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Effect of essential oils on the oxidative stability of sunflower oil during storage

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Abstract: The main non-microbial cause of quality deterioration in lipid-containing food is lipid oxidation, the process in which, simplifying, unsaturated fatty acids react with molecular oxygen via a free radical mechanism. The use of substances with antioxidant properties during the manufacturing process can minimize the extent of lipid oxidation.

This research aimed to determine the effect of selected essential oils on the quality and oxidative stability of sunflower oils. Sunflower oils were obtained by refining and cold-pressing and their quality and oxidative stability were studied during the different storage conditions: 20 °C and 5 °C at dark and with light exposure.

Obtained results suggested that the antimicrobial and antioxidant properties of essential oils can positively affect the oxidative stability and consequently the quality of sunflower oils during storage, and implicate with further application of essential oils in the edible vegetable oil industry.

Keywords: Essential oils; laurel; lipid oxidation; mint; rosemary; sage; sunflower oil.

1 Introduction

Edible vegetable oils, produced by a mechanical process known as cold-pressing from sunflower seeds, pumpkin, and olives are considered as functional food products. They are very attractive to consumers, primarily because of their nutritional value, pleasant aroma and taste, stability, and positive effect on health. In the context of oil properties, there are big differences in the quality of refined and

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cold-pressed oils. Refined oils have a lower nutritional value and a lower price on the market, while cold-pressed oils are highly valued, have a dedicated nutritional use, and, therefore, can be susceptible to adulteration with much cheaper refined vegetable oils (Gogolewski et al. 2000; Wroniak et al. 2008).

Due to the limited shelf life, undesirable changes in the composition of oils are possible, manifested by a decrease in nutritional value and changes in organoleptic properties. The above occurs due to spoilage of oil, resulting in the loss of essential fatty acids, vitamins, and provitamins. The most common types of spoilage are oxidative and hydrolytic spoilage, caused by high temperature, moisture, and light. The process of oxidation of vegetable oils is inevitable, and the duration of the process depends on the composition of the oil itself, and on the presence of factors that can accelerate or slow down the oxidation process. It can be slowed down and even prevented by the addition of antioxidants that can be found naturally in the oil. The best-known natural antioxidants are tocopherols, polyphenols, and sterols. Apart from them, carotenoids and chlorophylls, pigments found in oils, also play a role in preventing oxidation. The addition of essential oils, as natural antioxidants, aims to increase the oxidative stability of vegetable oils (Bauer and Garbe 1985; Kuštrak 2005; Van de Braak and Leijten 1999).

This study aimed to determine the effect of selected essential oilson the oxidative stability of sunflower oils. The effect of essential oils were tested on not refined cold pressed and fully refined oils during thedifferent storage conditions in order to distignish does the production method can affect the oxidative stability modulation by essential oils. Determination of the positive and negative effects of selected essential oils on lipid oxidation may be useful in the efforts of increasing the oxidation stability and the shelf-life of edible vegetable oils.

2 Materials and methods

Sunflower oils, refined and cold-pressed, were used for the analysis. The refined oil was bought at the market in the suburbs of the city of Sarajevo. Cold-pressed sunflower oil was procured from an individual producer. The essential oils of rosemary, laurel, sage, and mint, with which the vegetable oil samples were spiked, were obtained from an individual producer.

After the initial analyses, sunflower oil samples were poured into previously sterilized 500 mL volumetric flasks. Each essential oil was added, individually to each edible oil sample in concentrations of 0.1%, 0.5, and 1%. The air in the space above the surface of the oil was approximately 3% of the packaging volume. During the testing period, oils from newly opened flasks were analyzed each time. After the completed treatment (120 days), at different temperatures: 20 °C in dark and light, and at +5 °C, control analyses were performed to establish the changes in the quality and stability of the oil before the foreseen preservation process. Initial analyzes were performed on six samples (two samples of edible oil and four samples of essential oils) and 26 samples of treated oils were analyzed after 120 days of storage at three different storage temperatures, namely in dark and in light at 20 °C and a refrigerator at +5 °C, which makes in total 78 analyzed samples (Tables 1 and 2).

Table 1: Description and labels of tested sunflower oil samples (industrial producer).

	Sample type	Sample ID
Sunflower oils		
Industrial producer		
1	Refined sunflower oil	R-S
2	Refined sunflower oil with 0.1% rosemary essential oil	R-S-R-0.1
3	Refined sunflower oil with 0.5% rosemary essential oil	R-S-R-0.5
4	Refined sunflower oil with 1% rosemary essential oil	R-S-R-1
5	Refined sunflower oil with 0.1% essential oil of laurel	R-S-L-0.1
6	Refined sunflower oil with 0.5% essential oil of laurel	R-S-L-0.5
7	Refined sunflower oil with 1% essential oil of laurel	R-S-L-1
8	Refined sunflower oil with 0.1% essential oil of sage	R-S-S-0.1
9	Refined sunflower oil with 0.5% essential oil of sage	R-S-S-0.5
10	Refined sunflower oil with 1% essential oil of sage	R-S-S-1
11	Refined sunflower oil with 0.1% essential oil of mint	R-S-M-0.1
12	Refined sunflower oil with 0.5% essential oil of mint	R-S-M-0.5
13	Refined sunflower oil with 1% essential oil of mint	R-S-M-1

Table 2: Description and labels of tested sunflower oil samples (individual producer).

	Sample type	Sample ID
Sunflower oils		
Individual producer		
1	Cold-pressed sunflower oil	CP-S
2	Cold-pressed sunflower oil with 0.1% rosemary essential oil	CP-S-R-0.1
3	Cold-pressed sunflower oil with 0.5% rosemary essential oil	CP-S-R-0.5
4	Cold-pressed sunflower oil with 1% rosemary essential oil	CP-S-R-1
5	Cold-pressed sunflower oil with 0.1% essential oil of laurel	CP-S-L-0.1
6	Cold-pressed sunflower oil with 0.5% essential oil of laurel	CP-S-L-0.5
7	Cold-pressed sunflower oil with 1% essential oil of laurel	CP-S-L-1
8	Cold-pressed sunflower oil with 0.1% essential oil of sage	CP-S-S-0.1
9	Cold-pressed sunflower oil with 0.5% essential oil of sage	CP-S-S-0.5
10	Cold-pressed sunflower oil with 1% essential oil of sage	CP-S-S-1
11	Cold-pressed sunflower oil with 0.1% essential oil of mint	CP-S-M-0.1
12	Cold-pressed sunflower oil with 0.5% essential oil of mint	CP-S-M-0.5
13	Cold-pressed sunflower oil with 1% essential oil of mint	CP-S-M-1

2.1 Determination of chemical composition and oil quality

2.1.1 Composition of fatty acids: Methyl esters of fatty acids were analyzed by the standard method (BAS EN ISO 12966-2:2012), and the composition of fatty acids was determined by the gas chromatograph system SHIMADZU QP2010 (Shimadzu, Tokyo, Japan). The conditions of the chromatographic determination were as follows: Supelco SP-2560 capillary column 100 m 0.25 mm, df 0.20 µm, injector and detector temperatures 220 °C, column temperature 170 °C, sample volume 1 µL. Helium was used as the carrier gas, with a flow rate of 0.9 mL min^{-1}.

