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INVESTIGATION OF NANO-SILVER PARTICLES ADDITION IN THE OIL EXTRACTED FROM TWO TYPES OF ALMOND (AMYGDALUS SCOPARIA, AND AMYGDALUS LYCIOIDES) AND ITS EFFECT ON THE FATTY ACID PROFILES

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Abstract

Almond oil showed significantly higher unsaturated fatty acids compare to saturated fatty acids. They could be used in cosmetic products and human nutrition. In this work, amygdalus scoparia (A.Sc) and amygdalus lycioides (A.Ly) were utilized as the raw material for oil extraction. The Scanning Electron Microscope (SEM) assessed the efficiency of the oil extraction process. Since the evaluation of changes in fatty acids profile is of great importance in nutritional value of oils, researchers decided to evaluate their changes in presence of nano silver particles. Several methods have been proposed to extract oil from plants and oil seeds. In this study, the oil of two types of almonds, A.SC and A.LY, was extracted using Soxhlet extractor with hexane solvent. The amount of fatty acids profile in these types of oil was investigated employing Gas Chromatography (GC-FID). Adding different ratios of nano-silver particles to the oil, the effect of these adding particles on the fatty acids profile was evaluated. In each analysis of both almond oils, the amount of saturated fatty acids (SFA), total unsaturated fatty acids (TUFA), and iodine value (IV) were calculated. The experimental results confirmed that the overall increase in nano-silver particles has approximately increased TUFA. Moreover, the results also revealed that the highest amount of TUFA / SFA in each type of almond oil is associated with the use of 0.04 g nano silver powder in the oil samples. The obtained results could be utilized in scientific and food industry studies, particularly in examining the impact of heavy metals, such as silver effect on the oil industry. Since oleic and linoleic acid are essential for human body, it could be argued that these almond oil has a high nutritional value.

Keywords; Fatty acids, Amygdalus, nanosilver, Oil extraction.

Introduction

Almonds and their subgroups are dispersed as the major species in Zagros forests in Iran. The fruits of these species contain oils with remarkable nutritional properties that are familiar with the amount of extracted oil whose fatty acid profile plays a critical role in the optimal and economical utilization of forest. Almond seed could be used in many products, including bakeries and confectionery products. It is also very valuable due to its dietary, cosmetic and medicinal properties(Kornsteiner et al. 2006) [1]. In a study in 2008 on the chemical structure of A.Scoparia oil, it was concluded that regarding the USFA to SFA ratio, the oxidation index and the amount of tocopherol and phenolic compounds in A.Scopari oil were greater than olive oil(Farhoosh and Tavakoli 2008) [2]. Investigation of almond oil reveals high levels of unsaturated fatty acids (UFA) and small amounts of saturated fatty acids (SFA). The comparison between sweet and bitter almond implied that there were significant differences in their physical and chemical properties. Palmitic acid content of almond seed oil was reported to be 4.62-5.71% while the linoleic acid content was 18.86-22.3% and oleic acid content was 66.7-69.5%(Nabi

