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Scientific Research

Investigating the antioxidant properties of mustard seed sprout extracted with ultrasound and its effect on the oxidative stability of soybean oil

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ABSTRACT

The use of plant extracts containing natural antioxidants to increase the oxidative stability of edible oils has received more attention. The purpose of this research was to investigate the antioxidant properties of methanol and acetone extracts of Iranian mustard seed sprouts extracted by maceration and ultrasound methods and its effect on the oxidative stability of soybean oil. The antioxidant properties of the extracts were investigated by determining the total phenolic contents, DPPH free radical scavengering capacity and also the Ferric Reducing Power, and the stability of soybean oil samples containing 0.5% and 1% of the extracts was evaluated after oven test on the 5th, 10th, 15th, 20th, 25th, and 30th days of storage by peroxide, anisidine, thiobarbituric acid values and oxidative stability tests. The results showed that during the storage, the amount of peroxide, anisidine and thiobarbituric acid values of the samples increased and the oxidative stability index decreased significantly ($p \leq 0.05$). There was no statistically significant difference between the oxidative stability of samples containing methanolic and acetone extracts. Increasing the concentration of the extract significantly reduced the rate of oil oxidation ($p \leq 0.05$). The rate of decreasing stability in samples containing 1% concentration of ultrasound assisted extract due to having higher phenolic compounds and antioxidant properties was significantly slower compared to the control and other samples ($p \leq 0.05$). This extract was effective as a natural antioxidant in increasing the stability of soybean oil.

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1- Introduction

Today, many studies are focused on natural antioxidants. Plants contain many types of antioxidants. [1] Phenolic compounds have attracted a lot of attention because they play a protective role against damage caused by oxidative stress [2]. Extensive research has been done on these compounds and it has been confirmed that phenolic compounds have various biological activities and can be beneficial for human health. Consumption of these compounds are related to reducing the risk of cancer, heart diseases, diabetes, inhibiting the accumulation of plasma platelets, histamine secretion, as well as anti-bacterial, anti-viral, anti-inflammatory and anti-allergic activities [3]. These advantages are caused by the antioxidant properties of phenolic compounds; Therefore, it is important to identify, quantify and evaluate their antioxidant activities. mustard,herbal belonging to the family *Brassicaceae* is.Mainly three types of mustard are cultivated worldwide for their nutritional value: yellow or white mustard (*White mustard*), brown mustard (*Brassica juncea*) and black mustard (*Brassica nigra*). Mustard has been used as a medicinal plant and spice since the past, especially in Indian cuisine [4,5]. Mustard seeds are rich in elements and minerals, vitamins(A،E و K)، It contains carotenoids such as carotenes, zeaxanthin, lutein, flavonoids and phenolic compounds and has antimicrobial, stabilizing and antioxidant properties [6]. Numerous researches have shown that the flavonoids in mustard seeds can be effective in preventing or treating various diseases such as diabetes and other disorders related to oxidative stress [7]. The phenolic compounds of mustard seeds, which have antioxidant properties, include catechin, epicatechin, gallic acid, myristicin, quercetin, and rutin. [8] Germinated edible seeds have many biological activities and their antioxidant capacity after germination is significant. Attention increases [9]. In addition to germination, the extraction method with ultrasound leads to further extractioncome onNet oxidants are especially polyphenolic compounds and as a result increase the antioxidant activity of the extracts [10]. Mustard seeds are rich in biologically active compounds and have a reasonable price and considerable popularity all over the world. These characteristics have made mustard seeds an attractive subject for scientific research. All kinds of wild types of mustard that are scattered all over Iran show the high compatibility of this oil seed with the country's climate.[11]Iranian mustard is mainly cultivated in the southern regions of Iran and is traditionally used as a natural preservative [12].Despite this, according to the studies carried out,So far, there has been no study on the antioxidant properties of cultivated and native Iranian mustard seed sprout extract extracted by maceration method and treatment with ultrasound waves. Therefore, the present study was conducted for the first time with the aim of investigating the phenolic compounds and antioxidant capacity of methanolic and acetic extracts of Iranian mustard seeds and evaluating the effect of their use on the oxidative stability of soybean oil.

2- Materials and methods

2-1- Materials

Iranian mustard seeds*Brassica juncea*) It was obtained from Larestan in Fars province and soybean oil without antioxidants was obtained from Varamin No. 1 oil factory. The mentioned samples were kept at a temperature of 4 degrees Celsius and in a dark and dry place until the time of testing. All standards, chemicals and solvents used in this research were purchased from Sigma.

2-2-Methods

2-2-1- germination

Mustard seeds were carefully cleaned and their impurities were completely separated and after sterilization with 1.5% sodium hypochlorite solution, they were used for germination. Mustard germination stage based on the methodPerez-Balibrea et al. (2011) with slight changes. The seeds were washed with distilled water and soaked overnight in one liter of double distilled water. Hydrated seeds were placed in germination trays containing moist filter paper and transferred to the growth chamber. The sprouts were collected after 7 days and dried in an electric oven (Memmert, Germany) at 40 °C for 24 hours and then powdered.]13 [.