2.1.2 Free fatty acids: The acidity of the oils and the content of free fatty acids were determined by the standard method (BAS EN ISO 660:2010).

2.1.3 Water content: Determination of the water content in the tested samples was performed on the automatic humidity analyzer Sartorius MA 35 (Goettingen, Germany), according to the standard method (BAS EN ISO 662 2006)

2.1.4 Peroxide number: The degree of fat oxidation and monitoring spoilage and viability was determined by the peroxide number using the Wheeler method (BAS EN ISO 3960:2012).

2.1.5 Determination of sensory quality of the oil: After adding 20 mL of oil to each glass cup (50 mL), the samples were tempered for 30 min in an oven at 50 ± 1 °C and the characteristic sensory properties (color, smell, taste, and aroma) were descriptively determined by the committee that did the sensory analysis. A point system of analytical descriptive tests was used to characterize and evaluate the sensory properties of quality (Dimić and Turkulov 2000). Sensory analysis of the oil during storage was carried out identically as previously described. In the case of changes in aroma intensity and the appearance of an unusual taste, the sample was characterized as stale/aged. The intensity of the aroma was evaluated with points ranging from 0-not noticeable to 5-very pronounced (Table 3).

Table 3: Description of quality assessments of sensory properties.

Score	Description of properties
5	Exceptional, inherent (typical), the optimal level of quality OPTIMUM QUALITY
4	Noticeable deviations or slight defects in quality MINOR DEVIATIONS FROM OPTIMUM QUALITY
3	Defects or defects in quality DEVIATIONS FROM OPTIMAL QUALITY
2	Marked to very marked flaws or defects in quality SIGNIFICANT DEVIATIONS FROM OPTIMUM QUALITY
1	Very pronounced flaws or defects in quality SIGNIFICANT DEVIATIONS FROM OPTIMAL QUALITY
0	Completely changed, atypical properties UNACCEPTABLE PRODUCT QUALITY

2.1.6 Index of refraction: The refractometer ABBE 2WAJ (Bioevopeak Co., Ltd. Shangon, China) was used to test the index of refraction. Determination of light refraction and sample density was done according to the standard method (BAS EN ISO 6320:2006).

2.1.7 Heavy metals and metalloids: Analysis of metals was performed in AA-7000F Dual Atomizer System (Shimadzu, Tokyo, Japan). Analysis of Iron (Fe) and Copper (Cu), was done using the standard method for determination of trace elements in foodstuffs (BAS EN ISO 14084:2003), while the analysis of Arsenic (As) and Nickel (Ni) was done using the internal methods (IM-OP-5.4-01-15-1-S & IM-OP-5.4-01-16-1-S 2018a,b). Sample preparation was done by the standard method of microwave digestion (BAS EN 13805:2015).

2.1.8 Determination of microbiological stability: Microbiological analyzes of the samples were performed using standardized methods, for the presence of the following microorganisms: *Enterobacteriaceae* (BAS EN ISO 21528-2:2008); *Listeria monocytogenes* (BAS EN ISO 11290-1:2005); *Yeasts* (BAS EN ISO 21527-1:2008); Mold (BAS EN ISO 21527-1:2008) and Aerobic mesophilic bacteria (BAS EN ISO 4833-1:2014).

2.1.9 Quality of essential oils: Analyzes of essential oils were performed on a gas chromatograph with a mass spectrophotometer GC/MS QP2010 (Shimadzu, Tokyo, Japan) system, according to the standardized method (18. BAS EN ISO 12966-2:2012) and European Pharmacopoeia (Ph. Eur.) Essential Oils requirements (Council of Europe 2017). The following quality parameters were tested: appearance, colour, and smell; relative density; index of reflection; optical rotation. In addition to quality parameters, GC/MS analyzes of the composition of essential oils were done.

2.1.10 Statistical analysis: Statistical analysis was performed using IBM SPSS Statistics 25 Premium software (IBM, USA). The significance of the differences between the defined variables was tested with ANOVA, Tukey-Kramer test, and *t*-test.

3 Results and discussion

3.1 Analyzes of the composition of fatty acids

Standard type sunfower oil is made up of about 15% saturated and 85% unsaturated fatty acids. About 14–43% and 44–75% of the unsaturated fatty acids are oleic (C18:1) and linoleic acids (C18:2), respectively. Sunfower oil is not only one of the most important vegetable oils for human nutrition but also one of the best quality vegetable oils for its fatty acid composition (Akkaya 2018).

The composition and content of fatty acids in examined samples were in accordance with the national Roolbook for this seed oil (Table 4).

There was a certain increase in the content of saturated and unsaturated fatty acids in the samples spiked with essential oils, but no significant deviation was observed from the values established by the national Rulebook (Vije}e ministara

Table 4: Composition and content (%) of fatty acids of refined and cold-pressed oils and samples spiked with essential oils – initial values.

Sample ID	Fatty acids [%]									
	16:0	18:0	18:1	18:2	18:3	SFA	MUFA	PUFA	MUFA/SFA	PUFA/SFA
R-S	6.00	4.20	20.20	55.00	0.10	10.20*	20.20**	55.10**	1.98	5.40
R-S-R-0.1	6.20	4.50	25.00	59.00	0.15	10.70*	25.00**	59.15**	2.34	5.53
R-S-L-0.1	6.30	4.60	23.20	60.00	0.15	10.90*	23.20**	60.15**	2.13	5.52
R-S-S–0.1	6.30	4.70	26.70	57.00	0.18	11.00*	26.70**	57.18**	2.43	5.20
R-S-M-0.1	6.40	4.50	25.00	58.30	0.14	10.90*	25.00**	58.44**	2.29	5.36
R-S-R-0.5	6.50	4.80	29.00	64.20	0.20	11.30*	29.00**	64.40**	2.57	5.70
R-S-L-0.5	6.60	4.90	27.30	65.00	0.20	11.50*	27.30**	65.20**	2.37	5.67
R-S-S-0.5	6.60	4.80	28.70	60.00	0.20	11.40*	28.70**	60.20**	2.52	5.28
R-S-M-0.5	6.70	4.80	28.40	61.00	0.20	11.50*	28.40**	61.20**	2.47	5.32
R-S-R-1	6.70	5.00	31.00	66.30	0.25	11.70*	31.00**	66.55**	2.65	5.69
R-S-L-1	6.80	5.10	30.20	68.20	0.25	11.90*	30.20**	68.45**	2.54	5.75
R-S-S-1	6.90	5.00	34.00	67.30	0.27	11.90*	34.00**	67.57**	2.86	5.68
R-S-M-1	6.90	5.00	32.00	65.20	0.23	11.90*	32.00**	65.43**	2.69	5.50
CP-S	6.00	3.50	20.50	62.30	0.06	9.50*	20.50**	62.36**	2.16	6.56
CP-S-R-0.1	6.50	4.00	25.30	64.20	0.07	10.50*	25.30**	64.27**	2.41	6.12
CP-S-L-0.1	6.40	4.00	24.00	63.10	0.07	10.40*	24.00**	63.17**	2.31	6.07
CP-S-S-0.1	6.70	4.20	25.20	64.00	0.08	10.90*	25.20**	64.08**	2.31	5.88
CP-S-M-0.1	6.80	4.40	26.40	63.50	0.08	11.20*	26.40**	63.58**	2.36	5.68
CP-S-R-0.5	6.90	4.60	29.00	66.00	0.10	11.50*	29.00**	66.10**	2.52	5.75
CP-S-L-0.5	6.70	4.50	28.30	65.00	0.10	11.20*	28.30**	65.10**	2.53	5.81
CP-S-S-0.5	7.00	4.70	28.40	67.20	0.12	11.70*	28.40**	67.32**	2.43	5.75
CP-S-M-0.5	7.10	4.80	28.10	65.50	0.10	11.90*	28.10**	65.60**	2.36	5.51
CP-S-R-1	7.20	5.10	30.10	68.50	0.15	12.30*	30.10**	68.65**	2.45	5.58
CP-S-L-1	7.00	5.00	30.00	67.00	0.15	12.0*	30.00**	67.15**	2.50	5.59
CP-S-S-1	7.40	5.50	31.00	69.00	0.20	12.90*	31.00**	69.20**	2.40	5.36
CP-S-M-1	7.50	5.40	32.50	68.40	0.15	12.90*	32.50**	68.55**	2.52	5.31

SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA Polyunsaturated fatty acids. *Level of significance $p < 0.5$. **Level of significance $p < 0.01$. ***Level of significance $p < 0.001$.

Bosne i Hercegovine 2011). The highest ratios of the tested groups of fatty acids were in the PUFA/SFA ratio (5.20%–6.56%). Significantly lower values were found in the MUFA/SFA ratios (1.98%–2.86%). These relationships, in the samples that are spiked with essential oils, did not show significant differences concerning the method of oil production (industrial/individual). These results are in accordance with those outlinedby Krizmanić et al. (2013).

No statistical difference in the content of saturated fatty acids (16:0 and 18:0) in the oils of industrial and individual producers was found in the initial samples ($p < 0.5$) and neither in the samples spiked with essential oils ($p < 0.5$). On the other

hand, a highly statistically significant difference ($p < 0.001$) in the content of unsaturated fatty acids (18:1, 18:2 and 18:3) between initial industrial oil (75.3%) and individual producers (82.86%) samples was found as well as between samples spiked with essential oils ($p < 0.01$).

According to the results of fatty acids content in the samples spiked with essential oils and stored for 12 months in the dark at 20 °C (Table 5), both saturated and unsaturated fatty acids are increased, with no deviations from the values established in the national Rulebook (Vije}e ministara Bosne i Hercegovine 2011). PUFA/SFA showed the highest (5.15%–6.32%) and MUFA/SFA had the lowest ratios

Table 5: Composition and content (%) of fatty acids of refined and cold-pressed oils and samples spiked with essential oils stored in the dark for 12 months at a temperature of 20 °C.

Sample ID	Fatty acids [%]									
	16:0	18:0	18:1	18:2	18:3	SFA	MUFA	PUFA	MUFA/SFA	PUFA/SFA
R-S	6.20	4.50	22.80	56.50	0.12	10.70**	22.80*	56.62*	2.13	5.29
R-S-R-0.1	6.30	4.90	26.00	59.50	0.18	11.20**	26.00*	59.68*	2.32	5.33
R-S-L-0.1	6.80	4.90	29.50	64.80	0.23	11.70**	29.50*	65.03*	2.52	5.56
R-S-S-0.1	6.90	5.50	33.00	66.90	0.28	12.40**	33.00*	67.18*	2.66	5.42
R-S-M-0.1	6.30	4.60	24.00	61.00	0.18	10.90**	24.00*	61.18*	2.20	5.61
R-S-R-0.5	6.80	5.00	27.80	65.50	0.22	11.80**	27.80*	65.72*	2.36	5.57
R-S-L-0.5	7.00	5.60	30.50	68.90	0.28	12.60**	30.50*	69.18*	2.42	5.49
R-S-S-0.5	6.40	4.80	27.10	57.50	0.20	11.20**	27.10*	57.70*	2.42	5.15
R-S-M-0.5	6.80	5.00	29.50	60.70	0.25	11.80**	29.50*	60.95*	2.50	5.17
R-S-R-1	7.00	5.80	35.00	67.90	0.30	12.80**	35.00*	68.20*	2.73	5.33
R-S-L-1	6.40	4.50	25.50	58.90	0.16	10.90**	25.50*	59.06*	2.34	5.42
R-S-S-1	6.80	4.90	28.80	61.60	0.23	11.70**	28.80*	61.83*	2.46	5.28
R-S-M-1	7.30	5.30	32.30	65.80	0.27	12.60**	32.30*	66.07*	2.56	5.24
CP-S	6.50	3.80	21.60	65.00	0.08	10.30**	21.60*	65.08*	2.10	6.32
CP-S-R-0.1	6.90	4.10	26.00	66.50	0.09	11.00**	26.00*	66.59*	2.36	6.05
CP-S-L-0.1	7.10	4.70	30.10	68.30	0.13	11.80**	30.10*	68.43*	2.55	5.80
CP-S-S-0.1	7.40	5.20	32.00	70.10	0.17	12.60**	32.00*	70.27*	2.54	5.58
CP-S-M-0.1	6.70	4.20	25.00	65.30	0.08	10.90**	25.00*	65.38*	2.29	6.00
CP-S-R-0.5	6.90	4.80	29.50	69.00	0.11	11.70**	29.50*	69.11*	2.52	5.91
CP-S-L-0.5	7.20	5.10	33.00	70.20	0.16	12.30**	33.00*	70.36*	2.68	5.72
CP-S-S-0.5	6.90	4.50	26.10	65.90	0.10	11.40**	26.10*	66.00*	2.29	5.79
CP-S-M-0.5	7.20	4.90	28.90	69.50	0.15	12.10**	28.90*	69.65*	2.39	5.76
CP-S-R-1	7.50	5.60	32.50	71.00	0.21	13.10**	32.50*	71.21*	2.48	5.44
CP-S-L-1	7.00	4.60	27.00	65.50	0.10	11.60**	27.00*	65.60*	2.33	5.66
CP-S-S-1	7.15	4.90	28.90	67.20	0.15	12.05**	28.90*	67.35*	2.40	5.59
CP-S-M-1	7.55	5.80	33.50	69.00	0.20	13.35**	33.50*	69.20*	2.51	5.18

SFA, Saturated fatty acids; MUFA, Monounsaturated fetty acids; PUFA, Polyunsaturated fatty acids. *Level of significance $p < 0.5$; **Level of significance $p < 0.01$; ***Level of significance $p < 0.001$.

