Scientific paper

Influence of Sunflower Oil Qualities and Antioxidants on Oxidative Stability on Whey-Based Salad Dressings

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Abstract

The antioxidant effect of ascorbic acid and EDTA (ethylenediaminetetraacetic acid) in food emulsions, based on whey and sunflower oils with enhanced oleic acid, α- and β- tocopherol content, was not described up to now. Salad dressings based on cold-pressed high-oleic/α-, β- tocopherol sunflower oil were oxidatively stable after 3 months of storage at 25 °C regarding primary (peroxide value, PV) and secondary (hexanal) lipid oxidation products (PV = 0.34 mmol O_2 kg^{-1}, hexanal value = 1.54 mg kg^{-1}). Slight enhancement of PV and hexanal values was recorded in salad dressings prepared with cold-pressed medium-oleic/α-, β- tocopherol oil, after 3 months of storage at 25 °C, and was inhibited by ascorbic acid or EDTA. Ascorbic acid (0.50 g kg^{-1}) reduced PV by 80% and hexanal value by 32%. EDTA (0.075 g kg^{-1}) reduced PV by 60% and hexanal value by 27%. In salad dressings, containing linoleic/α- tocopherol sunflower oil, the antioxidant effects of ascorbic acid and EDTA were as following: ascorbic acid (0.25–4.00 g kg^{-1}) reduced PV by 83–100% and hexanal value by 82–73%; EDTA (0.075 g kg^{-1}) reduced PV by 75% and hexanal value by 76%, after 12 months of storage at 4 °C.

Keywords: High-oleic sunflower oils, tocopherols, emulsions, antioxidants

1. Introduction

Oxidation of unsaturated lipids in oil/water (o/w) emulsions or dispersions, such as milk, mayonnaise and salad dressings, is more complex and generally occurs faster than in bulk oils.[1] Antioxidants are widely employed to delay formation of ultimate oxidation products that could alter the taste and nutritional content of foods. There is a great interest in utilizing antioxidants from natural sources and oils rich in monounsaturated fatty acids, which resist oxidation for a longer period of time. Genetic alterations of sunflower oil quality, developed by conventional breeding, are the most promising regarding the oxidative stability and are directed both towards increase in oleic acid content and changes in tocopherol composition. Standard sunflower oil is composed of 55–65% linoleic acid (C18:2), 20–30% oleic acid (C18:1), 8–10% palmitic (C16:0) and stearic (C18:0) acid and traces of other higher fatty acids. Different modes of inheritance of high-oleic acid content, mainly dominant and determined by a low number of genes, have been reported so far in sunflower.[2] Tocopherol (vitamin E) complex of sunflower oil is known to predominantly (95%) contain the α- form. There is an inversion in vitamin and antioxidant activity in tocopherol forms. The ratio of vitamin to antioxidant activity has been estimated as 1.00, 0.50, 0.25, 0.01 and 1.0, 1.3, 1.8, 2.7 for α-, β-, γ- and δ- tocopherol, respectively. The changes in tocopherol composition were found to be controlled by two independent genes tph1 and tph 2.[3] Demurin *et al.*[4] found positive synergy between genes for high oleic acid content and those for different to-

copherol levels. Attempts are being made to obtain high-oleic sunflower hybrids with stable oleic acid content, modified tocopherol composition, resistance to main sunflower pathogens and good agronomic characteristics.[5,6] So far, limited research on the antioxidant activity of tocopherols (α, β, γ and δ) in o/w emulsions has been reported.[7,8,9,10] Oxidative stability of high-oleic oils with natural α- and β- tocopherol was not studied in emulsion systems up to now. Tocopherols are predominantly nonpolar chain-breaking antioxidants which were found to be more active in polar systems like o/w emulsions than in bulk oils, due to the location in the oil phase where the oxidation propagate, but sufficient surface activity to be oriented in the oil-water interface.[1] The phenomenon is known as "polar paradox" of inverse correlation between polarity of antioxidants and their efficacy in polar or nonpolar systems.[1] However, factors other than antioxidant partitioning (particle size, emulsion droplet charge, thickness of the emulsifier layer at the interface region and protein emulsifiers)[11] may also affect antioxidant activity in heterophasic systems like o/w emulsions, and different antioxidant mechanisms prevail in different kinds of food emulsions. Whey protein isolates (WPI) and sweet whey were found to stabilize salmon or corn o/w emulsions when pH was lower than pH of the whey proteins absorbed at the interface, presumably by producing cationic emulsion droplets that can repeal prooxidative metals. The antioxidant properties of sweet whey were even higher than those of WPI although positive charge of the emulsion droplets was higher when WPI was used as an emulsifier,[11,12] suggesting that sweet whey may be applied as an antioxidant as well as emulsifier. Ascorbic acid exerts its antioxidant activity as hydrophilic radical scavenger or O_2 scavenger, and is used in emulsion systems for its ability to regenerate tocopherol or act as metal chelator. However, ascorbic acid could also be a prooxidant, due to its ability to reduce transition metals, enhancing lipid oxidation via the breakdown of already existing lipid peroxides.[10] Addition of EDTA has been found to protect against formation of both peroxides and volatile secondary lipid oxidation products in mayonnaise, whey protein-based salad dressings and milk drinks, enriched in n-3 or n-6 PUFA, showing better antioxidant activity than nonpolar molecules ascorbyl palmitate and tocopherols,[10] contrary to the "polar paradox" hypothesis.[1] The strong antioxidant effect of EDTA in mayonnaise and salad dressings with polyunsaturated lipids has been ascribed to its ability to chelate iron from the egg yolk protein phosvitin or whey proteins located at the oil-water interface.[10]

The objective of this study was to assess the effects of ascorbic acid and EDTA, in sweet whey-based salad dressings, prepared with oils from medium-oleic and high-oleic sunflower hybrids with approximately 50% α- : 50% β- tocopherol content[5] or linoleic/α- tocopherol type of sunflower oil. Ascorbic acid was found to be a prooxidant in model emulsions[1] and food emulsions containing polyunsaturated lipids[10] but the effect may be different in food matrices containing less unsaturated lipids and modified tocopherol composition.