2-2-2- Preparation of methanolic and acetone extracts of mustard seeds

Extraction of methanolic and acetone extracts of edible mustard seeds by maceration method and using ultrasonic waves According to

MethodGharachorloo and colleagues (2012)With A few changes were made. Mustard seed powder with methanol solvents and acetone separately (5:1) mixed and the maceration and extraction of the extracts were done for 24 hours at room temperature with stirring. And then each of the mixtures containing methanol and acetone solvents was placed in an ultrasound bath (Oj Azma Plast, Iran) with a frequency of 20 kHz and a temperature of 25 degrees Celsius for 20 minutes. After completing the extraction, the extracts were centrifuged at a speed of 4000 rpm for 20 minutes.Behsan، Iran)and the upper phase was collected. Solvents using rotary evaporator under vacuum.Heidolph , Germany) and evaporated at a temperature of 40 degrees Celsius. The resulting concentrated extracts were stored in dark containers at a temperature of 4 degrees Celsius until consumption [14].

2-2-2-1- Extraction efficiency of mustard bud extract

The total amount of extracted mustard seed sprout extracts was calculated by taking into account the initial weight of the plate and the final weight containing the dry extract, and the extraction efficiency was determined in terms of percentage [15].

2-2-2-2- total phenolic compounds

The content of total phenolic compounds of mustard sprout extracts, according to the methodFernandez-Orozco**And**colleagues (2006) and measured using a spectrophotometer (Laviband, England) at a wavelength of 765 nm and the results were expressed in terms of milligrams of gallic acid per kilogram of extract [16].

2-2-2-3-Measuring the free radical inhibitory power DPPH[1]

Free radical inhibitory powerDPPH Mustard sprout extracts using the methodBrand-William and colleagues (1995) was measured with some changes. the amount of

[1]-2,2-Diphenyl-1-picrylhydrazyl

0.1 ml of the extract was diluted with 9.9 ml of distilled water and 0.1 ml of the resulting solution was diluted with 3.9 ml of methanol solution.DPPH mixed. The control sample contains 0.1 ml of methanol and 3.9 ml of methanolic solutionDPPH Was. Zero adjustment of the spectrophotometer (Lovebond, England) First, it was done with pure methanol, and then the absorption value of all samples was read after 30 minutes at a wavelength of 515 nm in a stable state. Finally, the percentage of free radical inhibitionDPPH **It was calculated by the extract with formula 1 where**And **control absorption and**As **sample absorption [**17**].**

Formula 1Inhibition (%) =[(Ac-As).Ac]×100

2-2-2-4-Measuring the reducing power of iron[2]FRAP)

To measure the reducing power of iron from the methodBenzi and Strain (1996) was used. working solutionFRAP by mixing 10 ml of 300 mmol acetate buffer (3.6 = pH), 1 millimole of 2,4,6-tri-2-pyridyl-S-Triazine(TPTZ) 10 mmol (dissolved in 40 mmol hydrochloric acid) and 1 ml of iron chloride 20 mmol were prepared daily. In the test tube, 0.02 ml of extract (1 mg/ml) or aqueous standard solution of iron sulfateFriday$_4$ (concentration 0.185-0.37 µmol/liter), 1 ml of working solution was added and mixed. The above mixture was placed at room temperature for 5 minutes. Then, the light absorption of the samples was read at 593 nm wavelength. The regenerative activity of the extract samples was calculated using the standard curve in terms of micromoles of iron per gram of the extract.[18]

2-3-Preparation and preparation of soybean oil samples containing extracted extracts for oven testing

Methanolic and acetone extracts of mustard seeds with a concentration of 0.5 and 1% (w/w) to 100 grams of refined soybean oil without added antioxidants and mixing by magnetic stirrer.Heidolph , Germany) was done for 15 minutes. Then, soybean oil samples containing different amounts of mustard seed sprout extract and soybean oil samples without extract (Table 1), according to the methodShakerardakani et al. (2019)in itMemmert, Germany) were placed and their stability was maintained on the 5th, 10th, 15th, 20th, 25th and 30th days using sub-evaluation tests.[19].

2-3-1- Oxidation stability index measurement

Oxidative stability index of soybean oil samples containing mustard seed extract using Ransimet device.Metrohm , Switzerland) according to the methodAOCS to numberCd 12b-92 At a temperature of 110 degrees Celsius, the amount of 2.5 grams of the sample and the air flow rate of 20 liters per hour were measured.[20].