(2.10%–2.73%) and these ratios did not show a significant difference in considering the method of oil production. Also, storage for 12 months in the dark at 20 °C did not affect the ratios between the PUFA/SFA and MUFA/SFA ratios. There was a high statistically significant difference ($p < 0.01$) in the content of saturated fatty acids (SFA) concerning the initial results and no statistically significant difference in the content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) compared to the initial results.

The addition of essential oils had the effect of increasing the content of both saturated and unsaturated fatty acids in the samples stored for 12 months in the light at 20 °C (Table 6), with no deviations from the established values according to the

Table 6: Composition and content (%) of fatty acids of refined and cold-pressed oils and samples spiked with essential oils stored in the light for 12 months in the light at a temperature of +20 °C.

Sample ID	Fatty acids [%]									
	16:0	18:0	18:1	18:2	18:3	SFA	MUFA	PUFA	MUFA/SFA	PUFA/SFA
R-S	6.20	4.50	21.00	57.50	0.12	10.70*	21.00*	57.62*	1.96	5.39
R-S-R-0.1	6.50	4.60	25.50	60.50	0.16	11.10*	25.50*	60.66*	2.30	5.46
R-S-L-0.1	6.80	4.90	30.00	65.00	0.22	11.70*	30.00*	65.22*	2.56	5.57
R-S-S-0.1	6.90	5.20	33.50	67.50	0.28	12.10*	33.50*	67.78*	2.77	5.60
R-S-M-0.1	6.30	4.80	24.00	61.50	0.16	11.10*	24.00*	61.66*	2.16	5.55
R-S-R-0.5	6.80	5.00	28.50	66.00	0.21	11.80*	28.50*	66.21*	2.42	5.61
R-S-L-0.5	7.00	5.20	31.00	69.50	0.25	12.20*	31.00*	69.75*	2.54	5.72
R-S-S-0.5	6.50	4.80	27.00	58.10	0.19	11.30*	27.00*	58.29*	2.39	5.16
R-S-M-0.5	6.80	4.90	29.50	62.00	0.22	11.70*	29.50*	62.22*	2.52	5.32
R-S-R-1	7.20	5.10	36.00	68.00	0.28	12.30*	36.00*	68.28*	2.93	5.55
R-S-L-1	6.50	4.50	28.50	59.00	0.15	11.00*	28.50*	59.15*	2.59	5.38
R-S-S-1	6.80	4.80	30.00	62.50	0.22	11.60*	30.00*	62.72*	2.59	5.41
R-S-M-1	7.00	5.10	33.80	66.00	0.25	12.10*	33.80*	66.25*	2.79	5.48
CP-S	6.00	3.80	20.60	62.50	0.07	9.80*	20.60*	62.57*	2.10	6.38
CP-S-R-0.1	6.50	4.00	25.80	64.50	0.08	10.50*	25.80*	64.58*	2.46	6.15
CP-S-L-0.1	7.00	4.70	29.30	66.50	0.12	11.70*	29.30*	66.62*	2.50	5.69
CP-S-S-0.1	7.30	5.20	30.50	68.80	0.17	12.50*	30.50*	68.97*	2.44	5.52
CP-S-M-0.1	6.40	4.10	24.20	63.40	0.08	10.50*	24.20*	63.48*	2.30	6.05
CP-S-R-0.5	6.70	4.60	28.50	65.30	0.11	11.30*	28.50*	65.41*	2.52	5.79
CP-S-L-0.5	7.10	5.10	30.10	67.60	0.16	12.20*	30.10*	67.76*	2.47	5.55
CP-S-S-0.5	6.80	4.30	25.30	64.30	0.09	11.10*	25.30*	64.39*	2.28	5.80
CP-S-M-0.5	7.10	4.75	28.50	67.50	0.13	11.85*	28.50*	67.63*	2.41	5.71
CP-S-R-1	7.50	5.55	31.20	69.20	0.22	13.05*	31.20*	69.42*	2.39	5.32
CP-S-L-1	6.80	4.50	26.50	63.80	0.10	11.30*	26.50*	63.9*	2.35	5.65
CP-S-S-1	7.10	4.90	28.20	65.80	0.13	12.00*	28.20*	65.93*	2.35	5.49
CP-S-M-1	7.50	5.50	32.60	68.50	0.18	13.00*	32.60*	68.68*	2.51	5.28

SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; *Level of significance $p < 0.5$; **Level of significance $p < 0.01$; ***Level of significance $p < 0.001$.

national Rulebook (Vije}e ministara Bosne i Hercegovine 2011). The highest ratios among the examined groups of fatty acids were for PUFA/SFA (5.16%–6.38%) and the lowest values were found in MUFA/SFA ratios (1.96%–2.93%) and there was no significant difference in these relations considering to the method of oil production. Also, storage of the samples for 12 months in the light at 20 °C did not affect the ratios between the groups.

There was no statistically significant difference in the content of saturated fatty acids (SFA) as well as in the content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in spiked and stored samples and the initial ones. Storage of the samples for 12 months in the light at a temperature of 20 °C, also, did not affect the content of SAFA, MUFA, and PUFA.

The content of saturated fatty acids (16:0 and 18:0), monounsaturated fatty acids (18:1) and polyunsaturated fatty acids (18:2 and 18:3) in the spiked samples stored for 12 months in dark at 5 °C were within the expected values according to the current Rulebook (Vije}e ministara Bosne i Hercegovine 2011) (Table 7). Also, established storage length and temperature did not affect the ratios between groups PUFA/SFA (5.22%–6.49%) and MUFA/SFA (5.22%–6.49%).

Moreover, the storage of 12 months at a temperature of 5 °C in dark did not affect the content of MUFA and PUFA.

3.2 Free fatty acids

Peroxides and hydroperoxides are the primary oxidation products of oxidative rancidity where oxygen reacts with unsaturated fatty acids in triglyceride in the presence of heat and light. This primary oxidation route can be inhibited by the intervention of antioxidants. In the case of the absence of such an intervention is not provided the secondary oxidation route starts producing aldehydes, and ketones, and termination occur at the free fatty acids formation stage (Nasirullah and Latha 2009). An increase in free fatty acids was observed in all spiked samples stored under different conditions (Table 8). The minimum value of free fatty acids was found in the samples origin from both refined and cold-pressed oils before treatment and it was 0.10% while a maximum value of free fatty acids was found in the cold-pressed oil samples with the addition of essential oils in the concentration of 1.0%, stored at a temperature of 20 °C in the dark and in the light, and in the refined oil samples spiked with laurel essential oil in concentration 1.0% stored at 5 °C and it was 0.45%.

A significant increase in free fatty acids was observed in all samples of refined oil spiked with essential oils and stored at a temperature of 20 °C in the light. This indicates that antioxidant components from essential oils, regardless of added concentration, did not affect the reduction of free fatty acid content.