2. Experimental

2. 1. Materials

Refined linoleic sunflower oil was obtained from local market. The characteristics of refined linoleic type sunflower oil were: 21.30% oleic acid : 67.40% linoleic acid and 560.00 mg L^{-1} α- tocopherol. Peroxide value, (PV), was 2.10 mmol O_2 kg^{-1}; hexanal value 2.60 mg kg^{-1} and acid value, (AV), 0.11). Cold-pressed oleic type sunflower oils (NSHOL2 and NSH2075) with modified β- tocopherol levels, were kindly donated by the Institute of Field and Vegetable Crops (Novi Sad, Serbia). The characteracteristics of NSHOL2 oil were: 89.40 % oleic acid : 5.00 % linoleic acid, 205.33 mg/L of α- : 206.67 mg/L of β- tocopherol, (PV 0.00 mmol O_2 kg^{-1}; hexanal value 1.69 mg kg^{-1}; AV 2.38). The characteristics of NSH2075 oil were: 40.30% oleic acid : 50.60% linoleic acid, 338.00 mg L^{-1} of α- : 217.33 mg L^{-1} of β- tocopherol, (PV 0.00 mmol O_2 kg^{-1}; hexanal value 1.69 mg kg^{-1}; AV 2.99). Dried sweet whey was from Novi Sad Dairy (Novi Sad, Serbia). Mustard, salt and vinegar (9%) were obtained from the local supermarket. K- sorbate and EDTA were from Merck (Darmstadt, Germany), Stabilizers Grinstead FF5103 (guar gum and xanthan gum) and Grinstead (guar gum and modified starch) were donated by Danisco, Cultor (Brabrand, Denmark). Ascorbic and citric acid and external standards for "headspace" gas chromatography mass spectrometry method (pentanal 97%, hexanal > 97%, heptanal 95% and octanal > 98%) were from Sigma-Aldrich (Steinheim, Germany), while nonanal 98% was from Supelco (Bellefonte, PA, USA).

2. 2. Preparation of Salad Dressings

Whey-based salad dressings similar to mayonnaise were prepared in 1.5 kg batches composed of refined non-hydrogenated linoleic sunflower oil or unrefined cold-pressed high-oleic sunflower oil (40%), sweet whey powder 12% (final whey protein concentration 1.2%), deionized water, salt 0.7%, mustard 3%, vinegar 3%, stabilizers 0.2%, K-sorbate 0.1%. Antioxidative agents: ascorbic acid (0.25–4.00 g kg^{-1}), citric acid (0.07 g kg^{-1}) or EDTA (0.075 g kg^{-1}) were added to the aqueous phase. The products were emulsified in Stephen UMCS electronics (Austria) at 3000 rpm and aliquots were stored in nontransparent jars at 4 °C or 25 °C.

2. 3. Separation of Salad Dressings for Determination of Oxidation Products

After a certain period of storage (0–12 months at 4 °C or 0–3 months at 25 °C), the samples of salad dres-

sings were frozen at –80 °C for ten days. Salad dressings were thawed at room temperature, centrifuged at 26100 × g for 30 minutes and analyzed for oxidative stability of separated oil phase.[13]

2. 4. Determination of Peroxide Values (PV), Acid Values (AV) and pH

The development of primary products of lipid oxidation was monitored through peroxide value in bulk oil or in the separated oil phase of the dressing.[14] The development of free fatty acids (FFA) was analyzed through acid value in bulk oil or in the separated oil phase of the dressing. AV was determined in the separated oil phase through titration with NaOH, using phenolphthalein as indicator.[15] pH of salad dressings was determined by using pH-meter (Consort, C 830, USA) with viscous emulsion electrode.

2. 5. Determination of Secondary Volatile Oxidation Products (Volatiles)