Table 1 The Cods of Treatments

Treatment code	Extraction Method	Extract Concentration
T1	Methanol	0.5
T2	Methanol	1
T3	Acetone	0.5
T4	Acetone	1
T5	Methanol/Ultrasound	0.5
T6	Methanol/Ultrasound	1
T7	Acetone/Ultrasound	0.5
Q8	Acetone/Ultrasound	1
T0(Blank)	----	----

2-3-2- Measurement of thiobarbituric acid index

The thiobarbituric acid index shows the milligrams of malondialdehyde present in 1000 grams of oil and indicates the secondary stages of fat oxidation and the presence of secondary oxidation compounds in the sample. To measure the index of thiobarbituric acid in a 250 ml Erlenmeyer flask, one gram of sample, one

[2]- Ferric Reducing Antioxidant Power

milliliter of 0.75% solution of thiobarbituric acid and 2 ml of 35% solution of trichloroacetic acid were added. The resulting mixture was placed in a boiling water bath for 20 minutes. After this period, the mixture was centrifuged at 3000 rpm for 3 minutes. The aqueous phase was taken out with a syringe and transferred to the spectrophotometer cell, and the absorption of the sample was measured with a spectrophotometer (Lavibond, England) at a wavelength of 532 nm, and the absorption value of the sample at the said wavelength was considered as an indicator of thiobarbituric acid.[22].

2-3-3- Measurement of peroxide number

Peroxide number is a measure to measure the oxidation rate of oils and fats. In this method, the amount of all substances that can oxidize potassium iodide in the test conditions.، It was determined in milliequivalents of peroxide per kilogram of fat sample. Evaluation of indic peroxide by iodometric method and according to the methodAOCS to numberCd 8-53 Done. 5 grams of oil sample was added in a 250 ml Erlenmeyer flask and 30 ml of a mixture of chloroform and acetic acid (3:2). After the sample was dissolved, 0.5 ml of saturated potassium iodide solution was added to it and left in the dark for 1 minute, then 30 ml of distilled water was added and sodium thiosulfate solution of 0.1 normal titer and the yellow color disappeared. After adding 2 ml of starch reagent and shaking, the titration continued until all the iodine released from the solvent reacted with the sodium thiosulfate solution and the blue color disappeared, and the peroxide index was calculated using formula 2 [20,21] [.

$$\text{عدد پراکسید} = \frac{(S - B) \times N \times 1000}{w}$$ Formula 2

B: The volume of sodium thiosulfate solution used in the control test (milliliters)

S: The volume of sodium thiosulfate solution used in the sample test (milliliters)

N: Normality of sodium thiosulfate solution

IN: sample weight (gram)

1000: conversion factor of gram to kilogram unit

2-3-4- Anisidine number

The basis of this test is the reaction of the aldehyde compounds of the oil in an acetic solution and anisidine reagent and then determining the absorbance by a spectrophotometer (Laviband, England) at a wavelength of 320 nm. The yellow color of the reaction products depends on the amount of aldehyde compounds present and their structure. The completely transparent sample was weighed with 0.001 g precision in a 25 ml balloon and a balloon with isooctane. Using spectrophotometry and a glass cell, absorption was determined at a wavelength of 350 nm.Ab). Using a pipette, 5 ml of the oil solution was transferred to a test tube with a lid. In another test tube with a lid, 5 ml of solvent was poured. 1 ml of anisidine reagent was added to each of these tubes and shaken well. After 10 minutes, absorb the solution of the first test tube with a glass tube E was determined at a wavelength of 350 nm and the anisidine number was calculated using formula 3.As)[22].

$$\rho - A.V. = \frac{(1.2\ As - Ab) \times 25}{m}$$ Formula 3

As: Absorption of fat solution after reaction with anisidine reagent

Ab: Absorption of fat solution

m: sample weight (gram)

2-3-5- Number of Tutox

Totox number is a measure of the overall oxidation of the oil and was calculated through formula 4]22 [:

Totox = 2×(PV) + AnV

PV: Peroxide number

AnV: Anisidine number

2-4- Statistical analysis

In this study, the tests were conducted in the form of a factorial experiment based on a completely randomized design with three replications. Analysis of the obtained results using variance analysis(ANOVA) and Duncan's multi-domain mean comparison test with the software SPSS 26.0، SAS9.4 And also drawing diagrams with the software2016 Excel Done. Duncan's test was used at the 95% confidence level to compare and detect the existence of a significant difference between the average results obtained.