and Issa 2015) [3] .
Fatty acids play pivotal roles in the body metabolism and the structure of important compounds, such as prostaglandins, leukotriene's and thromboxins. The most important food sources containing fatty acids are vegetable oils(Rustan and Drevon 2005) [4] . Herein, the two groups of essential fatty acids in the human body, omega-3 fatty acids and omega-6 fatty acids, are derived from alpha-linolenic acid (ALA) and linoleic acid (LA), respectively (Abbaszadeh et al. 2011) [5].
It is noteworthy to mention that lack of essential fatty acids in human diet, could lead to some problems, for instance cardiovascular disease, certain types of cancers, eczema, viral infections, diabetes-related neurological diseases, and Autoimmune disease (Jump et al. 2012) [6]. Several studies have been conducted on the impact of different extraction methods on the fatty acid profile. In a study, the effect of fermentation process on the amount of extracted fatty acids was analyzed, where white and brown rice samples were affected by fermentation process with lactobacillus paracasei. Afterwards, the amount of fatty acids in the fermented rice samples were compared to the control rice samples. The obtained results showed that both oleic acid and linoleic acid decreased during fermentation (Lee et al. 2018) [7]. The use of subcritical fluids in extraction processes might be a useful and practical technology in the oil industry. This solvent-extraction process could result in producing higher quality almond oils in the future (Qi et al. 2019) [8]. Of note, in the oil industry, in order to extract the fat, utilizing polar solvents as well as non-polar solvents are important due to water loss, protein denaturation, and the breakdown of hydrogen bonds between fat and protein. The higher is the ratio of non-polar to polar solvents, the more lipids is extracted. On the other hand, the solvents used should not react with the extracted fat (Čertík et al 1996) [9].
Moreover, several studies have been implemented on the oxidation and spoilage of oils, based on which the role of various substances and compounds in either increasing or decreasing the oxidizing properties of oils has been investigated. In a study in 2013 on the behavior of ascorbic acid and tocopherol on their antioxidant properties on unsaturated fatty acids (UFA), it can be observed that ascorbic acid activates the oxidation reaction at n-3HUFA in rich water emulsion. Additionally, Polyphenol and alpha-tocopherol compounds also act as the antioxidants (Jayasinghe et al. 2013) [10].
Investigating the properties of silver, it could be said that silver is an ancient antibiotic that has been widely employed for its special structure. The issue of silver toxicity has currently become one of the most controversial topics in the scientific community. The surface of the nano-silver can easily be released by oxidation with O_2 and other molecules in nature to release Ag^+, which is itself a famous toxic ion. As such, the toxicity of Nano-silver is directly related to the release of Ag^+ ions (McShan et al. 2014) [11].Other studies have been conducted extensively on the toxic properties of nano-silver, such as oxidative damage to molecular systems, which have assessed and reported its effects on molecular scales (Awasthi et al. 2013) [12].For note, the silver atoms on the nano-silver surface, when collided with oxygen molecules, can convert to silver oxide. In this situation, a collision with other redox-active compounds results in the formation of silver ionic form (Ag^+) (Kim et al. 2013; Li et al. 2013; Reidy et al. 2013; Unrine et al. 2012) [13-16].
Recently, considering changes in fatty acids profile and oxidation is very important in nutritional value of oils. A lot of research has been done to identify the factors affecting the profile of fatty acids in the oil production process. The objective of this paper was to explore the effect of nano-silver particles on the fatty acids profile of two kinds of extracted almond oil. As nano-silver compounds produce (Ag^+) ions, which are active and toxic compound, investigating their interaction with saturated and unsaturated fatty acids could be important and critical in the food and medical sciences. Thus, in this paper, the effect of these nano-silver particles on the amount of saturated fatty acids (SFA) and total unsaturated fatty acids (TUFA) as well as iodine value (IV) in oil extracted from two types of almonds (amygdalus scoparia and amygdalus lycioides) were analyzed.

Materials and Methods

Plant material and chemical composition

This study was conducted on two Iranian local almond fruits. Two types of local major almond cultivars (A.LY-A.SC) in Zagros region was collected in 2019. The considered species were gathered from different parts of the forest area in Kohgiluyeh. For this purpose, in each study area, five trees of each species were randomly chosen to produce fruit. Subsequently, 500g of almond was prepared from each tree on average. The collected samples were separately transferred to the laboratory to perform the oil

extraction. Having collected the samples, they were washed, cleaned and dried, and their shells were separated. Until the process started, the cleaned samples were stored in sealed plastic containers at a temperature of 4±1°C. Moisture content of almond seeds was measured with psychrometer (Sartorius - MA 100). The chemical components used in this study were mainly received from the German company Merck and their purity was controlled by gas chromatography. The obtained results confirm that the mass fraction of all chemicals used was greater than 0.99 (Mohsen-Nia and Paikar 2007) [17]. The standard mixture of methyl ester fatty acids was obtained from Company Supelco^{TM} (37 Component FAME Mix). The nano-silver powder was prepared by Nano Cid (Nano Nasb Pars Company).