Table 7: Composition and content (%) of fatty acids of refined and cold-pressed oils and samples spiked with essential oils stored in the dark for 12 months at 5 °C.

Sample ID	Fatty acids [%]									
	16:0	18:0	18:1	18:2	18:3	SFA	MUFA	PUFA	MUFA/SFA	PUFA/SFA
R-S	6.10	4.30	20.50	56.00	0.13	10.40*	20.50	56.13*	1.97	5.40
R-S-R-0.1	6.20	4.50	25.50	59.50	0.17	10.70*	25.50	59.67*	2.38	5.58
R-S-L-0.1	6.60	4.80	29.50	64.60	0.22	11.40*	29.50	64.82*	2.59	5.69
R-S-S-0.1	6.80	5.10	31.50	66.80	0.27	11.90*	31.50	67.07*	2.65	5.64
R-S-M-0.1	6.30	4.70	23.50	60.50	0.16	11.00*	23.50	60.66*	2.14	5.51
R-S-R-0.5	6.60	4.90	27.80	65.70	0.20	11.50*	27.80	65.90*	2.42	5.73
R-S-L-0.5	6.90	5.15	30.60	68.70	0.27	12.05*	30.60	68.97*	2.54	5.72
R-S-S-0.5	6.30	4.80	26.70	57.70	0.19	11.10*	26.70	57.89*	2.41	5.22
R-S-M-0.5	6.70	4.90	28.80	60.60	0.22	11.60*	28.80	60.82*	2.48	5.24
R-S-R-1	7.00	5.20	34.10	67.80	0.30	12.20*	34.10	68.10*	2.80	5.58
R-S-L-1	6.40	4.60	25.10	58.80	0.15	11.00*	25.10	58.95*	2.28	5.36
R-S-S-1	6.70	4.90	28.50	61.50	0.25	11.60*	28.50	61.75*	2.46	5.32
R-S-M-1	6.90	5.10	32.20	65.70	0.25	12.00*	32.20	65.95*	2.68	5.50
CP-S	6.10	3.60	20.80	62.88	0.07	9.70*	20.80	62.95*	2.14	6.49
CP-S-R-0.1	6.60	4.10	25.80	64.90	0.08	10.70*	25.80	64.98*	2.41	6.07
CP-S-L-0.1	7.00	4.80	29.50	66.70	0.11	11.80*	29.50	66.81*	2.50	5.66
CP-S-S-0.1	7.30	5.20	30.50	68.80	0.20	12.50*	30.50	69.00*	2.44	5.52
CP-S-M-0.1	6.50	4.15	24.20	63.50	0.08	10.65*	24.20	63.58*	2.27	5.97
CP-S-R-0.5	6.70	4.60	28.80	65.80	0.11	11.30*	28.80	65.91*	2.55	5.83
CP-S-L-0.5	7.10	5.15	30.50	67.90	0.15	12.25*	30.50	68.05*	2.49	5.56
CP-S-S-0.5	6.80	4.30	25.40	64.50	0.08	11.10*	25.40	64.58*	2.29	5.82
CP-S-M-0.5	7.10	4.80	28.90	67.70	0.15	11.90*	28.90	67.85*	2.43	5.70
CP-S-R-1	7.50	5.55	31.20	69.90	0.25	13.05*	31.20	70.15*	2.39	5.38
CP-S-L-1	6.80	4.40	26.80	63.60	0.10	11.20*	26.80	63.70*	2.39	5.69
CP-S-S-1	7.20	4.80	28.70	65.70	0.13	12.00*	28.70	65.83*	2.39	5.49
CP-S-M-1	7.50	5.45	32.80	68.50	0.18	12.95*	32.80	68.68*	2.53	5.30

SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; *Level of significance $p < 0.5$; **Level of significance $p < 0.01$; ***Level of significance $p < 0.001$.

In samples, of refined oilve with the addition of concentrations of 0.1%, 0.5%, and 1.0% of mint essential oil, a decrease in free fatty acids was observed at a temperature of 5 °C compared to the same sample before treatment and samples stored at a temperature of 20 °C in the dark and in the light. Refined sunflower oil with the addition of different types of essential oil and stored at 5 °C had shown better stability regardless of the conditions and strength of the added essential oil concentration. In the study conducted by Vujasinović et al. (2017), the content of free fatty acids in sunflower oil was higher compared to our results (0.40%–0.80%). Similar values are reported by Rauf et al. (2017), Abitogun et al. (2008) and Rabrenović (2011).

Table 8: Free fatty acids [%] in sunflower oil samples before and after specific treatment.

Sample ID	Free fatty acids [%]			
	Initial analysis	After 12 months at 20 °C in dark	After 12 months 20 °C in light	After 12 months at 5 °C
R-S	0.10	0.25	0.40	0.15
R-S-R-0.1	0.25	0.25	0.40	0.25
R-S-R-0.5	0.28	0.30	0.35	0.30
R-S-R-1	0.35	0.40	0.38	0.35
R-S-L-0.1	0.25	0.30	0.40	0.25
R-S-L-0.5	0.28	0.35	0.35	0.30
R-S-L-1	0.30	0.40	0.35	0.30
R-S-S-0.1	0.25	0.30	0.35	0.25
R-S-S-0.5	0.29	0.30	0.35	0.29
R-S-S-1	0.40	0.45	0.45	0.40
R-S-M-0.1	0.26	0.27	0.35	0.20
R-S-M-0.5	0.28	0.30	0.40	0.25
R-S-N-1	0.35	0.35	0.45	0.30
CP-S	0.10	0.15	0.25	0.15
CP-S-R-0.1	0.20	0.25	0.30	0.25
CP-S-R-0.5	0.25	0.30	0.35	0.28
CP-S-R-1	0.35	0.45	0.45	0.40
CP-S-L-0.1	0.15	0.20	0.25	0.20
CP-S-L-0.5	0.25	0.30	0.35	0.30
CP-S-L-1	0.40	0.45	0.40	0.45
CP-S-S-0.1	0.20	0.25	0.30	0.25
CP-S-S-0.5	0.25	0.30	0.30	0.30
CP-S-S-1	0.30	0.35	0.35	0.35
CP-S-M-0.1	0.18	0.20	0.25	0.20
CP-S-M-0.5	0.20	0.25	0.30	0.25
CP-S-M-1	0.25	0.30	0.35	0.30

There was a statistically significant difference in the content of free fatty acids, between spiked samples based on refined and cold-pressed sunflower oil, before treatment, as well as after storage for 12 months at 20 °C in the dark and in the light. Also, there was no statistically significant difference between samples before treatment and after storage at 5 °C (Table 9).

3.3 Water and volatile substances in the oil

Based on the water content values shown in Table 10, before treatment and 12 months after the storage in the different storage conditions, a certain increase in

Table 9: The effect of defined treatment on the content of free fatty acids.