3. Results and Discussion

1-3- The results of mustard seed extract evaluation tests

The amount of phenolic compounds, Free radical inhibitory powerDPPH AndThe reducing power of ironFRAP) and also the extraction efficiency of methanolic and acetone extracts of mustard seeds are presented in Table 2.EfficiencyExtraction of methanolic and acetone extracts respectively 0.02±10 and 0.05±It was 10 mg per gram of mustard seed powder, which was 0.04 in methanol/ultrasound and acetone/ultrasound extracts, respectively.±14 and 0.05±15 mg per gram increased. In other words, the use of ultrasonic waves in the extract extraction process increased the extraction efficiency by 40% and 50%, respectively, in methanolic and acetone extracts. Also, there was no significant difference between the total amount of phenolic compounds, the free radical inhibitory power and also the iron reducing power of methanolic and acetone extracts of mustard seed extract (0.05)≥*p*). However, the extracts of mustard seeds extracted with ultrasound had a higher amount of total phenolic compounds, free radical inhibitory power, and iron reducing power (0.05).≥*p*). The free radical inhibitory power in methanolic/ultrasound extracts of mustard seeds is 69% andThe regenerative power of ironμmol /g 77 was determined By using ultrasonic waves during extraction, due to the phenomenon of cavitation and increased destruction of the cell wall, the penetration of the solvent into the plant cells is increased and the diffusion of cell materials from the solid phase to the solvent is increased and the mass transfer is better.Dubieh et al. (2013), in their research results, reported that ultrasound treatment by reducing the process time can extract them from seeds without significant degradation of existing primary antioxidants.*Brassica juncea* to help [23]. Two et al. (2014), high amounts of polyphenol (mg GAE/g DW 15.726 ± 0.15) in methanolic extract*Brassica Juncea* reported [24].Dubieh et al. (2013), the amount of polyphenols*Brassica juncea* in the extraction method with water,mg SAE/g DW13/79 and in the ethanol extraction methodmg SAE/g DW reported 8/53 [24].Doinita Bors et al. (2017), Mizan

Phenolic compounds of 7-day-old brown mustard sproutsmg GAE/g DW 8/13 and reported its free radical inhibition rate of 59% [1].

Table 2 The total phenolic contents, DPPH free radical scavengering capacity, ferric reducing antioxidant power (FRAP) and *extraction efficiency of* mustard seed extract [a]

Test type	Extract type			
	Methanol extract	Acetone extract	Methanol/Ultrasonic extract	Ultrasonic/Acetone extract
Total phenolic contents (mg Gallic acid/ g)	435±0.02	434±0.01	434±0.02	475±0.03
DPPH free radical scavengering capacity (%)	65±0.04	66±0.03	69±0.04	68±0.04
Ferric reducing antioxidant power (FRAP) (μmol /g)	72±0.01	73±0.01	77±0.01	75±0.01
Extraction efficiency (mg extract/ g)	10±0.02	10±0.05	14±0.04	15±0.03

[a]Results are means of three different experiments

2-3- The results of stability evaluation tests of soybean oil samples

By examining the results of analysis of variance and also comparing the mean of oxidative stability index, it was observed that the effect of treatment, storage time and the interaction effect of treatment during storage time on the amount of oxidative stability index of soybean oil treatments were significant at the probability level of 0.05% (0.05).≥*p*). According to Figure 1, The amount of stability index on the fifth day of storage in all samples was equal to 11 hours And with the passage of time, the index of oxidative stability decreased so that the lowest level of stability was observed on the 30th day of storage. But the amount of oxidative stability index of soybean oil samples containing extract Methanol and acetone extracted with ultrasonic waves, were higher than soybean oil samples containing acetone and methanolic extracts (0.05≥*p*). Also, increasing the concentration of the extract from 0.5 to 1% led to an increase in oxidative stability.In higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups in the reaction medium, the probability of donating hydrogen to free radicals and, subsequently, the inhibitory power of the

3-2-1- Oxidative stability index

extract increases [25]. samples Contains a concentration of 1% methanol extract/ultrasoundT6(and Estonian/ultrasound)Q8) showed the highest stability (10 hours). has been reported that Extraction with the help of ultrasonic waves is more effective compared to the maceration method and leads to the acceleration of extract extraction and the release of more effective compounds, including phenolic compounds, which was consistent with the results of the present research.[26]. Based on previous research, it has been proven that the extract*Brassica juncea*As a natural antioxidant, compared to synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene, it works very effectively in preventing lipid oxidation [27,28].

There was no significant difference between the oxidative stability index of soybean treatments based on the type of acetone and methanol extract.Ngo et al. (2017), reported in their research results that methanol and acetone are among the solvents that have the highest extraction efficiency of solid materials from plants. Bioactive compound

Available in plants are diverse and have different polarity andThey are selectively more soluble in some solvents. Each group of these compounds has a different antioxidant power, the result of which determines the antioxidant capacity of the extract [29].In previous researches, higher amounts of extractable plant polyphenols have been reported in methanol extract compared to other solvents such as ethanol, diethyl ether and hexane.Dua et al. (2014), in their research results, stated that by using methanol, high amounts of polyphenols, especially caffeic acid and quercetin, can be extracted from fat-free mustard seeds.[24].

