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Effects of β-carotene on the thermal oxidation of fatty acids

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β-Carotene is one of the most important fat soluble pigment with well known antioxidant and provitamin A activities. It is used in industries as food colorant and a source of vitamin A. The thermal induced degradation during processing leads to color and properties losses. The thermal stability of the fatty acids composition of edible oils is of great importance to food manufacturers. Corn oil, rapeseed and sunflower oils were fortified with 50 to 300 µg/g of β-carotene and oxidized using Rancimat (air flow rate 20 L/h) at 110°C for 14 h. Fatty acid methyl esters (FAMEs) were measured using gas chromatography with Agilent-Technologies DB-Wax capillary column. It was found that by adding β-carotene (50 to 300 µg/g) to the corn, rapeseed and sunflower oils, no significant changes was observed in saturated fatty acids. Saturated fatty acids were relatively more protected in the presence of unsaturated fatty acids of similar carbon atoms and in the presence of β-carotene. The addition of β-carotene affected the composition of unsaturated fatty acids in the tested oils. Thus, β-carotene acts as a pro-oxidant in highly unsaturated sunflower oil. Unsaturated fatty acids are oxidized earlier and results to the formation of unpleasant flavor and consequent rancidity.

Key words: β-Carotene, lipids oxidation, thermal stability, edible oils, fatty acids composition.

INTRODUCTION

β-Carotene is one of the most important fat soluble food pigments. It plays a significant role in human health by acting as biological antioxidant, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen (Paiva and Russell, 1999; Montenegro et al., 2002) and is also a potential anti-carcinogen (Black et al., 2000). It is also one of the main additive and colorant in food products like margarine, buttery oils, bakery shortening, fried potatoes and popcorn oil (Goulson and Warthesen, 1999). β-Carotene is present in nearly all vegetable oils, including corn, groundnut, soybean, rapeseed, linseed, olive, barley, sunflower, cottonseed, sea buckthorn, palm and soybean oils (Puspitasari-Nienaber et al., 2002; Moreau et al., 2007). During the refining of edible oils, carotenoids are degraded and thus synthetic carotenoids are used to fulfill the requirement. β-Carotene has been shown to protect lipids from free radical auto-oxidation by reacting with peroxyl radicals, ultimately inhibiting propagation reactions and promoting termination of the oxidative chain reactions (Britton et al., 2008). Lee and Min (1988) observed the effects of 0, 5,

10 and 20 ppm of β-carotene on the oxidation of soybean oil/methylene chloride model system containing 4 ppm chlorophyll in light (4000 lux). β-Carotene was found to reduce the oxidation of soybean oil. However, Steensen and Min (2000) found that during auto-oxidation of soybean oil in the dark, thermal β-carotene degradation products act as pro-oxidants, and thermally degraded lycopene showed antioxidant activity in similar soybean oil systems. In addition, they also found that β-carotene as well as lycopene degradation products exposed to singlet oxygen oxidation under light neither increase nor decrease the oxidative stability of their respective soybean oil samples.

Due to the importance of β-carotene in industries as food colorant and a source of vitamin A, the thermally induced degradation and the resulting loss of color and properties like provitamin A and antioxidant activity, during processing is of great concern to the food manufacturers. In food industries and daily cooking one of the main processes is the thermal treatment of edible oils and carotenoids containing foods (Zeb, 2012). β-

Carotene was found to play significant role in the oxidation of triacylglycerols (Zeb and Murkovic, 2010, 2011). To the best of our knowledge, the effects of β-carotene on the changes in fatty acids composition of edible oils during control oxidation received little or no attention. This study mainly focused on the effects of β-carotene fortification on the thermal stability of individual fatty acid composition of corn, rapeseed and sunflower oils.

MATERIALS AND METHODS

All-E-β-carotene (97.0%) was purchased from Sigma Aldrich USA, while all other chemicals and reagents were of ACS grade. Corn (CO), rapeseed (RPO) and sunflower oils (SFO) were purchased at a local market in Graz, Austria.

Fortification and thermal oxidation

All-E-β-carotene was dissolved in acetone and added to the three tested oils in order to obtain concentrations in the range of 50 to 300 µg/g. The samples were then sonicated for 1 min and kept for 24 h under nitrogen before sealing airtight in a glass bottle. Acetone was evaporated from the fortified oils using nitrogen. All preparations were carried out in a nitrogen environment and in the absence of intense light (Zeb and Murkovic, 2010). From the stock of each β-carotene fortified oils, 5 g (±0.0050 mg) were put into the reaction vessel and oxidized using a Rancimat 679 (Metrohm AG, Switzerland). The air flow rate was set to 20 L/h and the temperature of the heating block was set to 110°C for 1 to 14 h. The samples were then stored at -20°C under nitrogen until further analysis.