Oil extraction

Herein, to extract oil from two kinds of almonds (A.LY and A.SC), Soxhlet extractor (model Buchi, B-811, Extraction System) and hexane solvent were employed. The almond seed samples were primarily grinded and then, they were separated by an 850 µm sieve. We weighed 10 g of almonds and then mixed them with about 140 ml of hexane solvent, and ultimately, the oil was extracted after 6 hours in the Soxhlet extractor. From each species (two kinds of almond oils), approximately four kind of oils were prepared in 1 ml. Afterwards, one sample was selected as a control, and added to the other samples containing 0.01, 0.02, and 0.04 g of nano-Silver powder. The combination was then stirred. The samples were kept at the same conditions and ambient temperature, and then the effect of nano silver particles on the fatty acid profile was investigated by fatty acid analysis.

Almond seed characterization

The structures of the two types of dried almond seeds, *Amygdalus scoparia* and *Amigdalus lycioides*, were analyzed before and after the extraction using Scanning Electron Microscope (SEM). This study investigated two types of almond seeds (before and after oil extraction). All samples were gold coated before being analyzed with a FEI Quanta Inspect F Scanning Electron Microscope (SEM).

Analysis process

The fatty acids have been analyzed with GC-FID after transmethylation with methanolic potassium hydroxide (KOH). In the following, 0.05 g of oil sample was weighed and saponified in 0.05 M sodium methoxide. The analysis was carried out using a 7890 B (Agilent) gas chromatography. The column, detectors, oven temperature and other device conditions are reported in Table 1. The mixed standard fatty acids (Supelco^{TM} 37 Component FAME Mix) were used simultaneously in the GC device and were then reported comparing the retention time of the sample peaks with the reference sample peaks of the final percentage below the peak level.

Table 1. Operating condition for gas chromatography measurements

Column	CP-Cil88,length=100m,0.23mm inner diameter and Film thickness=0.2 µm specification
Detector	FID-260^{o} C
Injector temperature	260^{o} C
Oven temperature planning	50 ^{o}C(5 min), 50-140 ^{o}C (heat rate 4 ^{o}C/min), 140 ^{o}C(5 min),140-240 ^{o}C (heat rate 4 ^{o}C/min),and 240 ^{o}C (10 min)
Carrier gas	Nitrogen
Flow rate	1.0 ml.min^{-1}
Split ratio	100:1

Characterization of fatty acids

The iodine value (IV) content, which indicates the unsaturated degree of fats and oils, as well as the ratio of oleic acid to linoleic acid (O / L) were calculated and reported in Tables 2 and 3 (Shin et al. 2012) [18].

IV= (% Palmitoleic Acid × 0.95) + (%Oleic Acid × 0.86) + (%linoleic Acid × 1.732) + (%Gadoleic Acid × 0.785) + (%Erucic Acid × 0.723)
O/L = %(Oleic Acid) / % (Linoleic Acid)

The SFA (Saturated Fatty Acids), MUFA (Mono Unsaturated Fatty Acids), PUFA (Poly Unsaturated Fatty Acids) and TUFA (Total Unsaturated Fatty Acids) were also represented in Tables 2 and 3.