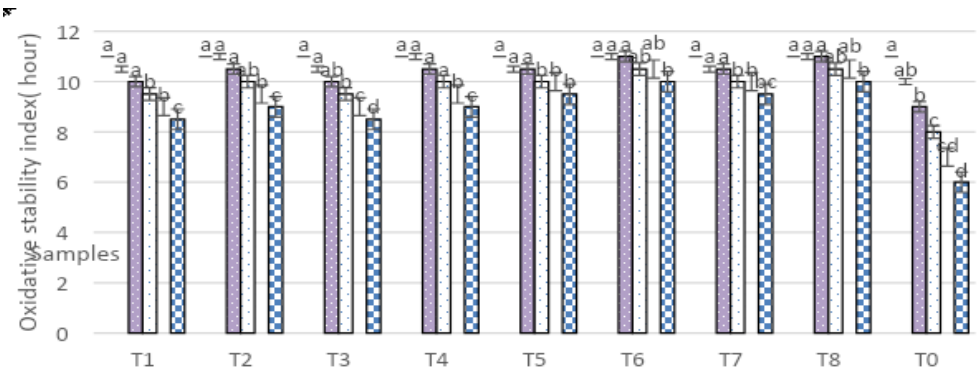

Fig 1 Comparison of the average of oxidative stability of soybean treatments during storage. Same letters indicate no statistical difference among the treatments while different letters indicates treatments have significantly difference ($p < 0.05$).Error bars represents standard deviations of mean values.

3-2-2- Thiobarbituric acid index

By examining the results of analysis of variance and also comparing the mean of thiobarbituric acid index, it was observed that the effect of treatment, storage time and the interaction effect of treatment during storage time on the amount of thiobarbituric acid index of soybean oil treatments were significant at the probability level of 0.05% (0.05).≥*p*). Due to theShape2، The amount of initial thiobarbituric acid on the fifth day of storage in all samples was 0.02 mg of malonaldehyde per kg. With Increased retention time, valuesIndicatorRenal thiobabituric acidSoybean oil samples increased significantly (0.05).≥*p*) and on the 30th day of storage, the highest amount of thiobarbituric acid was observed for all samples. This increase is attributed to the accumulation of products resulting from the breakdown of unsaturated fatty acids[28].. Therefore, the high level of this index in the oil indicates more oxidation of the oil and, as a result, less stability [22]. However, the trend of increasing thiobarbituric acid in soybean oil samples containing extract extracted with ultrasoundCompared with The control and soybean oil samples containing methanolic and acetone extracts were significantly slower (0.05).≥*p*) . Use of waves

ultrasoundBy reducing the extraction time and increasing the efficiency, it increases the extraction of antioxidants[23]. Also, by increasing the concentration from 0.5% to 1%, the increase of thiobarbutyric acid was slower, which is due to the presence of phenolic compounds and the increase in antioxidant activity of the extract extracted from mustard seeds [28]; So that on the 30th day of storage, the lowest amount of the indexThiobarbituric (mgMDA/kg16/0) In soybean oil samples containing 1% concentration of methanol extracts (ultrasound)T6) and Estonian/ultrasoundQ8) was observed.Antioxidants in mustard seed extract are effective in donating electrons, and this feature is involved in the antioxidant capacity of mustard seed [24]. There was no significant difference between the amount of thiobarbituric acid index of soybean treatments based on the type of acetone and methanol extract.Ribeiroet al. (2019), stated that the extraction efficiency of different solvents depends on the characteristics of the material and the internal linkage of the phenolic compounds in the product and compatibility with the chemical composition of the solvent used [30]. There are various compounds in mustard seeds that have antioxidant activity. oxidants are involved and these compounds have different solubilities and different antioxidant behavior. The most important parameter in ultrasonic extraction includes the ratio of solvent to substance, solvent composition and extraction temperature. Twenty minutes of using ultrasound is enough to maximize antioxidant extraction, and sonication for more than 30 minutes does not increase the concentration of phenols in the extracts [23].

