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Reducing the oxidation of cold pressed sunflower oil by adding rosemary or parsley

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Abstract
Cold-pressed sunflower oil is produced by pressing the highest quality sunflower seeds. They are less stable and easily oxidized. It is important to extend the shelf life of these oils by preventing and slowing down the oxidation process. Subject to this analysis was the "Super Vita" cold pressed sunflower oil, with and without adding rosemary and parsley. The degree of oxidation was determined through the peroxide value (PV), taking into volume of the oil, the storage time and the antioxidant properties of the additives. The oils were stored under the same conditions, at room temperature and in dark, over a four-week period. In oils without additives, continuous oxidation begins right after opening. Smaller volume oils have a higher peroxide value than higher volume oils. After the fourth week, the peroxide value of smaller volume oils without additives is above the permissible limitsin accordance with the Rulebook. Peroxide values in all analysed oils with added rosemary and parsley are exceptionally low and below the permissible limits according to the Rulebook. In oils with added rosemary, oxidation begins after the third week. In oils with added parsley, oxidation begins after the second week. The peroxide value is directly proportional to the period in storage of cold pressed oil, and inversely proportional to the volume of oil in the bottle. There is a difference of statistical significance between the peroxide value of oils without additives and the oils with added rosemary and parsley. Rosemary and parsley as natural antioxidants added to analyzed oils greatly reduce the oxidation process.

Keywords: cold pressed sunflower oil, oxidation, peroxide value, rosemary, parsley

Introduction

Vegetable oils are an important part of a quality diet due to the health benefits of essential fatty acids and the benefits of minor components such as tocopherols, sterols, phenolic compounds, squalene, carotenoids, and others (Dimić, 2005; Konuskan *et al*l 2018) [6, .14] Sunflowers are one of the most important oilseed crops. Sunflower oil contains high-value components such as liposoluble vitamins (A, D, E and K), phytosterols, natural pigments and phospholipids (1 to 5%) (Evrard *et al*l, 2007) [9]. Sunflower oil, which contains a high percentage of linoleic acid (omega 6) is one of the most common oils (Dimić, 2005; Dimić *et al*l 2005) [6]. Although edible oils are a rich source of fatty acids and bioactive components, processing and refining cause adecline in the nutritional content of oils. As a result, unprocessed cold-pressed oils are increasingly in demand (Dimić, 2005; Pal *et al*l 2015) [6, 20]. Cold-pressed and refined oils differ in their sensory attributes, chemical composition, nutritional value, and oxidative stability (Pavlovska *et al*l, 2016) [21]. In commercial refinement, major changes occur in the physical chemical properties and stability of the oil, and the effect on the composition of the fatty acids is negligible (Shah *et al*l, 2018) [24]. Cold-pressed oils, in addition to essential fatty acids, also contain antioxidants, such as vitamin E and selenium (Butinar *et al*l, 2011) [5]. Cold-pressed sunflower oil is valued for its favourable sensory attributes and high biological value (Dimić *et al*l, 2015; Ramadan, 2013) [6, 22]. Due to the high percentage of unsaturated fatty acids, cold-pressed oils are difficult to sustain, and easily oxidize and spoil (Dimić, 2005) [6].

The process of lipid oxidation in edible oils is influenced by many factors such as exposure to light, heat treatment, the presence of oxygen, period in storage and the presence of antioxidants (Moigradean *et al*l, 2014; Kockritz and Martin, 2008; Keszler *et al*l, 2000; Grosshagauer *et al*l, 2019; Valery *et al*l, 2020) [18, 12, 11, 26]. Peroxides and hydroperoxides, which are the main oxidizing products of oils, are denoted by a peroxide value (Valery *et al*l, 2020) [26].