Fatty acid analysis

The fatty acid was determined as FAME. FAMEs were prepared by the modified method as described in detail earlier (AOCS, 1998). Briefly, a sample of 20 mg (±0.5 mg) was measured in 20 ml vial and an aliquot of 40 µl of internal standard (C11:0, 5.65 g/100 g) was added. The solvent was evaporated and then 6 ml of 0.5 M methanolic NaOH were added and stirred at 80°C for 30 min. The samples were cooled and transmethylated using BF_3/methanol at 80°C for 15 min. After cooling, phases were separated using water and n-heptane. FAMEs were then separated on a GC (HP 5890) system using Agilent-Technologies DB-Wax capillary column (30 m long, with internal diameter of 0.25 mm and film thickness of 0.25 µm). The helium was used as carrier gas at a flow rate of 5.7 ml/min. Column oven temperature was programmed from 50 to 140°C with 15C/min increase, then with 8°C/min increase from 140 to 240°C. Initial and final hold up time was 1 and 8 min, respectively. The injection volume was 1 µl. FAMEs were than identified by comparing their relative and absolute retention times with those of authentic standards and also by GC/MS detection.

Data analysis

Results are expressed as the mean ± standard deviation of three to five independent experiments. The data were analyzed statistically by one way analysis of variance (ANOVA) using XLSTAT (Addinsoft, v. 7.5.2). Means were compared and significance was accepted at 5% level (P < 0.05) where necessary.

RESULTS AND DISCUSSION

Fatty acids composition

Fatty acids are the important constituents of the edible oils. Oxidation of the oils or fats destroys essential fatty acids and produces toxic compounds and oxidized polymers. Thus, oxidation is very important in terms of palatability, nutritional quality and toxicity of edible oils (Kamal-Eldin et al., 2003). In fats and oils, the fatty acids occur in saturated and un-saturated forms and commonly exist as triglycerides in nature. In these experiments, we studied the correlation of addition of β-carotene on the oxidation of saturated and unsaturated fatty acids of corn, rapeseed and sunflower oils.

Figure 1 shows the gas chromatography with flame ionization (GC-FID) chromatograms of the corn, rapeseed and sunflower oils. The major fatty acids in corn oils were palmitic acid (10.4 %), oleic acid (26.6 %) and linoleic acid (52.6 %). In rapeseed oil, the major fatty acids were palmitic acid (4.73 %), oleic acid (53.9 %), linoleic acid (19.2 %) and linolenic acid (7.09 %). Similarly, the content of palmitic acid was 5.94%, oleic acid was 23.9%, and there was relatively high level of linoleic acid (53.9 %) in sunflower oil as compared to the other two oils.

Saturated fatty acid oxidation

Saturated fatty acids are relatively more resistant to oxidation than unsaturated fats (Kamal-Eldin et al., 2003). The saturated fatty acids include myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid(C20:0) and behenic acid (C22:0) (Table 1). There was a small negative non-significant (P > 0.05) effect of added β-carotene on the myristic acid in corn oil, while in the case of rapeseed and sunflower oil, myristic acid was resistant against the thermal oxidation at 110°C. Palmitic acid was not affected by increasing β-carotene concentration. Allouche et al. (2007) showed that palmitic acid concentration increases with increasing duration of thermal oxidation of the virgin olive oil from Arbequina and Picual cultivars during heating at 180°C. In the present study, the concentration of stearic, arachidic and behenic acids did not changed with increased concentration of β-carotene. It was also observed that the presence of different concentration of each individual fatty acid in the three oils showed similar non-significant effects on the stability of the oils. Paradiso et al. (2010) showed that low amount of added free fatty acid caused increase in the oxidation of olive oil (Rancimat at 85°C, 20 L/h of air), while the addition of higher amount of fatty acid delayed the oxidation. The present results elaborate further by showing that the thermal stability of individual saturated fatty acid in different oil behaves relatively similar under similar conditions and are protected in β-
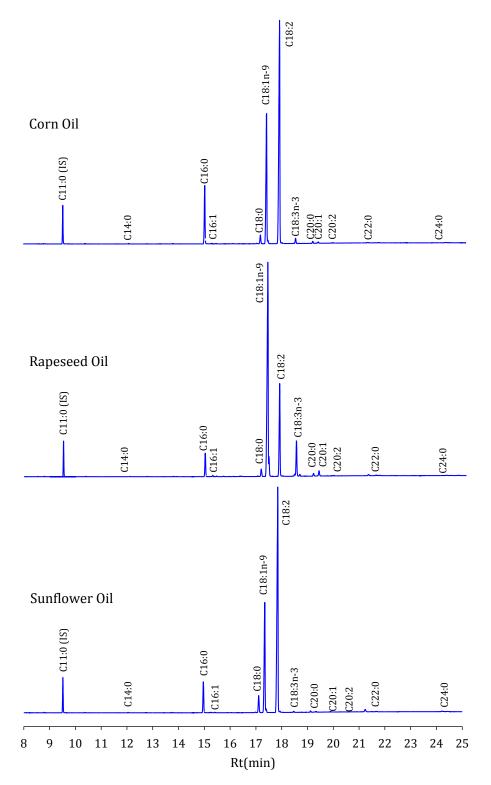
Figure 1. GC-FID chromatograms of the FAMEs of corn, rapeseed and sunflower oils.