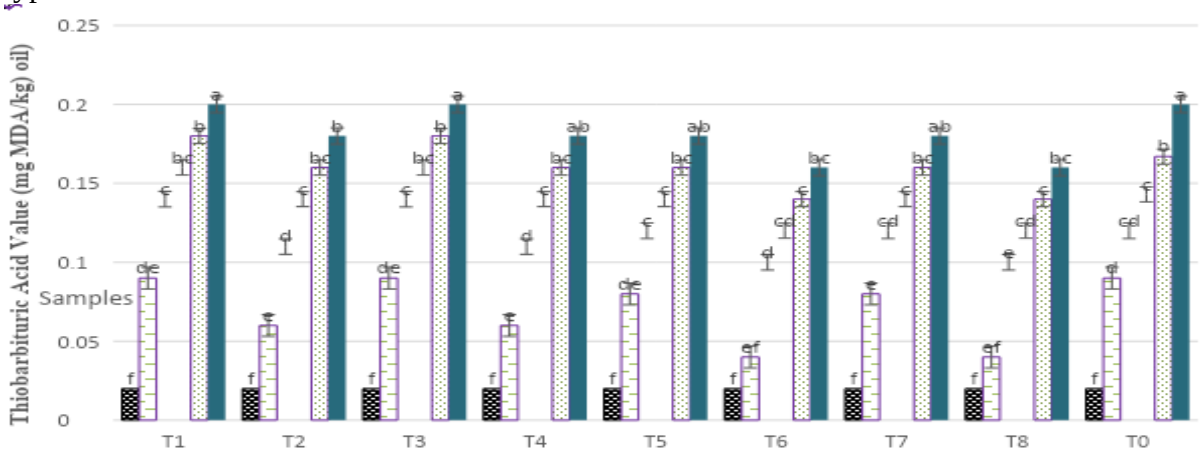

Fig 2 Comparison of the average Thiobarbituric acid value of soybean treatments during storage. Same letters indicate no statistical difference among the treatments while different letters indicates treatments have significantly difference ($p < 0.05$).Error bars represents standard deviations of mean values.

3-2-3 - number peroxide

By examining the results of analysis of variance and also comparing the average peroxide number, it was observed that the effect of treatment, storage time and effect

The interactions between treatments during storage on the amount of peroxide number of soybean oil treatments were significant at the probability level of 0.05% (0.05≥*p*). According to Figure 3,index Peroxide increased significantly in all samples during storage (0.05).≥*p*) which indicates the oxidation and formation of peroxides during storage [17] but increasing the concentration of mustard seed sprout extract from 0.5 to 1% and using ultrasound waves in the extraction significantly reduced the process of increasing the peroxide index (05 /0≥*p*). So that on the 30th day of storage, soybean oil samples containing 1% concentrations of methanolic/ultrasound extracts (T6) and Estonian/UltrasoundQ8) The lowest index compared to other samplesperoxide(mego$_2$/kg73/0) had. Peroxides are the primary oxidation products of unsaturated fatty acids. The peroxide index is used to evaluate primary oxidation [17]. The extracts added to soybean oil prevent oxidation and increase the stability of the oil due to their natural antioxidants.differencestatisticsThere was no significance between the amount of peroxide number of soybean treatments based on the type of methanolic and acetone extract.Salari et al. (2009), in the results of their research, stated that the polarity parameter plays a more important role in the extraction of phenolic compounds than the selectivity in solvent systems, because with this parameter being constant, no significant difference is observed between the treatments.[31]. Worker et al. (2014), in the investigation of the antioxidant effects of ethanolic and methanolic extracts of the savory plant on the antioxidant properties of soybean oil, found that the peroxide number and thiobarbituric acid index of soybean oil samples containing methanolic and ethanolic extracts during 49 days of storage significantly It was less than the control sample. Although the methanolic extract reduced the peroxide value more effectively compared to the ethanolic extract [32].

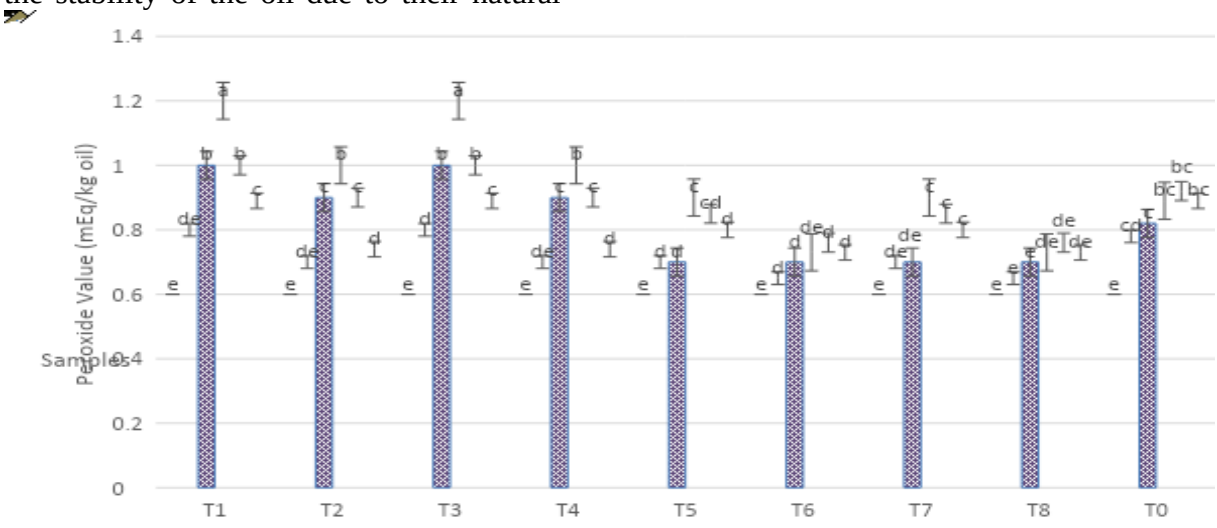

Fig 3 Comparison of the average peroxide value of soybean treatments during storage.

Same letters indicate no statistical difference among the treatments while different letters indicates treatments have significantly difference ($p < 0.05$).Error bars represents standard deviations of mean values.