The secondary volatile oxidation products of linoleic and oleic fatty acids in bulk oils or separated oil phase of salad dressings were monitored using "headspace" gas chromatography mass spectrometry detection (HS GC-MS).[16] Equipment included 6890N model gas chromatograph (Network GC System Agilent Technologies, USA), equipped with a mass-selective detector 5975B (Inert MSD Agilent Technologies, USA) headspace autosampler (CTC Analytics CombiPAL) and splitless injection (inlet temperature 225 °C, pressure 12.97 psi, injection volume 250 µl, syringe size 2.5 ml-HS). The oil samples (either bulk oil or separated oil phase of salad dressings), approximately 5g, were weighed into a vial (20 ml) which was then purged with helium for 3 minutes and sealed with a septum secured by aluminum cap. All analyses were made in triplicate. Samples were stored at –20 °C, for 10 days, defrosted before measuring at room temperature and placed in the headspace magazine. "Headspace" method includes: incubation temperature 180 °C, incubation time 600 s, syringe temperature 50 °C and agitator speed 500 rpm. All separations were carried out on capillary column HP-5MS, 5 % phenyl methyl siloxane (30m × 0.25 mm × 0.25 µm, Agilent Technologies, Cat No.19091S-433). Oven temperature program started at initial temperature 40 °C (for 8 min), rising at 25 °C/min to 150 °C (hold 2.00 min), rising at 30 °C/min to 200 °C (hold 0.00 min), rising at 30 °C/min to 280 °C (hold 0.00 min). Total run time was 18.73 min. Helium was used as a carrier gas at a constant rate 46 c ms^{-1}. MS detector was equipped with quadrupole analyzer operating in electron ionization mode. Ion source temperature was 230 °C, MS Quad temperature 150 °C, and solvent delay 1 min. Identification and quantification were performed in SCAN monitoring mode (low mass 35.0, high mass 450.0). The identities of the volatile oxidation compounds were confirmed by GC-MS and by external standards (pentanal, hexanal, heptanal, octanal and nonanal).

2. 6. Quantification of Antioxidative Effects

In order to quantify the effect of antioxidants on primary or secondary oxidation products, the maximal % inhibition obtained during storage period was calculated using the following equation:

$$\%\ Inhibition = [(control\ sample - sample\ with\ antioxidant)/control\ sample] \times 100$$

The control sample represents the measured values: peroxide value, PV (mmol $O_2\ kg^{-1}$) or concentration of volatiles, i.e. hexanal (mg kg^{-1}), without antioxidants.

2. 7. Statistical Analysis

Experimental results were expressed as means ± standard deviation (SD) of three parallel measurements. Statistical analysis was performed using Student *t* test. The significance was tested at $p < 0.05$ level.

3. Results and Discussion

3. 1. Validation of "Headspace" GC-MS Aldehyde Determination

Linearity of calibration was ensured by adding different amounts of aldehyde mixture in vial headspace. In the range of 0.1–12.0 µg g^{-1} (mg kg^{-1}) the equations had correlation coefficients (r^2) for pentanal, heptanal, hexanal, octanal and nonanal as shown in (Tabl. 1). The concentration of aldehydes was calculated from calibration curves as follows: concentration = intercept + slope × peak area (Tabl. 1).

Table 1. Parameters of calibration curves of external standards.

Aldehyde	Slope	Intercept	r^2
Pentanal	3.8E-06	0.03434	0.9998
Heptanal	4.3E-05	–0.0576	0.9999
Hexanal	5.2E-06	0.03172	0.9999
Octanal	3.6E-05	–0.0437	0.9998
Nonanal	2.5E-05	0.63577	0.9826

Repeatability of "headspace" sampling for all aldehydes was expressed as relative standard deviation (RSD) obtained from determination of the six replicates of standard mixture. Mean RSD of the "headspace" sampling of aldehyde mix was 20.72%.

Limits of quantification (LOQ) and determination (LOD) calculated as 10 or 3 standard deviations of the ba-

se line noise were 0.1 and 0.03 μg g^{-1} for all five aldehydes.

3. 2. Evaluation of the Influence of Ascorbic Acid and EDTA on Oxidative Stability of Salad Dressings Based on Sweet Whey and Different Types of Sunflower Oil

Salad dressings were based on sweet whey and different types of sunflower oil: refined linoleic acid/α- tocopherol oil (LO) or cold-pressed oils, obtained from two hybrids, with increased oleic acid and β- tocopherol content (approximately 50% α- : 50% β- tocopherol ratio).[5] Hybrids were designated as NSHOL2 and NSH2075 or as high-oleic (HO) and medium-oleic (MO) type, respectively.

In salad dressings (pH 4.5) based on LO, (SDL) primary lipid oxidation products were evaluated through peroxide value (PV) in a separated oil phase, during the 12 month period of storage at 4 °C. In control samples, PV enhanced rapidly during the first six months of storage and then enhanced more slowly (Fig. 1), probably due to formation of secondary oxidation products.[10] Ascorbic acid, in concentration of 0.25–4.00 g kg^{-1} induced a dose-dependent decrease on PV during the 12 months of storage. At a level of 0.25 g kg^{-1}, ascorbic acid induced 83% inhibition of PV. Levels of 0.50–4.00 g kg^{-1} induced 88–100% inhibition of PV (Fig. 1). EDTA (0.075 g kg^{-1}) induced 80 % reduction of PV after 3 months of storage at 4 °C (Fig. 3, control + EDTA) and 75% reduction of PV after 12 months of storage at 4 °C (data not shown).

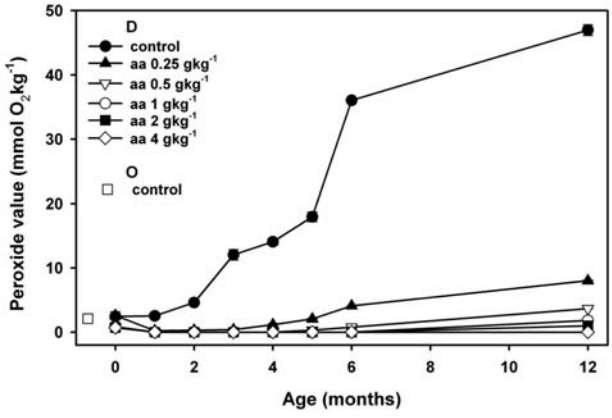

Figure 1. Effect of the concentration of ascorbic acid (0.25–4.00 g kg^{-1}) on the inhibition of lipid hydroperoxide formation, determined as peroxide value in salad dressings based on whey and linoleic acid/α- tocopherol type of sunflower oil (control), during storage at 4 °C. Data points represent means (n = 3) ± SD. Abbreviations: D, dressings; O, oil; aa, ascorbic acid.