Table 1. Effects of β-carotene (0 to 300 µg/g) on the saturated fatty acids composition of rapeseed, corn and sunflower oils.

Fatty acid (g/100 g)	Oil	β-Carotene (µg/g)							
		Fresh	00	50	100	150	200	250	300
	RPO	0.079 ± 0.022	0.078 ± 0.023	0.076 ± 0.025	0.077 ± 0.024	0.080 ± 0.024	0.081 ± 0.023	0.078 ± 0.024	0.080 ± 0.024
C14:0	CO	0.054 ± 0.010	0.052 ± 0.020	0.055 ± 0.020	0.055 ± 0.020	0.052 ± 0.020	0.052 ± 0.020	0.051 ± 0.021	0.035 ± 0.011
	SFO	0.30 ± 0.20	0.09 ± 0.02	0.09 ± 0.02	0.089 ±0.02	0.09 ± 0.02	0.088 ± 0.02	0.09 ± 0.03	0.087 ± 0.02
	RPO	4.73 ± 0.27	4.50 ± 0.36	4.45 ± 0.38	4.46 ± 0.4	4.52 ± 0.32	4.49 ± 0.28	4.41 ± 0.34	4.60 ± 0.31
C16:0	CO	10.4 ± 0.6	10.1 ± 0.6	10.3 ± 0.50	10.30 ± 0.6	10.21 ± 0.5	10.27 ± 0.4	10.4 ± 0.6	9.99 ± 0.50
	SFO	5.94 ± 0.4	5.88 ± 0.42	5.71 ± 0.47	5.82 ± 0.42	5.81 ± 0.43	5.70 ± 0.52	5.86 ± 0.48	5.82 ± 0.35
	RPO	1.76 ± 0.15	1.67 ± 0.18	1.68 ± 0.19	1.66 ± 0.19	1.68 ± 0.16	1.66 ± 0.15	1.63 ± 0.18	1.72 ± 0.16
C18:0	CO	1.77 ± 0.10	1.71 ± 0.10	1.75 ± 0.10	1.73 ± 0.10	1.73 ± 0.10	1.73 ± 0.10	1.77 ± 0.1	1.62 ± 0.09
	SFO	3.66 ± 0.30	3.61 ± 0.35	3.51 ± 0.34	3.57 ± 0.32	3.58 ± 0.34	3.51 ± 0.39	3.62 ±0.37	3.61 ± 0.27
	RPO	0.57 ± 0.03	0.52 ± 0.03	0.51 ± 0.04	0.52 ± 0.03	0.52 ± 0.03	0.52 ± 0.03	0.51 ± 0.04	0.54 ± 0.03
C20:0	CO	0.38 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.35 ± 0.01
	SFO	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.010	0.23 ± 0.01	0.23 ± 0.01
	RPO	0.37 ± 0.08	0.33 ± 0.08	0.33 ± 0.07	0.33 ± 0.08	0.33 ± 0.07	0.33 ± 0.08	0.32 ± 0.08	0.35 ± 0.07
C22:0	CO	0.17 ± 0.06	0.15 ± 0.05	0.14 ± 0.05	0.15 ± 0.05	0.15 ± 0.05	0.15 ± 0.05	0.15 ± 0.05	0.11 ± 0.01
	SFO	0.71 ± 0.10	0.66 ± 0.11	0.73 ± 0.13	0.64 ± 0.10	0.66 ± 0.10	0.65 ± 0.11	0.67 ± 0.11	0.67 ± 0.08

carotene. This means that saturated fatty acids containing β-carotene are stable at smaller to higher concentration when exposed to control oxidation at similar temperature.

