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Evaluation of oxidative stability of Iranian virgin walnut kernel (*Juglans regia L.*) oil enriched with ultrasound-assisted extracted Iranian germinated paddy rice extract

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ABSTRACT

The aim of this study was to evaluation of oxidative stability of Iranian virgin walnut kernel oil enriched with ultrasound-assisted extracted Iranian germinated paddy rice (GPR) extract under accelerated oxidation conditions. The powdered GPR sample was extracted with ethanol/H2O (70:30,V/V) using ultrasonic waves at the temperature of 50˚C and time of 30 minutes with the frequency of 40 KHZ and solid to solvent ratio of 1:20 (w/v) in an ultrasound water bath. The extraction yield (2.91%), DPPH radical scavenging activity (77.52%) and total phenolic contents (909.2 mg GAE/1000g DW) of GPR were determined. The protective effects of the GPR extract in stabilization of virgin walnut kernel oil at different concentrations (0 ppm,500 ppm,1000 ppm,1500 ppm,) were evaluated for a period of five days (120 h) by monitoring the peroxide, p-anisidine, totox values at an oven temperature of 90˚C (accelerated oxidation). Also, the oxidative stability index (OSI) was evaluated and compared with the synthetic antioxidant BHT (200 ppm). In oxidative stability tests of Iranian virgin walnut kernel oil supplemented with antioxidant extract, increasing the concentration of the extracts led to their better performance in delaying oxidative rancidity ($p<0.01$). According to the results, BHT (200 ppm) and the GPR extract (1500 ppm) showed the highest protective effects. In the rancimat test, the oxidative stability indices of the control sample, the sample of Iranian virgin walnut kernel oil containing 1500 ppm of GPR extract and BHT (200 ppm) were 3.25 h, 8.93 h and 12 h, respectively ($p<0.01$). Thus, BHT and GPR extract (1500 ppm) in comparison with the control sample, showed a significant effect on increasing the oxidative stability index of Iranian virgin walnut kernel oil. Therefore, GPR can be used as a source of antioxidant compounds in edible oils.

1-Introduction

Walnut kernels are a valuable source of polyunsaturated fatty acids (PUFA), which mainly consists of linoleic acid (47.4%) and alpha-linolenic acid (15.8%).[1].Walnut oil consumption leads to reduced levels of low-density lipoprotein.(LDL), increased high-density lipoprotein(HDL)It reduces total triglycerides, lowers blood cholesterol levels, and reduces the risk of cardiovascular disease.However, the presence of high levels of polyunsaturated fatty acids(PUFA), makes walnut oil susceptible to oxidation, whichIn addition to reducing economic value, it affects its quality parameters by reducing nutritional, sensory, and chemical properties.In order to delay the oxidation of edible oils, in addition to limiting oxidation-inducing factors such as light, oxygen, and high temperatures, the addition of antioxidants is also considered. .[2]Synthetic antioxidants such as butylated hydroxyanisole(THERE WERE), Butylated hydroxytoluene(BHT)Tertiary-butylhydroquinone(TBHQ)and propyl gallate(PG), are widely used in most countries. However, there are many concerns about their safety. Therefore, many studies have been conducted so far on the use of natural plant extracts with antioxidant activity in various edible oils. Plant extracts rich in phenolic compounds are extracted from herbaceous plants, cereals, leaves, vegetable and fruit waste, etc. .[3]