The analysis of secondary oxidation products in SDL was performed for selected concentrations of antioxidants. Low-molecular weight volatile aldehydes (oxidation products of linoleic and oleic acids) were analyzed by direct HS GC-MS,[16] using separated oil phase of salad dressings purged with helium, thus avoiding the need for non-oxidation products such as water, acetic acid and other volatile acids to be removed from the headspace.[17] Enhancement in secondary oxidation products in SDL was detected as formation of hexanal and pentanal, and inhibited by ascorbic acid and EDTA. Ascorbic acid (0.25, 0.50 and 1.00 g kg^{-1}) induced 82%, 76% and 75% reduction in hexanal value, respectively, (Fig. 2) and 73% at 2 and 4 g kg^{-1} of ascorbic acid (data not shown), after 12 months of storage. The maximal antioxidant effect on hexanal formation, at 12 months age, was at the lowest (0.25 g kg^{-1}) additional level of ascorbic acid, although the antioxidant effects of other four concentrations of ascorbic acid on PV were very similar. After 6 months of storage, the maximum effect was at the concentration of 0.5 g kg^{-1} of ascorbic acid (Fig. 2). Pentanal formation was approximately 50% reduced at all levels of ascorbic acid (data not shown). EDTA (0.075 g kg^{-1}) induced 76 % reduction of hexanal formation, after 12 months of storage at 4 °C (data not shown).

Increase in PV and volatile oxidation products was not observed in dressings based on high- and medium-oleic types of sunflower oils of α-,β- tocopherol type (SDHO and SDMO, respectively) during storage at 4 °C for three months (data not shown). Slight enhancement in PV (Fig. 3) and hexanal (Fig. 4 and Tabl. 2) was recorded in SDMO after 3 months of storage at 25 °C, and it was reduced by both ascorbic acid (0.50 g kg^{-1}) and EDTA (0.075 g kg^{-1}). Ascorbic acid was found to inhibit PV by 80% and EDTA by 60%. Hexanal formation was inhibited by 32% with ascorbic acid and by 27% with EDTA. Combination of ascorbic acid and EDTA had the reducing effect on PV in both SDL and SDMO, between the values for ascorbic acid and EDTA alone (data not shown). SDHO were oxidatively stable, with or without addition of antioxidants

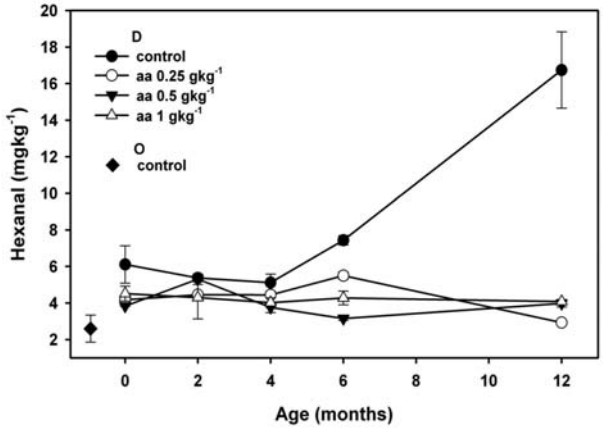

Figure 2. Effect of the concentration of ascorbic acid (0.25–1.00 g kg^{-1}) on inhibition of hexanal formation in salad dressings, based on whey and linoleic acid/α- tocopherol type of sunflower oil (control), during storage at 4 °C. Data points represent means (n = 3) ± SD. Abbreviations: D, dressings; O, oil; aa, ascorbic acid.

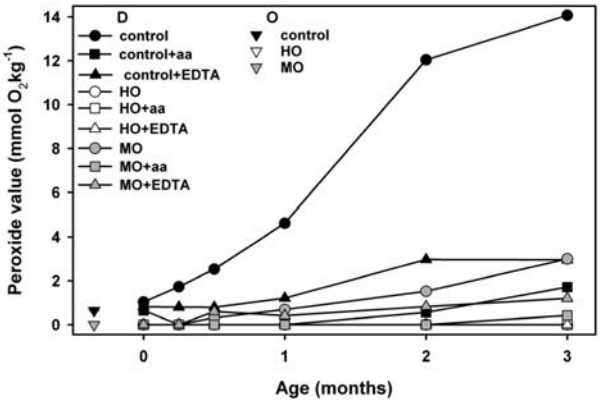

Figure 3. Antioxidant effect of ascorbic acid (0.50 g kg^{-1}) and EDTA (0.075 g kg^{-1}) on peroxide value, in salad dressings based on whey and MO or HO sunflower oil (SDMO and SDHO, respectively), during storage at 25 °C. The control was represented by linoleic/α- tocopherol sunflower oil- based dressing (stored at 4 °C). Data points represent means (n = 3) ± SD. Abbreviations: D, dressings; O, oil; aa, ascorbic acid; MO, medium-oleic/α-, β- tocopherol oil; HO, high-oleic/α-, β- tocopherol oil.