Unsaturated fatty acid oxidation

The unsaturated fatty acids were palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), ecosaenoic acid (C20:1) and ecosadienoic acid (C20:2). Among the unsaturated fatty acids, palmitoleic acid and oleic acid were stable towards increased β-carotene concentration, during thermal oxidation at 110°C in the Rancimat (Table 2). Linoleic acid in corn and rapeseed oils showed no significant (P > 0.05) effect, while sunflower oil showed a slight negative correlation. A significant reduction of oleic and linoleic acids was reported during thermo-oxidation of oils (Romero et al., 1998) and frying (Choe and Min, 2007). However, in our study, the stability of oleic and linoleic acid can be attributed to the decrement of linolenic acid or higher polyunsaturated fatty acid. Linolenic acid in rapeseed oil was oxidized more readily than in the case of corn and sunflower oils. Due to the presence of double bonds, it is well established that by increasing the heating time, unsaturated fatty acids are oxidized differently (Allouche et al., 2007). The various strengths of the hydrogen carbon bond of fatty acids explain the differences of oxidation rates of stearic, oleic, linoleic and linoleic acids during thermal oxidation (Marquez-Ruiz and Dobarganes, 1996). Eicosaenoic acid was found stable in all the three oils. In sunflower oil, the amount of eicosadienoic acid was affected by high concentration of carotene, while it was stable at concentration up to 250 µg/g with an

Table 2. Effects of β-carotene (0 to 300 µg/g) on the unsaturated fatty acids composition of rapeseed, corn and sunflower oils.

Fatty acid (g/100 g)	Oil	β-Carotene (µg/g)							
		Fresh	00	50	100	150	200	250	300
C16:1	RPO	0.22 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.200 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
	CO	0.096 ± 0.020	0.080 ± 0.010	0.081 ± 0.010	0.081 ± 0.010	0.076 ± 0.010	0.079 ± 0.010	0.080 ± 0.010	0.088 ± 0.010
	SFO	0.075 ± 0.010	0.079 ± 0.010	0.077 ± 0.010	0.26 ± 0.20	0.250 ± 0.200	0.076 ± 0.010	0.076 ± 0.010	0.250 ± 0.200
C18:1n-9	RPO	53.9 ± 0.72	51.2 ± 4.5	50.2 ± 4.6	50.6 ± 5.0	51.1 ± 4.0	50.4 ± 3.5	49.7 ± 4.4	52.3 ± 4.0
	CO	26.6 ± 1.90	24.5 ± 1.7	25.2 ± 1.6	24.9 ± 1.6	24.9 ± 1.5	24.9 ± 1.2	25.3 ± 1.7	23.9 ± 1.2
	SFO	23.9 ± 2.0	21.8 ± 1.9	21.3 ± 2.2	21.0 ± 1.6	21.0 ± 1.7	20.7 ± 2.0	21.9 ± 2.0	21.6 ± 1.4
C18:2	RPO	19.4 ± 1.3	13.4 ± 1.1	13.1 ± 1.1	13.0 ± 1.4	12.9 ± 0.9	12.6 ± 0.9	12.5 ± 1.0	12.2 ± 1.0
	CO	52.6 ± 3.7	38.2 ± 2.4	37.9 ± 2.3	37.1 ± 2.5	36.9 ± 2.2	36.4 ± 1.7	36.1 ± 2.5	35.8 ± 2.0
	SFO	53.9 ± 2.0	33.2 ± 2.7	31.2 ± 3.2	28.2 ± 2.2	27.9 ± 2.0	28.21 ± 2.40	32.8 ± 2.7	31.46 ± 2.00
C18:3n-3	RPO	7.09 ± 0.50	3.72 ± 0.33	3.58 ± 0.28	3.57 ± 0.45	3.53 ± 0.27	3.41 ± 0.26	3.37 ± 0.31	3.30 ± 0.34
	CO	0.99 ± 0.10	0.562 ± 0.060	0.595 ± 0.070	0.553 ± 0.050	0.569 ± 0.060	0.566 ± 0.070	0.574 ± 0.071	0.545 ± 0.041
	SFO	0.359 ± 0.10	0.180 ± 0.30	0.153 ± 0.10	0.144 ± 0.02	0.137 ± 0.07	0.128 ± 0.06	0.120 ± 0.08	0.117 ± 0.07
C20:1	RPO	1.18 ± 0.10	1.16 ± 0.13	1.15 ± 0.16	1.13 ± 0.16	1.15 ± 0.13	1.12 ± 0.11	1.13 ± 0.14	1.10 ± 0.14
	CO	0.347 ± 0.07	0.308 ± 0.01	0.312 ± 0.04	0.313 ± 0.04	0.315 ± 0.05	0.346 ± 0.03	0.295 ± 0.05	0.298 ± 0.01
	SFO	0.143 ± 0.01	0.249 ± 0.02	0.145 ± 0.04	0.237 ± 0.02	0.154 ± 0.06	0.136 ± 0.05	0.177 ± 0.05	0.187 ± 0.04
C20:2	RPO	0.17 ± 0.07	0.10 ± 0.07	0.10 ± 0.08	0.05 ± 0.02	0.05 ± 0.02	0.09 ± 0.06	0.090 ± 0.07	0.050 ± 0.02
	CO	0.21 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.071 ± 0.020	0.27 ± 0.20	0.089 ± 0.020	0.170 ± 0.060
	SFO	0.155 ± 0.040	0.142 ± 0.080	0.136 ± 0.080	0.133 ± 0.070	0.102 ± 0.020	0.093 ± 0.020	0.099 ± 0.020	0.109 ± 0.020