Table 2. Fatty acids composition of amygdalus lycioides almond oils

Parameter	Area % Amygdalus lycioides				
Fatty acids	Free nano silver	Nano silver 0.01g	Nano silver 0.02 g	Nano silver 0.04 g	P_valve
C 6:0	0.0	0.0	0.0	0.0	1.00^{**}
C 16:0	6.0 ± 0.11^{ac}	7.1 ± 0.15^{b}	6.1 ± 0.1^{c}	5.8 ± 0.2^{c}	0.036^{**}
C 16:1	0.2 ± 0.1	1.0 ± 0.1^{ab}	0.3 ± 0.05^{b}	0.2 ± 0.15^{b}	0.063^{**}
C 17:1	0.1 ± 0.08	0.1 ± 0.05	0.1 ± 0	0.1 ± 0.05	1.00^{**}
C 18:0	2.2 ± 0	2.3 ± 0.01	2.3 ± 0.05	2.2 ± 0.01	0.549^{*}
C 18:1 n9c	69.8 ± 0.2	69.9 ± 0.4	69.2 ± 0.1	70.0 ± 0.2	0.278^{*}
C 18:2 n6c	21.3 ± 0.3^{a}	19.0 ± 0.25^{b}	21.7 ± 0.3^{a}	21.4 ± 0.15^{a}	0.048^{**}
C 20:0	0.2 ± 0.02	0.2 ± 0.05	0.2 ± 0.02	0.2 ± 0.04	1.00^{*}
C 20:1	0.1 ± 0^{a}	0.2 ± 0.02^{b}	0.2 ± 0.03^{b}	0.1 ± 0.01^{a}	0.034^{**}
C 20:2	0.2 ± 0.03^{a}	0.1 ± 0.02^{a}	0.0^{a}	0.0^{a}	0.022^{**}
SFA	8.4 ± 0.1^{a}	9.6 ± 0.2^{b}	8.6 ± 0.21^{a}	8.2 ± 0.2^{a}	0.00^{*}
MUFA	70.2 ± 0.04^{a}	71.2 ± 0.08^{b}	69.8 ± 0.04^{a}	70.4 ± 0.04^{a}	0.001^{*}
PUFA	21.5 ± 0.2^{a}	19.1 ± 0.1^{b}	21.7 ± 0.25^{a}	21.4 ± 0.15^{a}	0.049^{**}
TUFA	91.7 ± 0.4^{a}	90.3 ± 0.3^{b}	91.5 ± 0.50^{a}	91.8 ± 0.4^{a}	0.007^{*}
TUFA/SFA	10.9 ± 0.4^{a}	9.4 ± 0.2^{b}	10.6 ± 0.4^{a}	11.2 ± 0.2^{a}	0.00^{*}
O/L	3.2 ± 0.15^{a}	3.6 ± 0.1^{a}	3.1 ± 0.2^{a}	3.2 ± 0.2^{a}	0.027^{*}
IV	97.1 ± 0.01^{a}	94.1 ± 0.02^{b}	97.5 ± 0.03^{c}	97.5 ± 0.01^{c}	0.025^{**}

Parameters in the same row followed by different Latin letters are significantly different according to tuky test (p=0.05)

Table 3. Fatty acids composition of amygdalus Scoparia almond oils

Parameter	Area % Amygdalus scoparia				
Fatty acids (%)	Free nano silver	Nano silver 0.01g	Nanosilver 0.02 g	Nano silver 0.04 g	P_ value
C 6:0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	1.00^{**}
C 16:0	5.7 ± 0.46	5.6 ± 0.3	6.1 ± 0.42	5.4 ± 0.38	0.249^{*}
C 16:1	0.2 ± 0.04^{a}	0.2 ± 0.05^{a}	0.3 ± 0.02^{a}	0.2 ± 0.04^{a}	0.032^{*}
C 17:1	0.1 ± 0	0.1 ± 0.02	0.1 ± 0	0.1 ± 0	0.088^{**}
C 18:0	1.9 ± 0.04^{abc}	1.8 ± 0.05^{b}	2.0 ± 0.02^{c}	1.8 ± 0.05^{ab}	0.001^{*}
C 18:1 n9c	70.7 ± 0.19^{ab}	71.4 ± 0.2^{b}	68.6 ± 0.23^{c}	72.3 ± 0.15^{db}	0.00^{*}
C 18:2 n6c	21.1 ± 0.1^{a}	20.6 ± 0.2^{b}	22.6 ± 0.1^{c}	19.8 ± 0.1^{d}	0.00^{*}
C 20:0	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.05	1.00^{**}
C 20:1	0.1 ± 0.04	0.1 ± 0	0.1 ± 0.03	0.2 ± 0.02	0.082^{**}
C 20:2	0.2 ± 0.01^{a}	0.0 ± 0^{b}	0.1 ± 0.04^{c}	0.0 ± 0^{b}	0.014^{**}
SFA	7.8 ± 0.02^{abcd}	7.6 ± 0.02^{bd}	8.3 ± 0.03^{c}	7.4 ± 0.04^{d}	0.002^{*}
MUFA	71.1 ± 0.15^{ab}	71.8 ± 0.3^{bd}	69.1 ± 0.2^{c}	72.8 ± 0.2^{d}	0.00^{*}