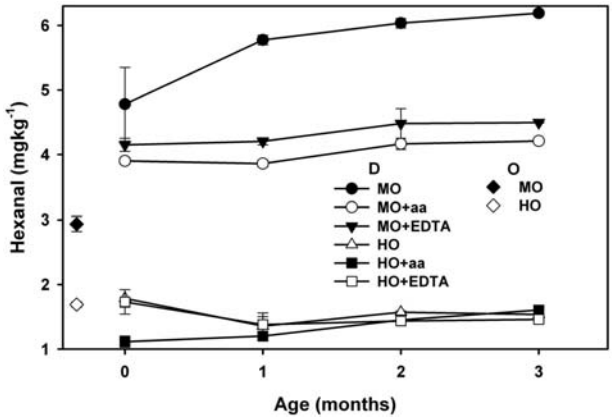

Figure 4. Antioxidant effect of ascorbic acid (0.50 g kg^{-1}) and EDTA (0.075 g kg^{-1}) on hexanal formation in salad dressings, based on whey and MO or HO sunflower oil, (SDMO and SDHO, respectively), during storage at 25 °C. Data points represent means (n = 3) ± SD. Abbreviations: D, dressings; O, oil; aa, ascorbic acid; MO, medium-oleic/α, β- tocopherol oil; HO, high-oleic/α, β- tocopherol oil.

(the enhancement of primary and secondary oxidation products was not observed), and both PV and volatile values were very low (Fig. 3. and Tabl. 2). SDL stored at 4 °C was used as control in this experiment, as SDL stored at 25 °C were not produced with antioxidants.

Direct comparison with equal quality MO- or HO-/α- tocopherol types of sunflower oil based-dressings was not possible. However, salad dressings based on commercial MO/α- tocopherol oil, during storage at 25 °C (data not shown), had almost equal PV as dressings based on refined L/α- tocopherol sunflower oil stored at 4 °C, i.e. the inferior quality to that of MO- and HO/α-, β- tocopherol sunflower oil-based dressings.

The acid value (AV) of separated oil phase of salad dressings and pH of the dressings were stable in samples stored at 4 °C, during the period of observation, independently of oil type. AV of SDL was 0.75, while the AV of SDMO and SDHO was 3.55 and 3.25, respectively. The AV was, however, slightly enhanced after 3 months at 25 °C in separated oil phase of both oleic-sunflower oil- based dressings: 11.75% in SDMO and 8% in SDHO, which correlated with pH decrease from 4.55 to 4.35. The enhancement of AV may be influenced by fatty acid hydrolysis, as well as by organic acids enhancement in oil phase. The data obtained in this study for salad dressings with 40% oil are in accordance to those obtained by Karas *et al.*[18] for light mayonnaise with 49% oil, where it was found that increase in AV corresponded to decrease in pH, after storage of 2 months on either 5–8 °C or 20–25 °C, i.e. the cold-stored samples had higher pH, lower AV and lower PV than the samples stored at room temperature.

Linoleic acid oxidation products are 9- and 13-hydroperoxides which decompose to produce mainly hydrocarbons and aldehydes,[19] but the major product that increases in content during storage is hexanal, which had become known as indicator of rancidity.[20] 13-hydroperoxides of linoleic acid are decomposed predominantly into pentane, pentanal and hexanal[20,21] and also octane and pentanol.[22] 9-hydroperoxides of linoleic acid decompose predominantly into 2,4 decadienal, 3-nonenal heptanal, 2-heptenal.[22] Oleic acid hydroperoxides (8-, 9-, 10- and 11-hydroperoxides) have octanal, nonanal, 2-undecenal and 2-decenal as main decomposition products.[22] Low-molecular weight volatile aldehydes (oxidation products of linoleic and oleic acids): pentanal, hexanal, heptanal, octanal and nonanal, which we have opted to analyze in this study, create oxidative rancid flavor and, among them, hexanal, octanal and nonanal are the major flavor compounds.[23] Increase in volatile oxidation products during storage was observed mainly as hexanal formation and it is represented in chromatograms of sunflower oil dressings for both SDL (Fig. 5) and SDHO (Fig. 6). BOHO and SDHO (Fig.6) had significantly higher nonanal content than BOL and SDL (Fig. 5 and Tabl. 2).

The observed heptanal, octanal and nonanal concentrations were lower in BOL than in BOMO and BOHO sunflower oils. It was also observed that peroxides and all analyzed aldehydes were more pronounced in SDL, compared to BOL, on the 0^{th} day. Greater o/w interface ratio in emulsion compared to bulk oil[1] and aeration during the homogenization of emulsion could facilitate oxidation, inducing enhancement in peroxide and aldehyde content in SDL, but not in the more stable SDHO (Tabl. 2). In SDMO, hexanal, octanal and nonanal contents were enhanced compared to BOMO on the 0^{th} day, while the concentration of pentanal and heptanal did not increase.

Ascorbic acid had the antioxidant effect in both LO- and MO- based dressings. Ascorbic acid reduced the pero-

Table 2. Effect of storage (at 4 °C and 25 °C) on concentration of individual volatiles (mg kg^{-1} oil) in bulk oil or salad dressings based on whey and different types of sunflower oil: linoleic/α- tocopherol, medium-oleic/α-, β- tocopherol and high-oleic/α-, β- tocopherol oil.