It is particularly important to reduce the oxidation of unsaturated fatty acid chains, which causesrancidity and spoilage of vegetable oils, which reduces their quality. Oxidation by-products are created that adversely affect the organoleptic properties of oils (Kockritz and Martin, 2008; Keszler *et al*l 2000) [13, 12]. Oxidation by-products are very harmful to human health and are the cause of many diseases (Saad *et al*l 2006; Barrera *et al*l 2018) [23, 2]. The process of autoxidation cannot be completely prevented, but it can be delayed and reduced by the addition of anti-oxidants that can donate hydrogen atoms to free radicals and thus become stable products. To protect against oxidation, natural and synthetic antioxidants are added to the oils (Barrera, 2012; Eftinzjijoska and Pavlovska, 2019; Mezza *et al*l, 2018) [8, 17]. Nowadays, natural antioxidants from herbs and spices are increasingly being used. Of the natural antioxidants, rosemary is widely accepted as one of the most antioxidant spices (Eftinzjijoska and Pavlovska, 2019; Martinez *et al*, 2013) [8, 16]. Antioxidant activity is due to several components of the rosemary extract, which mainly belong to the group of phenolic acids, flavonoid diterpenes and triterpenes (Martinez *et al*, 2013) [16]. Rosemary essential oil has antibacterial, antifungal and antioxidant effects and is used to prevent cancer (Wang *et al*l, 2018) [27]. Decreased lipid oxidation is due to the high content of carnosine and rosemary acid, which is twice as active as synthetic antioxidants (Genena *et al*l 2008) [10]. Parsley is an important plant because it has a great antioxidant effect (Bozhko *et al*l, 2018) [4]. The most important active

components of parsley are flavonoids, coumarins, and vitamin C. Flavonoids have anti-inflammatory, antioxidant, and anticancer effects (Andrei *et al*, 2018; Trifunschi and Ardelean, 2012).

Materials and Methods

Materials

Cold pressed sunflower oil from the manufacturer "Super Vita", produced in the Republic of Macedonia, was analysed. Twelve 750 mL volume bottles of oil were used. The packaging of the oils is transparent and plastic. The oils were selected randomly from markets in the city of Bitola in the Republic of Macedonia. During the entire analysis period, the oils were stored at room temperature and in dark. The peroxide value was determined, which is an indicator of the degree of spoilage or oxidation of the oil. This was determined in accordance with the ISO 3960: 1998 method. The volume of the oils analysed was varied in different bottles. Four of the bottles were filled with 750 mL volume (full bottle), four bottles with 560 mL (¾ bottle), four bottles with 370 mL (½ bottle) and four bottles with 190 mL (¼ bottle). Four bottles of different volumes were stored without additives. 20 g of fresh rosemary was added to 4 bottles of different volumes and 20 g of fresh parsley was added to four bottles of different volumes. Spices were weighed on a digital scale with an accuracy of 0.01 g (WTC 2000, WL-210-0001). The peroxide value of all oils was determined, i.e. the oils without additives and the oils with the addition of rosemary or parsley after a period of one, two, three and four weeks.

Determination of peroxide value

In accordance with the Rulebook on requirements regarding the quality of vegetable oils and vegetable fats, margarine, mayonnaise and related products of the Republic of Macedonia, the peroxide value in unrefined oils should be up to 7.5 mmol O_2/kg (Official Journal of R. Macedonia No. 127, 2012).

Peroxides in an acidic environment oxidize iodide to iodine, which is then titrated with a sodium thiosulphate solution:

$$(R - O_2) + 2KI + 2CH_3COOH = (R - O) + I_2 + 2\ CH_3COOH + 2H_2O$$

$$I_2 + Na_2S_2O_3 = 2\ NaI + Na_2S_4O_6$$

The peroxide value (PV) is expressed as the number millilitres of sodium thiosulfate solution with a concentration of 0.01 mol/L, which is used for titration of iodine released from potassium iodide due of the presence of peroxides in oil. The peroxide value expressed as millimoles per kg is calculated using the formula:

$$PV\ (mmol\ O_2/kg) = \frac{(v_1 - v_0)xfx5}{m}$$

Where

- V_1-volume of the $Na_2S_2O_3$ solution with a concentration of 0.01 mol/L, used for the titration of the sample (in mL);
- V_0-volume of the $Na_2S_2O_3$ solution with a concentration of 0.01 mol/L, used for the titration of the sample blank (in mL);
- F-$Na_2S_2O_3$ solution factor. If the concentration is exactly 0.01 mol/L, then f=1;
- m-mass of the test sample, in grams (determined with a scale).