Storage conditions	Samples	
	q	*p*
Initial analysis versus at 20 °C in dark	7.11	<0.001***
Initial analysis versus at 20 °C in light	13.70	<0.001***
Initial analysis versus at 5 °C	3.13	>0.05
At 20 °C in dark versus at 20 °C in light	6.60	<0.001***
At 20 °C in dark versus at 5 °C	3.98	<0.05*
At 20 °C in light versus at 5 °C	10.58	<0.001***

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

Table 10: Water content in sunflower oil before treatment and after 12 months of storage with the addition of different essential oils.

Sample ID	Water content [%]			
	Before the treatment	After 12 months of storing at 20 °C in dark	After 12 months of storing at 20 °C in light	After 12 months of storing at 5 °C
R-S	0.01	0.01	0.03	0.02
R-S-R-0.1	0.10	0.10	0.15	0.12
R-S-R-0.5	0.18	0.17	0.20	0.20
R-S-R-1	0.25	0.24	0.25	0.30
R-S-L-0.1	0.14	0.15	0.15	0.15
R-S-L-0.5	0.17	0.15	0.19	0.20
R-S-L-1	0.19	0.18	0.20	0.20
R-S-S-0.1	0.18	0.18	0.20	0.19
R-S-S-0.5	0.19	0.17	0.20	0.19
R-S-S-1	0.30	0.28	0.32	0.30
R-S-M-0.1	0.15	0.15	0.1	0.15
R-S-M-0.5	0.19	0.18	0.18	0.19
R-S-M-1	0.25	0.20	0.20	0.25
CP-S	0.20	0.18	0.15	0.15
CP-S-R-0.1	0.25	0.22	0.20	0.20
CP-S-R-0.5	0.30	0.25	0.25	0.25
CP-S-R-1	0.35	0.30	0.30	0.30
CP-S-L-0.1	0.28	0.25	0.25	0.25
CP-S-L-0.5	0.30	0.25	0.25	0.25
CP-S-L-1	0.35	0.30	0.30	0.30
CP-S-S-0.1	0.25	0.21	0.24	0.20
CP-S-S-0.5	0.29	0.25	0.25	0.25
CP-S-S-1	0.35	0.30	0.30	0.30
CP-S-M-0.1	0.25	0.23	0.25	0.23
CP-S-M-0.5	0.30	0.25	0.28	0.26
CP-S-M-1	0.35	0.35	0.32	0.31

water content could be observed. The minimum water content (0.01%) was found in the refined sunflower oil sample origin from the industrial producer before the treatment, while the maximum value (0.35%) was in the cold-pressed oil sample spiked with 1% of essential oil, as well as with a cold-pressed sample spiked with 1% of mint oil and stored at a temperature of 20 °C in the dark.

No significant increase in water content was observed considering the initial results and results obtained after 12 months of storage in different conditions. These results agreed with that reported by Rauf et al. (2017).

3.4 Peroxide number of oil

As it have been already mentioned the oxidation process of oils is complex and peroxide number is applicable for the assessment of peroxides formation in the early stages of oxidation (Popa et al. 2017). In this study, an increased value of the peroxide number was observed in all spiked samples except for the sample of refined sunflower oil with the addition of 1% laurel oil (Table 11).

The minimum value of 0.11 mmol O_2 kg^{-1} of oil was in initial sample of refined oil spiked with 1% of laurel oil, while the maximum value of 8.20 mmol O_2 kg^{-1} oil was in cold-pressed sample spiked with the same essential oil and the same concentration and stored at a temperature of 20 °C in the light.

An increase in the peroxide number was recorded in all samples in proportion to the increase in the concentration of essential oils. The greatest increase in peroxide numbers was observed in samples stored at 20 °C in the light. This could mean that the antioxidant components from the essential oils, regardless of the added concentration, did not affect the reduction of the peroxide number content. Abitogun et al. (2008) indicated slightly higher values of peroxide numbers in sunflower oils compared to this study (12.6 mmol O_2 kg^{-1}).

Statistically, a significant difference was found in the value of the peroxide numbers between samples prepared with refined and samples prepared with unrefined sunflower oils, in the initial analyzes and after 12 months of storage at 20 °C in the light and at 5 °C in the refrigerator ($p < 0.001$).

3.5 Sensory quality of the oil

As reported, the essential oils of spices and herbs are able to be employed in the catering/cosmetic industry since they can release a variety of tempting aromas and pleasant flavors. Therefore, the odor of sunflower oil samples could be influenced by the flavor of added essential oils (Meng et al. 2021). The tested samples were initially

Table 11: Peroxide number oil samples before treatment and after storage of 12 months with the addition of different essential oils and concentrations.

Sample ID	Peroxide number [mmol O_2 kg^{-1}]			
	Initial analyses	After 12 months of storing at 20 °C in dark	After 12 months of storing at 20 °C in light	After 12 months of storing at 5 °C
R-S	0.12	4.50	5.60	1.00
R-S-R-0.1	0.66	2.80	4.60	2.00
R-S-R-0.5	1.55	4.50	5.60	3.00
R-S-R-1	2.40	6.00	6.70	3.00
R-S-L-0.1	0.11	3.50	4.80	1.80
R-S-L-0.5	1.34	4.05	5.80	4.00
R-S-L-1	3.50	6.30	6.40	4.60
R-S-S-0.1	0.67	4.10	5.50	2.20
R-S-S-0.5	1.78	4.70	6.40	3.10
R-S-S-1	3.20	6.50	6.60	4.50
R-S-M-0.1	0.70	3.80	4.80	1.90
R-S-M-0.5	1.80	4.50	5.50	2.50
R-S-M-1	2.80	5.80	6.00	3.40
CP-S	1.20	2.00	4.50	1.90
CP-S-R-0.1	2.20	2.50	6.50	2.50
CP-S-R-0.5	4.10	4.90	7.50	5.50
CP-S-R-1	5.70	6.50	8.00	6.00
CP-S-L-0.1	2.00	2.50	4.00	2.50
CP-S-L-0.5	5.10	6.00	7.10	5.90
CP-S-L-1	7.00	7.50	8.20	7.50
CP-S-S-0.1	3.10	3.80	6.30	4.50
CP-S-S-0.5	5.10	5.90	7.50	6.50
CP-S-S-1	7.10	7.90	7.90	7.90
CP-S-S-0.1	2.50	3.00	6.00	4.00
CP-S-M-0.5	3.10	4.80	7.00	5.00
CP-S-M-1	5.00	5.20	7.50	6.10

evaluated with a score of five for all the tested characteristics (colour, odor, taste) and there were no observed significant changes in them after storing in determining condition. On the other hand, in the samples spiked with the essential oils, changes in taste and odor were determined with the evaluation grade 3. The colour in the same samples retained the appropriate properties and was evaluated with grade 5 meaning there were no deviations in quality. The spiked samples stored at the defined storing condition showed changes in sensory quality. In general, in all samples, the changes are especially noticeable in the colour of the oil, where the samples were rated with 4.

Somewhat bigger changes were reflected in the taste and smell of the oil and the samples were graded with 3, indicating deviations from the optimal quality.