Oil type	Age (month)	T (°C)	Pentanal (mg kg^{-1})	Hexanal (mg kg^{-1})	Heptanal) (mg kg^{-1})	Octanal (mg kg^{-1})	nonanal (mg kg^{-1})
BOL	0		1.41±0.19	2.60±0.74	0.13±0.03	0.08±0.03	0.35±0.11
SDL	0		2.04±0.11	6.07±1.06	1.08±0.37	0.60±0.12	1.02±0.18
SDL	12	4	3.99±1.23	16.74±2.09	0.55±0.07	0.31±0.09	0.75±0.31
BOMO	0		2.68±0.03	2.93±0.12	0.21±0.01	0.17±0.01	0.71±0.06
SDMO	0		2.26±0.09	4.78±0.57	0.20±0.01	0.22±0.01	1.10±0.14
SDMO	3	25	2.03±0.06	6.19±0.02	0.26±0.01	0.22±0.01	1.14±0.04
BOHO	0		1.27±0.11	1.69±0.04	0.37±0.04	0.49±0.03	2.28±0.11
SDHO	0		1.17±0.01	1.78±0.13	0.30±0.05	0.58±0.12	2.22±0.33
SDHO	3	25	1.21±0.01	1.54±0.06	0.28±0.01	0.40±0.04	1.65±0.11

xide value in a dosage-dependent way in LO-based dressings. This was also observed in mayonnaise and salad dressings enriched with polyunsaturated oils.[10,24] In contrast, the reducing effect on hexanal formation at the final time point, after 12 months of storage at 4 °C, was maximal at the lowest concentration of ascorbic acid. The effect of ascorbic acid in polyunsaturated lipids- enriched food emulsions was proposed to be on decomposition of peroxides and accumulation of secondary lipid oxidation products.[10] Maximal inhibition of hexanal formation (82%) obtained in SDL, at lowest level of ascorbic acid (0.25 g kg^{-1}), is suggestive of accumulation of ions of prooxidative metals in aqueous phase of the emulsion with increasing ascorbic acid levels,[25] but this is not supported with antagonistic effect of combination of ascorbic acid and EDTA on PV. Peroxide and hexanal contents of SDL and SDMO were however negatively influenced at all additional levels of ascorbic acid at the end of the storage, suggesting prevalence of antioxidant reaction mechanisms of ascorbic acid in whey and sunflower oil-based dressings. Strong antioxidant effect of ascorbic acid and ascorbyl palmitate on both hydroperoxides and hexanal formation in 40% o/w emulsion containing sunflower oil was also recorded by van Ruth *et al.* [26]

Addition of EDTA in concentration of 0.075 g kg^{-1} induced 80% and 60% inhibition of PV after 3 months of storage, in SDL and SDMO, respectively, which is comparable to the inhibition of peroxide formation found in mayonnaise and salad dressings enriched with n-3 polyunsaturated lipids.[10,26,27] Hexanal formation was also inhibited in SDL (after 12 months of storage at 4 °C) and SDMO (after 3 months of storage at 25 °C). According to literature,[10,27,28] EDTA prevented formation of n-3 and n-6 PUFA secondary oxidation products in whey and milk-based emulsions, enriched with n-3 polyunsaturated lipids or modified sunflower oil. The antioxidant effect of EDTA on volatile formation was influenced by emulsifiers, presence of n-3 unsaturated fatty acids, iron content and low pH. Protein-bound metal ions combined with low pH were suggested to be the most important factors of oxidation in mayonnaise and whey protein emulsified salad dressings enriched with fish oil, and EDTA was found to be the best antioxidant in these food systems.[10] EDTA is a synthetic metal-chelator

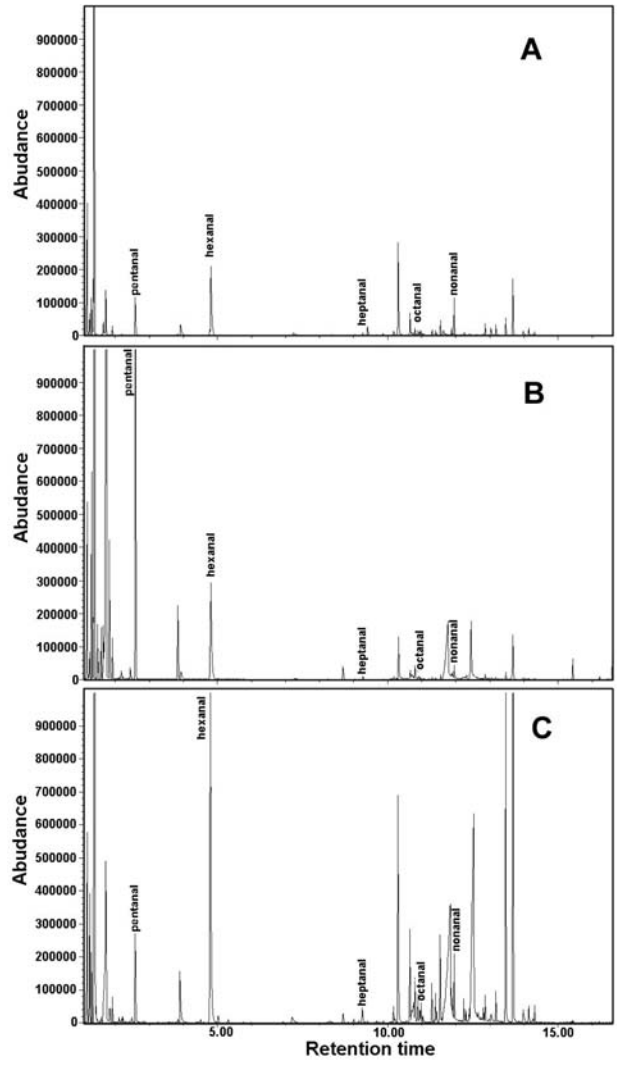

Figure 5. HS GC-MS chromatograms of volatiles in: A. bulk linoleic/α- tocopherol sunflower oil (BOL), 0th month; B. separated oil phase of salad dressing based on the same oil and whey (SDL), 0th month; C. separated oil phase of SDL, after storage of 12 months at 4 °C.