increased stability at 300 µg/g. The increase in the stability of linolenic acid towards oxidation in the presence of β-carotene showed a similar response as given by the aqueous micellar solutions of linolenic acids and β-carotene (Nagler et al., 2003). However, the overall change in the concentration of linolenic acid was not statistically significant (P > 0.05). Generally, edible oils with a higher degree of unsaturation are oxidized more readily than saturated oils (Parker et al., 2003). By increasing the degree of unsaturation, both the rate of formation and the amount of primary oxidation products increase (Martin-Polvillo et al., 2004). These results show a similar behavior from C18:0 to C20:2. It was also observed that there (C18:1) composition in all the three oils under control and β-carotene addition. Linoleic acid (C18:2) was oxidized significantly in the control oxidized samples, and its stability was higher in corn and sunflower oil samples. Linolenic acid (C18:3) concentration was depleted by 50% in all the three cases, however, no significant changes were observed by increasing β-carotene concentration in rapeseed and corn oils. However, the contents were further depleted in sunflower oil by increasing β-carotene concentration. The most probable reason is the presence of high amounts as compared to other oils. Similarly, different behaviors were shown by the C20:1 and C20:2 fatty acids upon thermal oxidation in the control and β-carotene addition.

An interesting correlation was found by plotting the changes in the contents of linolenic acid and stearic acid in the sunflower oil samples with β-carotene (Figure 2). It was observed that a positive correlation was present between the three components. It shows that stearic acid stability increases when a similar carbon chain fatty acid (linolenic acid) is thermally oxidized. Recent results show that β-carotene has different effects and reactivity toward the formation of different oxidized species in olive oil under similar

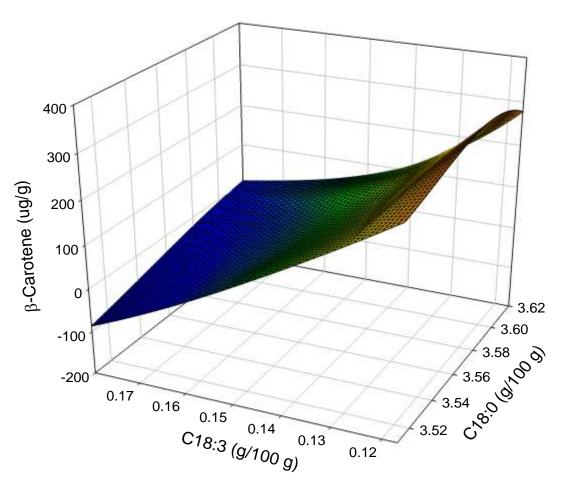**Figure 2.** Correlation of β-carotene addition, and changes in the concentration of stearic and linolenic acids during thermal oxidation of sunflower oil at 110°C in the Rancimat.

conditions (Zeb and Murkovic, 2011). This reflects the importance of saturated fatty acid presence during thermal oxidation.

Conclusion

The different fatty acids in edible oils and their relation to thermal oxidation are very important in food industries as well as our daily life. This study shows that by adding β-carotene (50 to 300 μg/g) to corn, rapeseed and sunflower oils, saturated and unsaturated fatty acids showed different behavior towards thermal oxidation. In saturated fatty acids, no significant change in the oxidation behavior was observed, while addition of β-carotene affected unsaturated fatty acid in the three oils. β-Carotene acts as a pro-oxidant in highly unsaturated oils such as sunflower oil. Saturated fatty acids are relatively more protected during thermal oxidation, especially when highly unsaturated fatty acids are available. Unsaturated fatty acids are thus oxidized earlier and results to the formation of unpleasant flavor

and consequent rancidity. Thus, using β-carotene in food industries should be monitored strictly to avoid loss of food and consequent economy.

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