Statistical analysis

The statistical processing of the data obtained from the testing was done by applying statistical correlation and a Student's t-test. The statistical processing was done using Microsoft Excel 2007 (Levine *et al* 2008).

Results and discussion

Peroxide value of cold pressed sunflower oil without additives

The peroxide value that is a parameter for oil stability, in cold pressed sunflower oils with different volumes, without additives and stored for 4 weeks is given in Figure 1. The obtained results are compared with the maximum permissible values (MPV) for cold pressed oil provided in the Rulebook on requirements regarding the quality of vegetable oils and vegetable fats, margarine, mayonnaise and related products of the R. Macedonia (Official Journal of R. Macedonia No. 127, 2012).

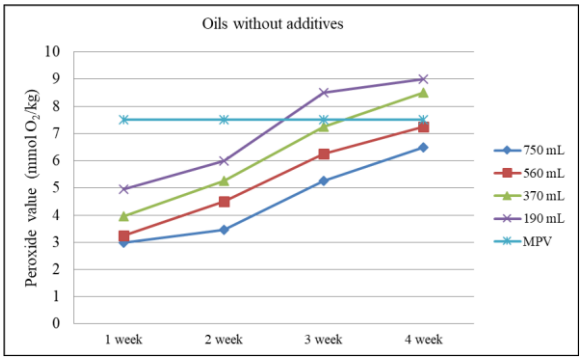

Fig 1: Peroxide value of cold pressed sunflower oils depending on the storage period and oil volume

Figure 1 shows that the peroxide value of all oils without additives, after one week of storage, is within the permissible limits according to the Rulebook. In the oil with the largest volume (750 mL) the peroxide value is the lowest, 2.9 mmol O_2/kg while in the oil with the smallest volume (190 mL) the peroxide value is the highest, at 4.9 mmol O_2/kg. After two weeks of storage, it can be seen that the oils have oxidized, but it is within the permissible limits according to the Rulebook. The oil with the largest volume (750 mL) has the lowest peroxide value of 3.5 mmol O_2/kg, and the oil with the smallest volume has the highest peroxide value of 5.9 mmol O_2/kg. After three weeks, the peroxide value of the oils with a volume of 750 mL, 560 mL and 370 mL was within the permissible limits, but the oil with a volume of 190 mL has a peroxide value of 8.5 mmol O_2/kg, which is one unit higher than the permissible limits according to the Rulebook, i.e. 7.5 mmol O_2/kg. After four weeks, the peroxide value of oils with a volume of 750 mL and 560 mL was within the permissible limits, but for oils with a volume of 370 mL and 190 mL it was above the permissible limits. In the oils with a volume of 370 mL the peroxide value was 8.5 mmol O_2/kg, in the oils with a volume of 190 mL the peroxide value was 9 mmol O_2/kg. This means that oils with a volume of 370 mL and 190 mL are not safe to consume four weeks after being opened.

The peroxide value is directly proportional to the storage time of cold pressed oil, i.e. as the storage time increases, so does the peroxide value. The amount of oil in the bottle is inversely proportional to the degree of oxidation, i.e. oils with a larger volume have a lower peroxide value while lower volume oils have a higher peroxide value due to the increased empty space in the oxygen-filled packaging.

Increased oxidation is affected by oxygen but also by already created free radicals that cause an oxidation chain reaction. Over time, these free radicals increase and continue to oxidize together with the oxygen.

Peroxide value of cold pressed sunflower oil with added rosemary

The peroxide values of the analyzed sunflower oil with added rosemary, compared with MPV, are given in Figure 2.