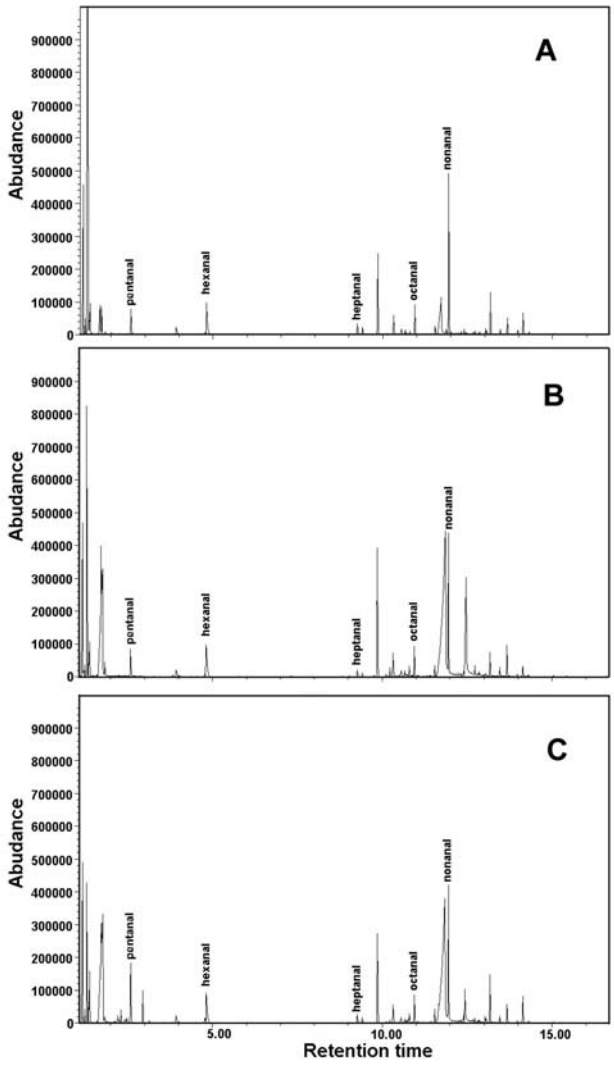

Figure 6. HS GC-MS chromatograms of volatiles in: A. high-oleic/α,β tocopherol sunflower oil (BOHO), 0th month. B. separated oil phase of salad dressing based on the same oil and whey (SDHO), 0th month. C. separated oil phase of SDHO, after storage of 3 months at 25 °C.

and therefore it is desirable to replace it with a naturally occurring one, like lactoferrin[29] or ascorbic acid. In our study, the best antioxidant effect regarding the primary and the secondary lipid oxidation products was obtained with ascorbic acid, which may be proposed as antioxidant in whey and sunflower oil-based dressings.

4. Conclusions

High-oleic acid content and tocopherols[30,7–10,31] are the most important genetically alternated qualities,[2–6] responsible for oxidative stability of sunflower oil. Whey-based salad dressings containing 40% cold-pressed high-oleic/α-, β- tocopherol sunflower oil were found to be oxidatively stable for 3 months at both 4 °C and 25 °C (peroxide value, PV 0.00–0.34 mmol O_2 kg^{-1}, hexanal 1.78–1.54 mg kg^{-1}), without the addition of antioxidants. Slight increase in primary and secondary oxidation products was recorded in dressings based on cold-pressed medium-oleic/α-, β- tocopherol sunflower oil at 25 °C after 3 months of storage, and it was reduced by both ascorbic acid (0.5 g kg^{-1}) or EDTA (0.075 g kg^{-1}). Ascorbic acid reduced PV by 80% and hexanal value by 32%, while EDTA reduced PV by 60% and hexanal value by 27 %. In linoleic/α- tocopherol type of salad dressings ascorbic acid (0.25–4.00 g kg^{-1}) induced the dose dependent reduction (83–100%) in PV and 82–73 reduction in hexanal value, while EDTA (0.075 g kg^{-1}) induced induced 75% reduction in PV and 76% reduction in hexanal value, after 12 months of storage at 4 °C. Briefly: the results showed that cold-pressed high- or medium- oleic sunflower oils with increased β- tocopherol content were of superior oxidative quality than linoleic/α- tocopherol oil in whey-based salad dressings. Ascorbic acid and EDTA efficiently prevented oxidation in medium-oleic/α-, β- tocopherol and linoleic/α- tocopherol oil- based dressings.

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6. Abbreviations

LO, linoleic/α- tocopherol oil; MO, medium-oleic/α-,β- tocopherol oil; HO, high-oleic/α-, β- tocopherol oil; BOL, bulk oil of linoleic/α- tocopherol type; BOMO, bulk oil of medium-oleic/α-, β- tocopherol type; BOHO, bulk oil of high oleic/α-, β- tocopherol type; SDL, salad dressing with linoleic/α- tocopherol oil; SDMO, salad dressing with medium oleic/α-, β- tocopherol oil; SDHO, salad dressing with high-oleic/α-, β- tocopherol oil; D, dressings; O, oil; aa, ascorbic acid; PUFA, polyunsaturated fatty acids.