Whole rice, including brown rice and paddy rice, is a good source of antioxidants, especially in the bran and husk parts. Lipophilic antioxidants such as tocopherols, tocotrienols, and oryzanol, and phenolic compounds such as phenolic acids and flavonoids are abundant in whole rice. Phenolic compounds have shown a wide range of biological activities such as antioxidant, antimicrobial, antiviral, and anti-inflammatory activities and promote human health. Given that the food consumption pattern in the world is changing rapidly today and the desire to consume healthy foods has increased, sprouting cereal grains and legumes has been recognized as an economical method to increase the nutritional value of these grains. This method increases bioactive compounds, including compounds with antioxidant properties such aspolyphenols, vitamins and gamma-aminobutyric acid, as well as increasing the antioxidant capacity of cereal grains[4].Sprouted rice grains have been used to produce high value-added products with improved biological, nutritional, and physicochemical activities. .[5,6,7,8,9]Some researchers have investigated changes in rice grain quality in terms of its nutritional, physicochemical, and antioxidant properties after germination. .[10,11,12,13, 14,15] Germination of cereal grains increases various bioactive compounds, including polyphenols, vitamins, gamma-aminobutyric acid, and increases the antioxidant capacity of cereal grains.Therefore, sprouting can be used to increase bioactive compounds.[16,17,18].Wu and Colleagues (2022) evaluated the effect of germination on the content of phytic acid, phytase, gamma-aminobutyric acid, gamma-oryzanol, phenolics, flavonoids and antioxidant capacity of rice and brown rice. The content of gamma-aminobutyric acid and phytase activity in germinated rice was determined to be lower than that of germinated brown rice. The antioxidant activities in germinated rice were higher than those of brown rice during the germination process.[19]. Chinmaand colleagues(2024) investigated the effect of germination on the technological-functional properties, nutritional composition and health-promoting compounds of rice grains and its products. Germination appears to be a suitable bioprocessing method to improve nutritional quality and increase bioactive compounds and modify the technological-functional properties of rice grains for diverse food applications, improving global nutrition and food safety.[20] .

As previously mentionedWalnut kernel oil is in high demand among consumers due to its favorable sensory and nutritional properties,

but it is easily oxidized, becomes rancid, and changes flavor. Given that research onAntioxidant capacity and amount of phenolic compoundsGerminated Iranian rice paddy extract and its effect on enhancing the oxidative stability of edible oils are not available, therefore the purpose of this research isExtraction of germinated Iranian rice paddy extract using ultrasound waves andThe antioxidant effect was evaluated on virgin Iranian walnut kernel oil extracted by cold pressing.

2- Materials and methods

2-1- Materials

SolventsAnd the chemicals used were of laboratory purity.

2-1- 1-Preparing Iranian walnut kernels

Walnuts from the Lower Tarom region of QazvinThe walnut kernels were then separated from the brown skin until the oil was extracted and the relevant tests were performed.In polyethylene plastics in the refrigerator at a temperature C ˚4It was kept.

2-1-2-Preparation of rice and paddy sample**Its germination**

Paddy riceThe Hashemi variety was purchased from the Kamashal region of Kiashahr, Astaneh Ashrafieh, Gilan.Sample**Paddy rice**First cleaned and washed, then soaked in water at room temperature.°C 35- C° 30 for 6 hours at room temperature (°C28) was soaked. The samples were then placed in a damp cloth and left at room temperature for 48 hours without exposure to light for germination.[21]. Sample**Paddy rice**Sprouted and dried at room temperature and away from sunlight.Then, using a home electric grinder (MJ-W176P, Panasonic, Japan) It was powdered. To make the particle size uniform, it was sieved with a 40 mesh sieve and stored in a glass container with a lid in a refrigerator at room temperature until the experiment.˚C 4 was maintained.

2-1-3-Ultrasound-assisted extraction of antioxidant extract from germinated rice paddy

Extraction of antioxidant extract from the sample Paddy riceGerminated in an ultrasound bath(WUC-D10H, Wiseclean, Korea) At temperature˚C50, time 30 minutes and frequency 40 kHz with solventsafeEthanol/water (70:30 v/v) with a solids to solvent ratio of 1:20 (w/v). Glass containers with lids were used to prevent and minimize solvent evaporation loss. After extraction, the extract was filtered through Whatman filter paper No. 1 and the extract was concentrated using a rotary evaporator. (Laborota 4000, Heidolph, Germany) In˚CThe concentrated and dried extract was stored in light-protected containers at 40 °C.˚C4 was kept until further tests.[22].