3-2-4- Anisidine number

By examining the results of analysis of variance and also comparing the mean anisidine number, it was observed that the effect of treatment, storage time and the interaction effect of treatment on the amount of anisidine number of soybean oil treatments were significant at the probability level of 0.05% (0.05).≥*p*). According to Figure 4The initial anisidine level of all soybean oil samples was 0.2, which increased significantly with increasing storage time.(05/0≥*p*) However, this increasing trend was slower in soybean oil samples containing methanol/ultrasound and acetone/ultrasound extracts, and the lowest amount of anisidine number (0.6) on the 30th day of storage in the treatmentsT5,T6,T7,T8 was observed.

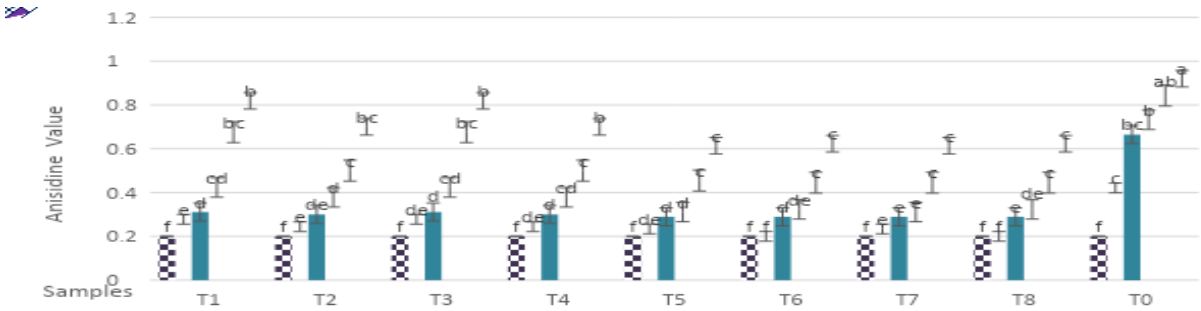

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Fig4. Comparison of the average anisidine value of soybean treatments during storage. Same letters indicate no statistical difference among the treatments while different letters indicates treatments have significantly difference (p < 0.05).Error bars represents standard deviations of mean values.

Anisidine number as an approximate estimate of the shelf life of freshly processed oil and It is used to control products caused by secondary oxidation, and after thirty days of storage under accelerated oxidation conditions, the amount of anisidine number was lower than the maximum allowed amount (10) [17].

There was no significant difference between the amount of anisidine number of soybean treatments based on the type of methanolic and acetone extract.Salari et al. (2009), reported in their research results that extraction systems containing acetone perform better in qualitative extraction and extraction systems containing methanol perform better in quantitative extraction. Optimizing the structure and type of solvent can play an effective role in better extraction of antioxidant compounds [31].It has been reported that methanolic and aqueous extracts of various plants have better radical scavenging activity compared to dichloromethane or ethyl acetate extracts, this indicates the polar nature of antioxidant biomolecules [33]. The increase of polyphenols and antioxidant activity after germination in this research was consistent with previous findings [34].

The results of research by Dua et al. (2011) showed that antioxidants*Juncea Brassica* It effectively prevents lipid oxidation either by chelating metals or by inhibiting diffusion reactions. Metal chelation plays a very important role in the antioxidant mechanism because it reduces the metal concentration that catalyzes lipid peroxidation. Inhibition of lipid peroxidation by polyphenols from other plant sources has also been reported [24].

3-2-5- Number of Tutox Sample soybean oil

By examining the results of analysis of variance and also comparing the average number of Totox, it was observed that the effect of treatment, storage time and the interaction effect of treatment in storage time on the Totox number of soybean oil treatments were significant at the level of 0.05% (0.05).≥*p*). According to Figure 5, the amount of Totox number showed a significant increase during the storage time (0.05).≥*p*(But this increase in soybean oil samples containing 1% concentration of methanolic/ultrasonic mustard seed extracts)T6) and Estonian/UltrasoundQ8) It was done at a slower pace. So that on the thirtieth day of storage, the lowest amount of Totox number (2.083) in the samplesT6 AndQ8 seen. A significant difference was observed by increasing the concentration from 0.5 to 1%. There was no significant difference between the Totox number of soybean treatments based on the type of methanolic and acetone extracts.Totox number includes both peroxide and anisidine indices, so it is considered a more suitable index for measuring oil quality and its value should be less than 10 [17]. The peroxide value changes over time as peroxides are produced and decomposed. The Totox number has the advantage of providing complete information about the oxidation state of the oil, and in calculating the Totox number, the primary products (anisidine number) and secondary oxidation products (peroxide number) are considered [35].. Two et al. (2014) reported thatThe protective effect of methanolic extract of defatted mustard seeds shows that antioxidants of mustard seeds can effectively deactivate existing hydroxyl radicals [24].