7. References

1. E. N. Frankel, S.-W. Huang, J. Kanner, J. B. German, *J. Agric. Food Chem.*, **1994**, *42*, 1054–1059.
2. B. Perez-Vich, R. Garces, J. M. Fernandez-Martinez, *Theor. Appl. Genet*., *99*, **1999**, 663–669.
3. Ya. N. Demurin, S. G. Efimenko, T. M. Peretyagina, *Helia*, **2006**, *29*, 55–62.
4. Ya. N. Demurin, D. Skoric, D. Karlovic, *Plant breeding*, **1995**, *115*, 33–36.
5. D. Škorić, S. Jocić, N. Lečić, Z. Sakač, *Helia*, **2007**, *30*, 205–212.

6. M. Pacureanu-Joita, D. Stanciu, E. Petcu, S. Raranciuc, J. Sorega, *Romanian Agric. Res.*, **2005**, *22*, 23–26.
7. K.-H.Wagner, B. Isnardy, I. Elmadfa, *Eur. J. Lipid Sci. Technol*., **2004**, *106*, 44–51.
8. J. Velasco, M. C. Dobarganes, G. Marquez-Ruiz, *Eur. J. Lipid Sci. Technol*., **2004**, *106*, 325–333
9. S.-W. Huang, E. N. Frankel, J. B., German, *J. Agric. Food Chem*., **1994,** *42*, 2108–2114.
10. C. Jacobsen, M. Bruni Let, N. Skall Nielsen, A. S. Meyer, *Food Sci. Technol*., **2008**, *19*, 70–93.
11. M. Hu, D. J. Mc.Clements, E. A. Decker, *J. Agric. Food Chem*., **2003**B, 51, 1435–1439
12. M. Hu, D. J. Mc.Clements, E. A. Decker, *J. Agric. Food Chem*. **2003**A, 51, 1666–1700.
13. C. Jacobsen, A.S. Meyer, J. A. Adler-Nissen., *J. Food Lipids*, **1998**, *5*, 87–101.
14. International Standard Organisation, ISO 3960:**1998**. Animal and vegetable fats and oils-Determination of peroxide value.
15. International Standard Organisation, ISO 660:**1996**. Animal and vegetable fats and oils–Determination of acide value and acidity.
16. J. M. Snyder, E. N. Frankel and E. Selke, *J. Am. Oil. Chem. Soc.*, **1985**, *62*, 1675–1679.
17. K. Hartvigsen, P. Lund, L. F. Hansen, G. Holmer, *J. Agric. Food Chem*., **2000**, *48*, 4858–4867.
18. R. Karas, M. Skvarča and B. Žlender, *Food Technol. Biotechnol*, **2002**, *40*, 119–127
19. E. N. Frankel, *J. Sci. Food Agric*., **1991**, *54*, 495–511.
20. G. Garcia-Llatas, M. J. Lagarda, F. Romero, P. Abellan, R. Farre, Food Chem., **2006**, *101*, 1078–1086.
21. J. P. Roozen, E. N. Frankel, J. E. Kinsella, *Food Chem*., **1994**, *50*, 39–43.
22. A. Keszler, T. Kriska and A. Nemeth, *J. Agric. Food Chem*, **2000**, *48*, 5981–5985.
23. K. J. H.Warner, P. S. Dimick, G. R. Zeigler, R. O. Mumma and R. Hollender, *J. Food Sci.*, **1996**, *61*, 469–472.
24. C. Jacobsen, M. Timm and A. S. Meyer, *J. Agric. Food Chem.*, **2001,** *49*, 3947–3956.
25. C. Jacobsen, J. A. Adler-Nielsen and A. S. Meyer, *J. Agric. Food Chem.*, **1999,** *47*, 4917–4926.
26. S. M. van Ruth, J. P. Roozen, M. A. Posthumus, F. J. H. M. Hansen, *JAOCS*, **1999**, *76*, 1375–1381.
27. D. Đorđević, D. Kimm, D. J. Mc Clemens, and E. A Decker, *J. Food Sci*., **2004** (part A), *69*, C351–C355.
28. D. Đorđević, D. J. Mc Clemens, and E. A. Decker, *J. Food Sci*., **2004** (part B), *69*, C356–C352.
29. N. S. Nielsen, A. Petersen, A. S. Meyer, M. Timm-Heinrich, and C. Jacobsen, *J. Agric. Food Chem*., **2004**, *52*, 7690–7699.
30. J. P. Rozen, E. N. Frankel, and J. E. Kinsela, *Food Chem.*, **1994**, *50*, 39–43
31. V. Martincic and J. Golob, *Acta Chim. Slov.* **2001**, *48*, 575–584.

Povzetek

Prvič je opisan vpliv dodatka askorbinske kisline in EDTA na oksidativno stabilnost solatnih prelivov pripravljenih iz sirotke in sončničnih olj z različnimi vsebnostmi oleinske kisline in tokoferolov. Dodatek askorbinske kisline ali EDTA solatne prelive pripravljene iz sončničnih olj z nižjimi vsebnostmi oleinske kisline in tokoferolov varuje pred oksidacijo. V primeru uporabe sončničnih olj z višjimi vsebnostmi oleinske kisline in tokoferolov pa dodatki niso potrebni.