3.6 Index of refraction

The refractive index is considered one of the most important physical characteristics, as it is useful for estimating the degree of their saturation as well as for identification processing purposes, establishing their purity, and observing the progress reaction such as catalytic hydrogenation, oxidation, and isomerization (Abd-ElGhany et al. 2010). An increase in the value of the index of refraction was observed in all spiked samples. The minimum value of 1.462 was determined in the refined and samples prepared with unrefined sunflower oils before the treatment. The maximum value of 1.476 was for individual sunflower oil samples with the addition of 1% sage essential oil, after 12 months of storage at a temperature of 20 °C in the dark and at 5 °C in a refrigerator (Table 12).

There was an increase in the value of the index of refraction in all samples in proportion to the increase in the concentration of added essential oils. The biggest increase was in all oil samples from refined producers with different additions of essential oils and different concentrations stored at a temperature of 20 °C in the light indicating that the antioxidant components from the essential oils, regardless of the added concentration, still influenced the index of refraction. The results of the analysis of the index of refraction in this study showed slightly lower values compared to the results presented in the study by Abitogun et al. (2008), where the mean value was 1.475. There was no statistically significant difference in the value of the index of refraction between samples originating from refined and samples prepared with unrefined sunflower oils, regardless of the storage conditions.

3.7 Heavy metals and metalloids

The results of heavy metals content in all analyzed samples were in the line with the acceptable value according to the national Rulebook (Vije}e ministara Bosne i Hercegovine 2011) and consistent with the results reported by Farzin et al. (2014) and Hashemi et al. (2017) (Table 13).

The results of the analysis, show that the method of production of sunflower oil has a highly significant effect ($p < 0.001$) on the content of As, Cu, Fe, and Ni. On the other hand, the addition of essential oils, in different concentrations (0.1%, 0.5%, and 1%), did not affect the content of As, Cu, Fe, or Ni, regardless of the method of production.

Table 12: Index of refraction in the samples before treatment and after 12 months with the addition of different essential oils and concentrations.

Sample ID	Index of refraction [*n*]			
	Initial analyses	After 12 months of storing at 20 °C in dark	After 12 months of storing at 20 °C in light	After 12 months of storing at 5 °C
R-S	1.462	1.468	1.469	1.465
R-S-R-0.1	1.468	1.468	1.474	1.468
R-S-R-0.5	1.468	1.468	1.474	1.468
R-S-R-1	1.468	1.470	1.470	1.469
R-S-L-0.1	1.465	1.468	1.469	1.467
R-S-L-0.5	1.467	1.467	1.469	1.468
R-S-L-1	1.468	1.469	1.470	1.469
R-S-S-0.1	1.467	1.469	1.470	1.468
R-S-S-0.5	1.467	1.468	1.469	1.468
R-S-S-1	1.468	1.470	1.469	1.468
R-S-M-0.1	1.466	1.467	1.469	1.468
R-S-M-0.5	1.467	1.468	1.470	1.468
R-S-M-1	1.468	1.470	1.471	1.468
CP-S	1.462	1.465	1.467	1.463
CP-S-R-0.1	1.465	1.466	1.468	1.465
CP-S-R-0.5	1.468	1.469	1.470	1.468
CP-S-R-1	1.470	1.473	1.473	1.472
CP-S-L-0.1	1.463	1.464	1.467	1.465
CP-S-L-0.5	1.467	1.468	1.470	1.468
CP-S-L-1	1.472	1.475	1.473	1.475
CP-S-S-0.1	1.464	1.466	1.468	1.465
CP-S-S-0.5	1.467	1.469	1.469	1.468
CP-S-S-1	1.474	1.476	1.475	1.476
CP-S-M-0.1	1.464	1.467	1.468	1.466
CP-S-M-0.5	1.466	1.468	1.469	1.467
CP-S-M-1	1.468	1.470	1.472	1.468

3.8 Microbiological correctness

In the initial analyses, the presence of *Enterobacteriaceae*, *L. monocytogenes*, yeasts, and molds have not been established. The total number of aerobic bacteria isolated in all tested samples with and without the addition of essential oils in different concentrations was in the amount of 10 cfu g^{-1} (mL) for industrial producer samples and 25 cfu/g (mL) for individual producer samples. After storage for 12 months in three different conditions (20 °C in the light, 20 °C in the dark and 5 °C in the refrigerator) in

Table 13: Content of heavy metals and metalloids in sunflower oil – initial analysis.

Sample ID	Heavy metals content [mg kg^{-1}]			
	Arsenic (As)	Copper (Cu)	Iron (Fe)	Nickel (Ni)
R-S	0.0053	0.0102	0.8903	0.0580
R-S-R-0.1	0.0055	0.0100	0.9021	0.0600
R-S-R-0.5	0.0054	0.0098	0.9009	0.0563
R-S-R-1	0.0057	0.0093	0.9121	0.0566
R-S-L-0.1	0.0055	0.0103	0.9043	0.0587
R-S-L-0.5	0.0057	0.0108	0.9031	0.0556
R-S-L-1	0.0059	0.0097	0.9008	0.0576
R-S-S-0.1	0.0058	0.0099	0.8804	0.0590
R-S-S-0.5	0.0057	0.0100	0.9052	0.0542
R-S-S-1	0.0059	0.0104	0.8900	0.0566
R-S-M-0.1	0.0060	0.0102	0.9000	0.0500
R-S-M-0.5	0.0048	0.0099	0.9026	0.0548
R-S-M-1	0.0052	0.0094	0.9066	0.0576
CP-S	0.0085	0.0090	1.1001	0.1211
CP-S-R-0.1	0.0085	0.0089	1.1098	0.1254
CP-S-R-0.5	0.0091	0.0091	1.1000	0.1297
CP-S-R-1	0.0088	0.0092	1.1064	0.1265
CP-S-L-0.1	0.0078	0.0100	1.1092	0.1200
CP-S-L-0.5	0.0099	0.0094	1.1032	0.1254
CP-S-L-1	0.0081	0.0092	1.1098	0.1198
CP-S-S-0.1	0.0080	0.0094	1.0960	0.1230
CP-S-S-0.5	0.0085	0.0090	1.0031	0.1232
CP-S-S-1	0.0083	0.0095	1.1087	0.1221
CP-S-M-0.1	0.0078	0.0088	1.0978	0.1290
CP-S-M-0.5	0.0080	0.0090	1.0087	0.1209
CP-S-M-1	0.0077	0.0088	1.0048	0.1254

all tested samples of sunflower oil, no tested microorganisms were isolated. These results are following the results reported by Pešić-Mikulec (2005). Similar values, i.e. trace microorganisms in sunflower oils are also reported by Pavlović (2006)..

3.9 Gas chromatography-mass spectrometry of essential oils

Analysis of the physicochemical constants of the given essential oil showed that there were certain minimal deviations from the prescribed values set in the Ph. Eur. (Council of Europe 2017). With rosemary essential oil, deviations were observed in

relative density and optical rotation, with sage essential oil, deviations were observed in relative density and the index of refraction, while with mint essential oil, the deviation was observed in relative density. These quality parameters are approximately the same as those specified for the given type of oil by Smelčerović et al. (2016).