2-2- Tests

2-2-1- Rice paddy extract tests

2-2-1-1-Extraction efficiency of antioxidant extract

The extraction efficiency (%, w/w) was determined based on the following equation:

$$yield\ (\%) = \frac{W2}{W1} \times 100$$

in which(W2) The weight of the resulting extract and(W1) The weight of the sample.

2-2-1-2- Amount of total phenolic compounds in antioxidant extractTotal phenolic content (TPC)

The total amount of phenolic compounds in the extract Sample Paddy riceSprouted Through colorimetry to. The Folin-Ciocaltio method is examined. According to the method of McDonald et al. (2001) ، 5/0 1 ml of the extract was mixed well with 5 ml of Folin-Ciocaltio reagent (diluted 10 times with distilled water) and 4 ml of 1 M sodium carbonate solution. The mixture was kept at room temperature for 15 minutes. Then the absorbance of the solution was read by spectrophotometer at a wavelength of 765 nm. The total amount of phenolic compounds was expressed as mg/g of dry matter using a standard curve based on gallic acid.[23].

2-2-1-3- Antioxidant activity, free radical scavengingDPPHAntioxidant extract

Free radical scavenging activityDPPHAccording to the methodBrand-Williams and colleagues(1995) Determined with some modifications. 5/0ml of different extract concentrationsSample Paddy riceSprouted (mg/ml1، mg/ml5/2 andmg/ml 5) with5/2 ml of solution5/0 Millimolar methanol solutionDPPHIt was mixed. Then the mixtureMix thoroughly with a shaker and serve.30The cells were incubated for 10 minutes in the dark at room temperature. Light absorption in517Nanometers against a control using a spectrophotometerUV–Vis The results were measured in terms of percentage of radical inhibition.DPPHCalculated according to the following equation:[24].

$$Inhibition\ DPPH\% = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100$$

in which(Abs control)AttractSolutionDPPH Without extract (control solution)،(Abs sample)AttractIn the presence of extract.

2-2-2-Iranian walnut kernel tests

2-2-2-1-Determining the moisture content of walnut kernels

The moisture content of the sample was measured by gravimetric method until constant weight was reached in an oven at a temperature ofC ° 100Calculated.[25]

2-2-2-2-Determining the percentage of walnut kernel oil

Determining the percentage of oil in the sampleWalnut kernelBy Soxhlet method and based on standardISO To the number659It happened..[26]

2-2-3-Extraction of walnut kernel oil by cold pressing method

Oil Walnut kernelBy cold press machine(NF1000,Karapress,Iran)With temperatureC °50- C°40 was extracted. After centrifugation at 4500 rpm for 20 minutes to remove suspended solid particles, the samples were stored in dark glass bottles with lids for one day until the time of the experiments at room temperature.C° 4 was kept.[27]

2-2-4- Study of physicochemical properties of Iranian walnut kernel virgin oil

Oil paint by Lavibond machine(Tintometer Model F, Lovibond PFX 995) Determined.[28] Index of Idi [29] and soap index[30]It was determined. To determine the amount and composition of fatty acids, first methylation of the oil sample was performed.[31]Then, the fatty acids in the sample were identified and quantified by gas chromatography.[32]The specifications and conditions of the gas chromatography device were as follows::

(Nexis 2030, SHIMADZU, Japan)Obviously Flame ionization device(FID)[1]، Capillary columnDikmacap-2330 Made of glass, 60 meters long and with an internal diameter of۲۰0.0 mm, hydrogen gas with a purity of 999.99% as carrier gas and flow rate of 2 ml/min, injection site temperature˚C 250، Detector temperature ˚C 260، Sample injection volume 1 microliter, device split ratio 1 to 60 and the device's temperature program: initial temperature˚C 60 and remaining for 2 minutes at the same temperature, then with a gradient/min ˚C10Reaching DMay˚C 200، Then with gradient/min˚ C5 Reaching temperature˚C 240, remaining for 7 minutes at the same temperature to remove all fatty acids from the column.