PUFA	21.3 ±0.15[a]	20.6± 0.2[b]	22.7 ± 0.2[c]	19.8 ±0.15[d]	0.00*
TUFA	92.4±0.05[ab]	92.4±0.02[b]	91.8±0.04[c]	92.6 ±0.05[b]	0.001*
TUFA/SFA	11.8 ± 0.2[ab]	12.1 ± 0.1[b]	11.0 ± 0.5[c]	12.5 ±0.25[b]	0.000*
O/L	3.3 ± 0.15[a]	3.4 ± 0.1[a]	3.0 ± 0.12[a]	3.6 ± 0.2[a]	0.007*
IV	97.6±0.01[ab]	97.3±0.05[bc]	98.5±0.05[b]	96.8± 0.07[c]	0.00*

*oneway-anova
**kruskal-wallis

Statistical analysis
The experimental data were analyzed using SPSS 16 software. For data analysis, the normality of data was determined with Kolmogorov-Smirnov normality test. Besides, the equality of variances was assessed utilizing Leven test. One-way analysis of variance (ANOVA) for normal distribution data and kruskal-wallis test for abnormal distribution data were employed to compare the mean of samples. Tukey's tests were used to compare the means at the confidence level of 5%. The effect of different concentrations of nano-silver was investigated on the fatty acid profile and iodine value.

Result and Discussion
Silver nanoparticles are of oxidative properties and are also effective in oxidative corrosion. These particles also tend to affect double-bonded compounds. In view of these properties of nano-silver particles, we investigated the effect of these particles on the fatty acid profile. In this regard, two types of almond oil were extracted with the help of hexane solvent and soxhlet. By examining the effect of nano-silver particles on these oils, we considered its effect on fatty acid profiles, including saturated and unsaturated fatty acids.

Percentage of almond oil and moisture content
Samples of the two almond seeds were collected and sent to the laboratory. After the seeds of these grains were separated, the moisture and fat of these grains were analyzed. The humidity level in both samples ranged from 4-5% while the fat extracted was in the range of 37% to 53%.

Nano composition addition
The amount of fatty acids profile in the absence of nano-silver, and in the presence of nano-silver particles was calculated to be 0.01, 0.02 and 0.04 g in 1 cc of oil samples for almond A. Ly, in Table 2, for almonds A.SC, in Table 3. Respectively. As shown in Tables 2 and 3, fatty acids C 18:2 n6c and C 20:2 were significantly different in both almonds. Linoleic acid (omega-6) was significantly different in both almonds at the presence of different amounts of nano-silver. The ratio of TUFA/SFA in oil is usually considered as a measure of the unsaturated oils and fats and their tendency to participate in the lipid oxidation process. In almond Amygdalus Scoparia this ratio sees the highest amount (12.5±0.25) in the presence of 0.04g nano-silver. Meanwhile, there is a significant difference in Amygdalus Scoparia in the presence of 0.01, 0.02 and 0.0 g nano-silver. On the other side, in almond Amygdalus Lycioides TUFA/SFA ratio has the highest value (11.2 ±0.02) in the presence of 0.04g nano-silver, so it has a significant difference with Amygdalus Lycioides in the presence of 0.01, 0.02 and 0.0 g nano-silver. Since linoleic acid (omega-6) has a double bond, the effect of nano-silver addition on this type of fatty acid is of great significance. Also, in account of the low amount of linolenic acid (omega-9), that has a three double bond in the oils of these two types of almonds, it could be said that these oils are more resistant to oxidation and ionization process.

Structural investigation
The powder of almond seeds A.LY and A.SC was analyzed by SEM (Scanning Electron Microscope) at a magnification of 10 μm. As shown in Figure 1, the images of each almond ahead of the oil extraction process are compressed and interlocked with Soxhlet.
After the oil extraction process using hexane solvent and Soxhlet extractor, vacuum and non-uniform structure could be easily observed. This is because of the efficiency of the process of extraction and

separation of oil from the almond species.