Table 14 shows the results of the GC-MS analysis of essential oils, with the percentage representation of certain identified substances.

The rosemary essential oil is composed mainly of the monoterpenes (99%): *oxides*, which include cineole (8.6%) and camphor (8.8%), *hydrocarbons* characterized by pinenes (a-pinene 14% and b-pinene 12%) and camphene (11.2%) and *alcohols* dominantly presented by borneol (4.75%) and a-terpinol (1.11%). There was some deviation in the rosemary essential oil composition in comparison to determined values by Ph. Eur. (Council of Europe 2017) which refer to the sufficient content of α-pinene, 1,8-cineole, limonene, and camphor and significantly higher content of β-pinene, borneol, and bornyl acetate. A similar composition of rosemary essential oil is presented in the study by Miladi et al. (2013).

The composition of the laurel essential oil was within the values specified in other studies. The obtained results were consistent with that reported by Derwich et al. (2009).

The sage essential oil consists dominantly of monoterpenes, α-thujone (16.50%), camphor (45.00%), and 1,8-cineole (8.3%). Monoterpene carbohydrates were represented in the form of α-pinene (2.30%), β-pinene (2.30%), camphene (8.50%), and limonene (3.75%). The most significant alcohol component was borneol (1.80%). The concentration of borneol acetate oxide was 5.5%. Sesquiterpenes were present in the form of α-humulene (2.50%), while other components were below 1%. There were certain deviations from the prescribed norms in the Pharmacopoeia in the content of α-pinene, sabinene, 1,8-cineole, and camphor. Despite this, the tested oil satisfies and fulfills the high-quality criteria for a given type of essential oil and indicates that it is Dalmatian sage due to the specific percentage ratio of thujone and cineole as well as the total content of ketones calculated as thujone. Similar results are presented in the study of Tosun et al. (2014). The most dominant components in mint essential oil were fractions of monoterpene cyclic alcohols in the form of menthol (41.50%), menthone (31.11%), isomenthone (9.64%), and 3-octanol (6.50%). Oxide fraction is mainly present in the form of 1.8 cineol (5.82%). The fraction of monoterpene hydrocarbons are characterized by pinenes (a-pinene 2.20% and b-pinene 1.20%). Mint essential oil fully corresponds to the quality parameters given in the monograph of mint essential oil in the European Pharmacopoeia (Council of Europe 2017).

Table 14: Composition of essential oils.

Rosemary essential oil		Laurel essential oil		Sage essential oil		Mint essential oil	
[%]	Components	[%]	Components	[%]	Components	[%]	Components
0.01	Tricyclene	2.10	α-Terpinene	0.20	*cis*-salvene	0.24	Butanal-3-methyl
0.05	α-Thujene	2.49	Terpinen-4-ol	0.01	Sabinyl acetate	0.46	3-Hexen-1-ol
14.00	α-Pinene	1.90	Linalool	0.03	Linalyl acetate	2.20	α-Pinene
11.22	Camphene	51.00	1.8-Cineole	0.14	α-Thujene	0.90	Camphene
12.00	β-Pinene	1.20	α-Terpineol	0.20	Tricyclene	1.20	β-Pinene
3.30	β-Myrcene	6.00	Sabinen	2.30	α-Pinene	0.46	Octen-3-ol
7.90	α-Phellandrene	1.90	Bornyl acetate	8.50	Camphene	41.50	Menthole
1.11	α-Terpineol	0.10	Terpinolene	0.05	Sabinen	2.20	β-Myrcene
1.75	*p*-Cymene	1.75	Methyl-eugenol	2.30	β-Pinene	6.50	3-Octanole
2.00	Trifluoroacetyl-α-terpineol	8.90	α-Terpinyl acetate	0.30	Linalool	31.11	Menthone
8.60	1.8-Cineole	0.10	Germacrene D	0.25	α-Terpinen	0.50	Phellandrene
0.05	Limonene	5.15	Limonene	0.24	*p*-Cymene	0.70	Terpinene
1.90	Carene	1.25	α-Phellandrene	3.75	Limonene	3.40	Limonene
1.40	*cis*-α-terpineol	3.80	α-Pinene	8.30	1.8-Cineole	5.82	1.8-Cineole
0.01	Terpinolene	3.10	β-Pinene	0.45	γ-Terpinene	1.44	*cis*-ocimen
0.01	Cineole	1.10	Myrcene	1.80	Borneol	0.40	Tepinolene
0.05	Linalool	0.15	(Z)-3-Hexenol	16.50	α-Thujone	9.64	Isomenthone
3.40	2-Bornanon	0.10	Camphene	0.50	Thujone	3.44	Menthofuran
8.80	Camphor	1.00	*o*-Terpinene	45.00	Camphor	0.54	Carvone
3.40	Isoborneol	0.12	*p*-Cymene	0.40	Terpinen-4-ol	2.56	Pulegone
4.75	Borneol	0.50	Eugenol	0.55	α-terpineol	4.42	Mentyl acetate
0.10	*p*-Menthol	0.55	β-sabinene	5.50	Bornyl acetata	0.12	Isopulegone
6.50	Bornyl acetate	0.45	3-Carene	1.55	*trans*-β-caryophyllene	0.80	*Trans*-β-caryophyllene
1.35	Verbenone	0.30	Isobornyl acetate	2.50	α-Humulene	0.30	α-Kadinen
		0.20	α-Thujone				
		0.20	β-Elemol				

4 Conclusions

Adding the essential oil to the sunflower oil did not affect the fatty acid compositions during the selected storage condition, as well as water content, microbiological stability, or heavy metals and metalloids contents. However, the index of refraction showed an increment in value in all samples after adding the essential oils. The same is found regarding the peroxide number except for the sample of refined sunflower oil with the addition of 1% laurel oil. This founding can indicate that essential oils may have acted as prooxidants. In the context of free fatty acids, there were found statistical differences in decreasing the free fatty acids values between the non-treat and samples spiked with mint essential oil stored at for 12 months at 5 °C in the dark, as well as between the cold-pressed and refined oil samples spiked with all types of essential oils and stored for 12 months at 20 °C in the dark and in the light. Regarding the sensory quality of the oil, in the samples spiked with the essential oils, changes in taste, color, and smell were determined initially and during the defined storing condition. The quality of the selected essential oils manly fulfilled the quality criteria requested according to the Council of Europe (2017).

Based on the obtained results, the possibility of further use of certain essential oils in terms of improving the properties and oxidative stability of edible vegetable oils, and their further application in the oil industry, could be assumed but more extensive research is needed in order to establish the proper type of essential oil as well as its concentration to secure the antioxidative effect of added essential oil and minimize the undesirable effect on sensory properties of sunflower oil. Consideration should include the evaluation of the potentially toxic effects of essential oil such as allergy, hepato or neurotoxicity.

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