2-2-5- Investigating the oxidation process through oven heating

Samples of virgin walnut kernel oil enriched with different levels of antioxidant extract of germinated rice paddy (ppm 1500، ppm 1000، ppm500 andppm0) and synthetic antioxidantsBHT (ppm200) individually packaged in suitable glass containers and

1-Flame Ionization Detector

placed in an oven at a temperature of 5± C°90 were kept.Oil stability to oxidation of both4 Once an hour during a period5Fasting (120 hours) with calculation of acid index values [33]، Peroxide index [34]، Para-anisidine index [35] And Andis Totox[36]It was evaluated.Totox index based on equationBelowDetermined.

TV=OFF+2PV

in which(OF) Para-anisidine indexand(PV) The peroxide index is .

2-2-6-Evaluation of oxidative stability index(OSI)

Oxidative stability index by Rencimet device(Metrohm 743,Switzerland) It was determined. The test was performed separately with 3 grams of virgin oil sample.Walnut kernelContainsppm 1500 Antioxidant extractSprouted rice paddy(Based on the results of the oil stability test during oven-drying), the oil sample containedppm200Synthetic antioxidantBHTand control sample (Does not contain any antioxidants.) inTemperature°C110 and air flow speedL/h 20 Done. [37].

2-3-AnalysisStatistical

All experiments were performed in triplicate. Results are expressed as the average of three replicates and standard deviation. Then, analysis of variance was performed.(ANOVA)One-way analysis was performed and finally, to determine a significant difference at the level01/0>pDuncan's multiple range test was used. Analyses were performed using software SPSS 22and drawing graphs using softwareExcelDone.

3-Results and Discussion

3-1-Extraction efficiency, amount of total phenolic compounds and Antioxidant activity, radical scavengingDPPH Sprouted rice paddy extract

Extraction efficiency, total phenolic compounds content and activityAntioxidant, radical scavengerDPPH Sprouted rice paddy extract It is shown in Table 1.

Table 1- Means and standard errors of extraction yield,total phenolic contents and %DPPH of Iranian germinated paddy rice extract

Factor	Result
Extraction yield (g/100g DW)	0.09 ± 2.91
Total Phenolic Content (mg GAE /100g DW)	4.61± 909.2
DPPH radical scavenging activity (%)	1.22 ± 77.52

In this study, extraction efficiency, total phenolic compounds content andAmount Antioxidant activity, radical scavengingDPPH ExtractExtracted germinated rice paddyBy ultrasound method, respectivelyg/100g DW 91/2، mg GAE/1000g DW2/909 and۲۲/1 ± 52/77% was determined.The extraction efficiency and composition of the extract depend on the extraction method and the polarity of the solvent. An efficient method should have characteristics such as shortening the extraction time, reducing solvent consumption, increasing extraction efficiency, and improving the quality of the extracts. Many studies،The effectiveness of ultrasound technique in increasing the extraction efficiency of phenolic compounds from plant extracts Compared to other extraction methods, extraction enhancement is primarily related to the phenomenon of cavitation, which involves the formation, growth, and collapse of microbubbles within the liquid phase when exposed to ultrasonic waves.Research has shown thatUltrasound