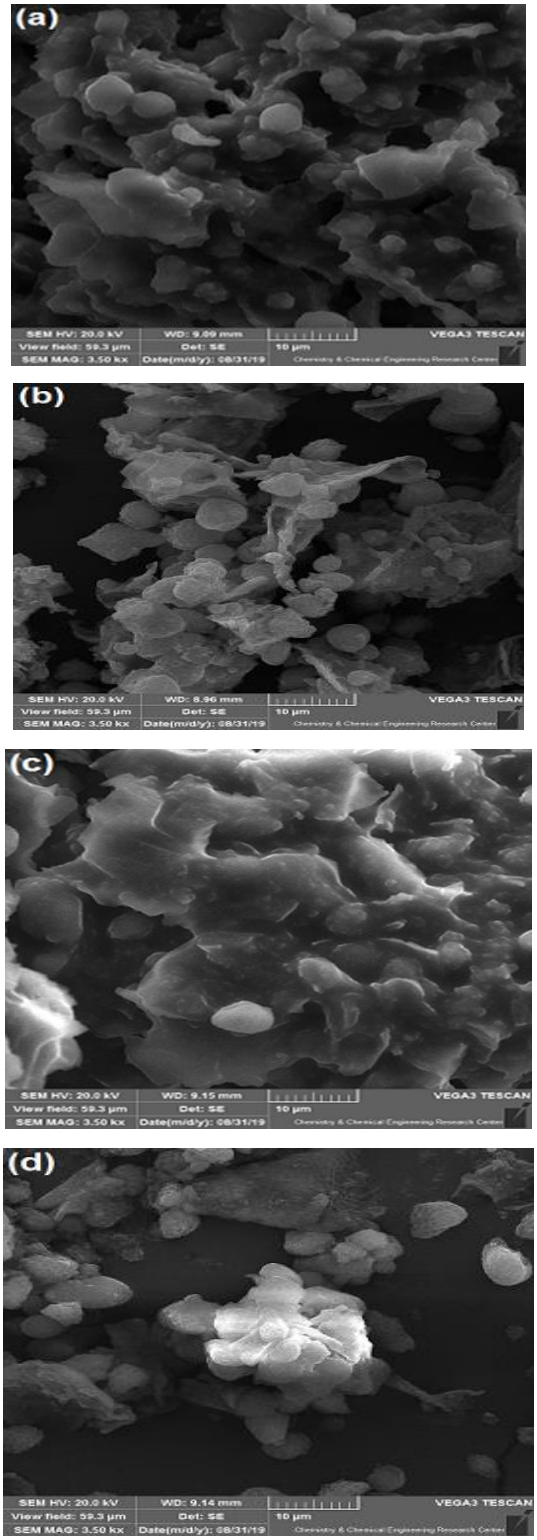

Figure 1. SEM image of the almond seeds: (a) A.Sc before Soxhlet extraction; (b) A.Sc after Soxhlet extraction; (c) A.Ly before Soxhlet extraction; (d) A.Ly after Soxhlet extraction

Fatty acid analysis

As shown in Table 2, the highest amount of TUFA / SFA belongs to the A.SC oil sample in the presence of 0.04 g nano-silver (12.5%). Meanwhile, the lowest amount of TUFA / SFA in A.SC oil belonged to 0.02 g nano-silver in the oil.

Furthermore, in this table, the highest iodine value was observed in extracted almond oil from A.SC of 0.02 grams of nano-silver. The simultaneous comparison of SFA, MUFA, PUFA, and TUFA in A.SC almond oil is reported in Figure 2 as bar graphs. In Table 3, the parameters of A.LY almonds are reported. As could be seen in this table, the highest TUFA / SFA ratio belongs to 0.04 g of nano silver in A.LY oil (11.2%). In contrast, the lowest amount of TUFA / SFA in A.LY almond oil was related to 0.01 g of nano-silver in this type of oil. (9.4%). According to the data in this table, the highest iodine value (IV) belongs 0.04 grams of nano-silver in A.LY almond oil (7.5%).