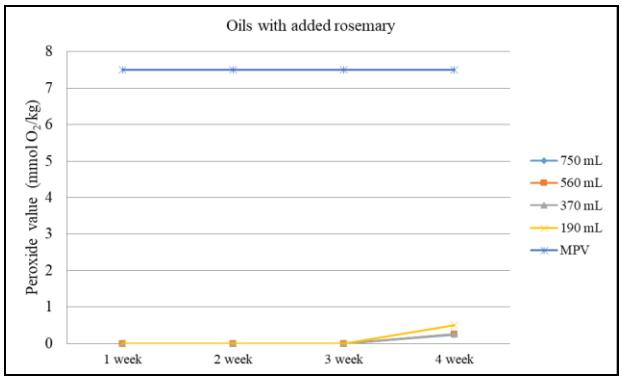

Fig 2: Peroxide value of cold pressed sunflower oils with added rosemary, depending on the time and volume of the oil

Figure 2 shows that after one, two and three weeks of storage, the peroxide value of all oils with added rosemary is 0 mmol O_2/kg, i.e. there is no oxidation regardless of the volume of oil in the bottle. This means that rosemary antioxidants protect the oil from oxidation and no oxidation has occurred at all for three weeks. After 4 weeks, the testing showed that oxidation has started and in the oil with the largest volume (750 mL) the peroxide value is 0.2 mmol O_2/kg, and in the oil with the smallest volume (190 mL) it is 0.5 mmol O_2/kg. The volume of the oil is inversely proportional to the peroxide value. Increasing the storage time increases the peroxide value. After 4 weeks of storage, oils with added rosemary have an exceptionally low peroxide value below 1 mmol O_2/kg, which means that rosemary is a powerful antioxidant that protects the oil from spoilage.

Peroxide value of cold pressed sunflower oil with added parsley

Figure 3 shows the peroxide values of the analyzed oils with the addition of parsley and compared with MPV.

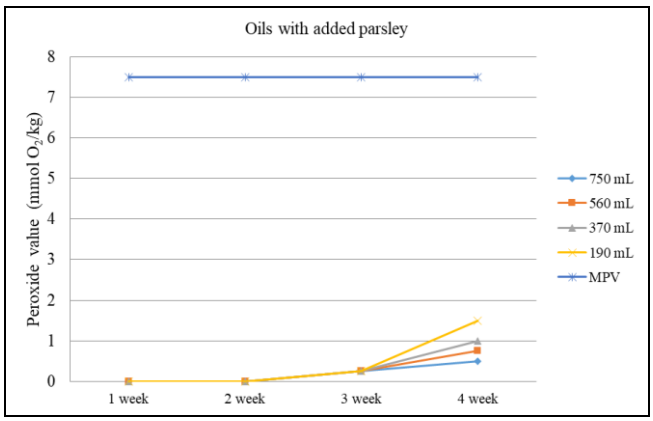

Fig 3: Peroxide value of cold pressed sunflower oils with added parsley, depending on the time and volume of the oil

Figure 3 shows that no oxidation occurred after two weeks, i.e. the peroxide value is 0 mmol O_2/kg. Oxidation begins after 3 weeks in all oils and the peroxide value is below 0.25 mmol O_2/kg. After 4 weeks the oxidation process continues, and the smallest volume of oil has a peroxide value of 1.5 mmol O_2/kg. The peroxide value for all analysed oils is within the permissible limits et out in the Rulebook (7.5 mmol O_2/kg). Parsley antioxidants store lipid peroxidation oil. The volume of the oil with the addition of parsley is inversely proportional to the peroxide value. The peroxide value is directly proportional to the storage time.

Statistical data processing

From the obtained results, correlation and student t-test between oils without additives and oils with addition of rosemary and parsley were calculated. The results of the correlation are given in Table 1, and from the student test in Table 2 and Table 3.

Table 1: Correlation between peroxide values obtained for cold pressed sunflower oil without and with the addition of rosemary and parsley

Correlation between PV in oils without and with additives	Correlation coefficient
Correlation between PV in oils without additives and oils with rosemary	0.7291
Correlation between PV in oils without additives and oils with parsley	0.8739

The results obtained in Table 1 show that the correlation coefficient is higher in parsley oil, compared to rosemary oil. The oils with added parsley have a strong positive correlation with oils without additives. Between the oils with added rosemary there is a strong positive correlation with oils without additives, but weaker than the correlation with added parsley.

