16] I. Konopka, M. Tańska, D. Rotkiewicz, and M. Zachodna, Bromat. Chem. Toksykol., Supplement, 343 (2003)
[17] A. Jarosławska, A. Sokół-Łętowska, and J. Oszmiański, Żywność, **30**, 99 (2002)
[18] A. Jarosławska, A. Sokół-Łętowska, and J. Oszmiański, Żywność, **35**, 77 (2003)
[19] C. Banias, V. Oreopoulou, and C. Thomopoulos, JAOCS, **69**, 520 (1992)
[20] C. Hall III and S. Cuppett, JAOCS, **70**, 477 (1993)
[21] E. Szukalska and B. Drozdowski, Przem. Spoż., **47**, 108 (1993)