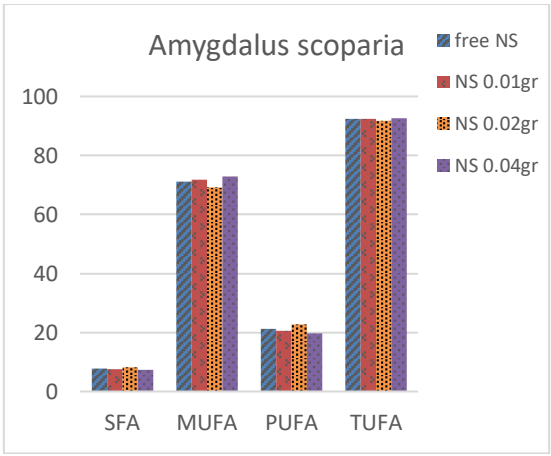

Figure 2. Simultaneous comparison of SFA, MUFA, PUFA, and TUFA for amygdalus scoparia (NS=Nano Silver)

Simultaneous review

The simultaneous comparison of SFA, MUFA, PUFA, and TUFA is illustrated and reported for A.LY in Fig. 3. In Fig. 4, the iodine value is reported simultaneously for two almond types A.LY and A.SC, and the effect of the presence of nano-silver particles in this form is comparable. As can be seen in Fig. 4, the highest value of IV (iodine value) in both almonds was attributed to the use of silver nanoparticles at the rate of 0.02 g in 1 ml oil. Statistically, in both almonds, significant differences were found in iodine value- O/L ratio and TUFA/SFA, TUFA, PUFA, MUFA, SFA amount.

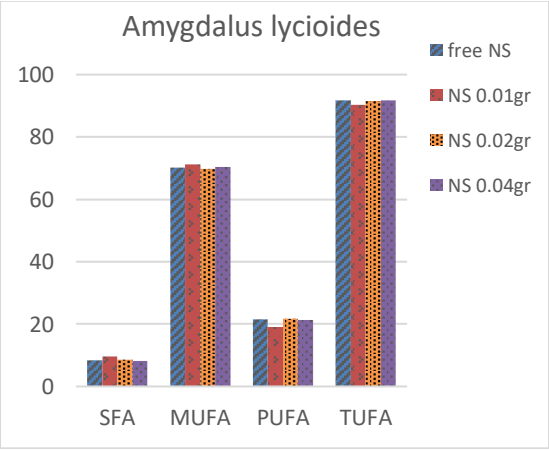

Figure 3. Simultaneous comparison of SFA, MUFA, PUFA, and TUFA for amygdalus lycioides (NS=Nano Silver)

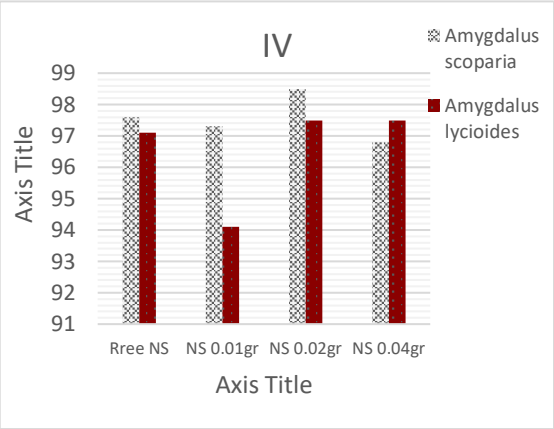

Figure 4. Simultaneous comparison of iodine value for two almond types A.LY and A.SC

Conclucion

In the present paper, the analysis of the fatty acid profile of A.LY and A.SC oils was reported for the first time, while they were in the presence of nano silver particles. As nano silver particles could finally lead to creating Ag^{+}, the effect of this ion on the fatty acid profile has been shown to some extent increase TUFA (total unsaturated fatty acids) level.

Owing to the fact that the presence of nano-silver compound leads to significant changes in iodine value, TUFA and SFA, the data of this research can be useful in examining the impact of heavy metals, such as silver effect on the oil industry. Since the amount of fatty acid oleic and linoleic acid in these two almonds is considerable (above 90%) and because of the very small amount of linolenic acid, these oils could be classified in linoleic-olic oil group. Oleic and linoleic acid are essential for the human body; therefore, it can be argued that these almond oil has a high nutritional value. In the end, the findings of this study can be utilized in scientific and food industry studies, particularly in studies on oils.

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