To calculate the t-test, a null and an alternative hypothesis are set:

- The null hypothesis for the obtained peroxide value of the "Super Vita" oils with out additive and the oils with the addition of spices is set out in the following form: H_0:μ = 6,4418;
- The alternative hypothesis is set out in the following form: H_A:μ ≠ 6,4418
- The average peroxide value of oils without additives is 6,4418 mmol O_2/kg;
- Limit p-value (risk level error) =α/2 = 0,01/2 = 0,005;
- Certainty level (P) = 95%.

Table 2: t-test between oils with and without added rosemary

Super Vita Oil	Oils without additives	Oils with rosemary
Mean value	5.8030	0.0772
t – test value	6.21199	
Critical value for t	2.44690	
Obtained p – value	0.000803	

From the obtained data it can be concluded that 6.21199 > 2.776. This means that the null hypothesis that there is no difference between oils with and without rosemary stored under the same conditions is rejected. The resulting p-value is 0.000803 < the limit value of p is 0.005, thus it can be concluded that the peroxide values between the samples of oils without and with the addition of rosemary are statistically significantly different.

Table 3: t-test between oils with and without added parsly

Super Vita Oil	Oils without additives	Oils with parsley
Mean value	5.8030	0.2951
t – test value	5.8308	
Critical value for t	2.4469	
Obtained p – value	0.00112	

From the obtained data it can be concluded that 5.8308>2.776. This means that the null hypothesis that there is no difference between oils with and without parsley stored under the same conditions is rejected. The obtained p-value 0.00112 < the limit p-value 0.005 and can thus be concluded that the peroxide values between the samples of oils without and with added parsley are statistically significantly different.

The p-value obtained for rosemary (0.000803) is lower than the obtained p-value of parsley (0.00112), which means that there is a greater difference between oils with and without added rosemary and oils with and without added parsley. This means that rosemary has a greater antioxidant ability than parsley. Rosemary has greater antioxidant power to reduce lipid peroxidation compared to parsley, but both spices have shown significant antioxidant activity during the 4 week storage period.

Conclusion

Cold-pressed sunflower oils from the manufacturer "Super Vita" were analysed. 12 bottles of oil with a volume of 750 mL, 560 mL, 370 mL, and 190 mL were analysed. 4 oils of different volumes were stored without additives. 20 g of fresh rosemary was added to 4 oils of different volumes and 20 g of fresh parsley was added to 4 oils of different volumes as an antioxidant to increase their stability. The peroxide values as determined, which is a measure of oil stability, and the analyses were performed after one, two, three and four weeks.

From the results obtained we can see that in oils without additives, continuous oxidation begins right after opening. Smaller volume oils have a higher peroxide value than larger volume oils. After the fourth week, low-volume non-additive oils (370 mL and 190 mL) have a peroxide number above the MPV, which means they are not safe to eat. Oils withot additives and larger volume (560 mL and 750 mL) are safe to eat even after 4 weeks of storage. Peroxide values in all analysed oils with added rosemary and parsley are exceptionally low and below the MPV according to the Rulebook. This means that oils with the addition of rosemary or parsley in different volumes are safe to consume and after 4 weeks of storage. After four weeks, the peroxide value of oils without additives is much higher than that of the oils with additives. Rosemary and parsley antioxidants prevent and decrease the oxidation process. Rosemary contains a large group of phenolic acids, flavonoid diterpenes and triterpenes that have a pronounced antioxidant effect. Parsley has a great antioxidant effect due to the content of flavonoids, coumarins and vitamin C. As the storage time increases and the volume of the oil decreases, the peroxide value in the oils without the additive increases a lot. In oils with supplements, this effect is exceedingly small. The correlation between the oils without the additive and the oils with the additive confirms the antioxidant effect of the spices. From the applied Student's t-test it can be concluded that when adding fresh rosemary and parsley to oils, the peroxide value is statistically significantly different from the peroxide value of oils without the addition of spices that are stored under the same conditions.

Rosemary and parsley as natural antioxidants added to oils greatly reduce the oxidation process and increase stability compared to oils without added antioxidants.

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