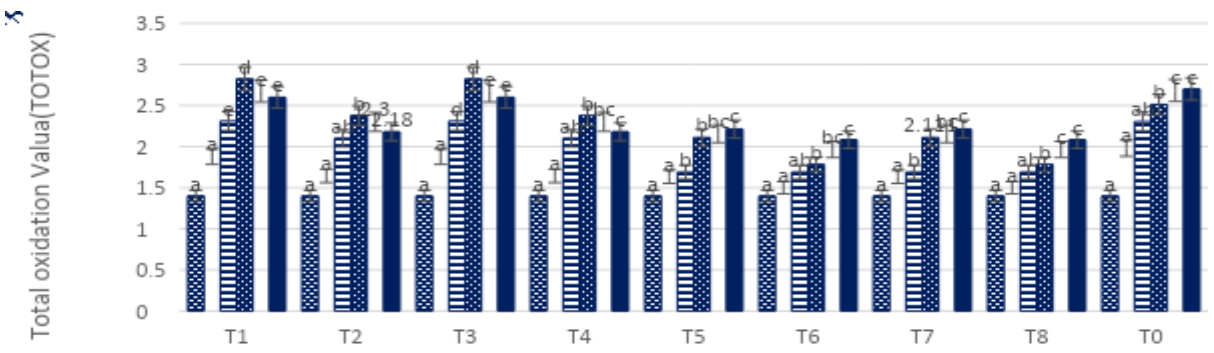

Fig 5 Comparison of the average TOTOX value of soybean treatments during storage. Same letters indicate no statistical difference among the treatments while different letters indicates treatments have significantly difference ($p < 0.05$).Error bars represents standard deviations of mean values.

4 - Conclusion

Considering the lack of reports on antioxidant activity and phenolic compounds of Iranian mustard sprouts, this study shows promising results. Antioxidant effects of mustard seed sprout extract Extracted with methanol, acetone and ultrasound In soybean oil, it was measured by different methods and it was found that due to the richness of this extract in phenolic compounds and having antioxidant properties, it effectively prevented the production of primary and secondary oxidation products during storage, which with a decrease in the amount peroxide numbers, anisidine, totox, thiobarbituric acid index,And the increase in the oxidative stability index of the oil samples was confirmed. The samples containing 1% concentration of methanol/ultrasound and acetone/ultrasound extracts were the best samples in terms of oxidative stability. seed sproutMustard is a valuable source of bioactive compounds and natural antioxidants that can be used in the formulation of edible oil or for the purpose of enriching and developing innovative food products due to its beneficial effects on human health.

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بررسی خواص آنتی اکسیدانی عصاره جوانه دانه خردل استخراج شده با امواج فراصوت و اثر آن بر پایداری اکسایشی روغن سویا

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چکیده

امروزه استفاده از عصاره های گیاهی حاوی آنتی اکسیدان های طبیعی برای افزایش پایداری اکسایشی روغن های خوراکی بیش از پیش مورد توجه قرار گرفته است. هدف از این تحقیق بررسی خواص آنتی اکسیدانی عصاره متانولی واستونی جوانه دانه خردل ایرانی استخراج شده به روش ماسراسیون و امواج فراصوت و اثر آن در پایداری اکسایشی روغن سویا بود. خصوصیات آنتی اکسیدانی عصاره‌ها با تعیین میزان کل ترکیبات فنولی، قدرت مهارکنندگی رادیکال آزادDPPH و همچنین قدرت احیاء کنندگی آهن بررسی شد و پایداری نمونه‌های روغن سویا حاوی غلظت ۰/۵ و ۱ درصد عصاره ها پس از آون گذاری در روزهای پنجم، دهم، پانزدهم، بیستم، بیست و پنجم و سی ام نگهداری، با انجام آزمون‌های تعیین شاخص پایداری اکسایشی، عدد پراکسید، عدد آنیزیدین، شاخص تیوباربیتوریک اسید مورد ارزیابی قرار گرفت. نتایج نشان داد که طی دوره نگهداری میزان پراکسید، آنیزیدین و شاخص تیوباربیتوریک اسید نمونه ها افزایش و شاخص پایداری اکسایشی به طور قابل توجهی کاهش یافت($p\leq$۰/۰۵). اختلاف آماری معنی داری بین پایداری اکسایشی نمونه های حاوی عصاره متانولی و استونی وجود نداشت. افزایش غلظت عصاره، سرعت اکسیداسیون روغن را به طور معنی داری کاهش داد($p\leq$۰/۰۵). روند کاهش پایداری در نمونه های حاوی غلظت ۱ درصد عصاره متانولی واستونی استخراج شده با امواج فراصوت به دلیل دارا بودن ترکیبات فنولی و خواص آنتی اکسیدانی بالاتر در مقایسه با شاهد و سایر نمونه ها به طور قابل توجهی کند تر بود($p\leq$۰/۰۵) و این عصاره به عنوان یک آنتی اکسیدان طبیعی در افزایش پایداری روغن سویا موثر واقع شد.