methodNot only does it increase the extraction efficiency of polyphenols, but it also leads to the preservation of the biological activity of polyphenolic extracts and their higher efficacy compared toMethodClassic extraction methods such as maceration and Soxhlet extraction are used. Parameters such as temperature, frequency, power, solvent type, and solvent-to-material ratio affect the composition of the phenolic extract. And its effectiveness (antioxidant, anticancer and antimicrobial properties) is impressive. Also, non-toxic, food-grade and environmentally friendly solvents such as ethanol, isopropanol and-n Butanol by the United States Food and Drug Administration(FDA)It is recommended for extraction purposes. Water and ethanol mixtures are commonly used for the extraction of phenolic compounds from plant materials. The reason for this is that, in addition to being non-toxic and having food grade, a wide range of phenolic compounds are soluble in water-ethanol mixtures. Therefore, in this study, ethanol/water 70:30 was used as the extraction solvent for antioxidant extracts. Extraction temperatures higher than˚C 50 causes the destruction and decomposition of polyphenols in the extract. Low frequencies up to 40 kHz are also more effective in extraction. Usually, the extraction efficiency of polyphenols increases with increasing power and frequency (up to a threshold), but beyond the aforementioned threshold, no significant increase in efficiency is observed. High power and frequency lead to the creation of hydroxyl free radicals and the destruction of polyphenols. Therefore, in order to achieve high results and efficiency in ultrasound-assisted extraction, the correct selection of parameters will be of particular importance. [22]. The propagation of ultrasound waves causes cavitation and increased pressure, more pores and greater penetration. As a result, the extraction efficiency increases.[38, 39].

Artichokeset al. (2015) showed that the use of ultrasonic waves in the temperature rangeC°45 toC°60 can increase the extraction efficiency of bioactive compounds. This is because the thermal effects and cavitation created in the liquid phase during ultrasound waves lead to the destruction of the cell wall and the reduction of particle size, ultimately increasing the extraction efficiency of bioactive compounds. However, the use of high temperatures will lead to solvent evaporation and damage to the bioactive compounds.[40].

RascalandWorker (2017) Extraction of antioxidant compounds from sprouted chickpeas by ultrasoundinvestigated and showed that ExtractionWith the help of ultrasound waves,More efficient than conventional extraction techniques[41] .

Studies show that the germination process causes the accumulation of secondary metabolites, some of which are known as antioxidants and can inactivate unstable free radicals..[15] Most research shows that germination leads to improved capacity. Antioxidant The seeds will be.[17,40, 42] Germination can alter the antioxidant activity of many edible seeds, due to an increase in antioxidant compounds, including vitamins and phenolic compounds. Researchers have shown that germinationSeeds,ContentTPCThey increase. .[43,44]The high levels of phenolic compounds in germinated rice paddy extract lead to an increase in the radical inhibition effect. DPPH It can be.

3-2- Moisture content and oil content of Iranian walnut kernels

Table 2 It shows the moisture content and oil content of Iranian walnut kernels.

In this research**Moisture content of walnut kernel sample 2**3.7±1/3% and the rate**Oil extraction efficiency of walnut kernel sample by Soxhlet method 35/2**±74% was determined. In international standards, the moisture content of walnut kernels is determined as 3%-5% and the oil content of walnut kernels is determined as 71%-62%.[45] .

Gaoet al. (2021) reported the yield of walnut oil extracted by cold pressing method. **25/1**±47.04% determined[46].

3-3- Physicochemical properties of virgin oilIranian walnut kernels

Table 2 also Physicochemical properties of virgin oilIranian walnut kernels Included The oil color shows the peroxide index, acid index, iodine index, soap index and oxidative stability index. Physicochemical properties of virgin walnut kernel oil extracted by cold pressing method, including oil color (70 yellow) and (30 red), peroxide index (meq O_2/kg oil0.39), acid index (mg KOH/g oil 06/0)، Iodine index (gI_2/100g oil 69/151), Soap Index (mg KOH/g oil 191.96) and oxidative stability index (3.24 hours) were determined.

Table 2- Means and standard errors of moisture and oil contents of walnut and physicochemical properties of walnut kernel oil

Factor	Result
Moisture content (%)	3.7±1.2
Oil content (%)	74±2.35
Color (yellow units)	70
Color (Red units)	30
Iodine Value (g I_2/100g oil)	151.69±0.06
Saponification Value (mg KOH/g oil)	191.96±0.02
Oxidative Stability Index (h)	3.24

3-4- Fatty acid profile of virgin oilIranian walnut kernels

Figure 1 Fatty acid profile of virgin oilIranian walnut kernels**shows.**

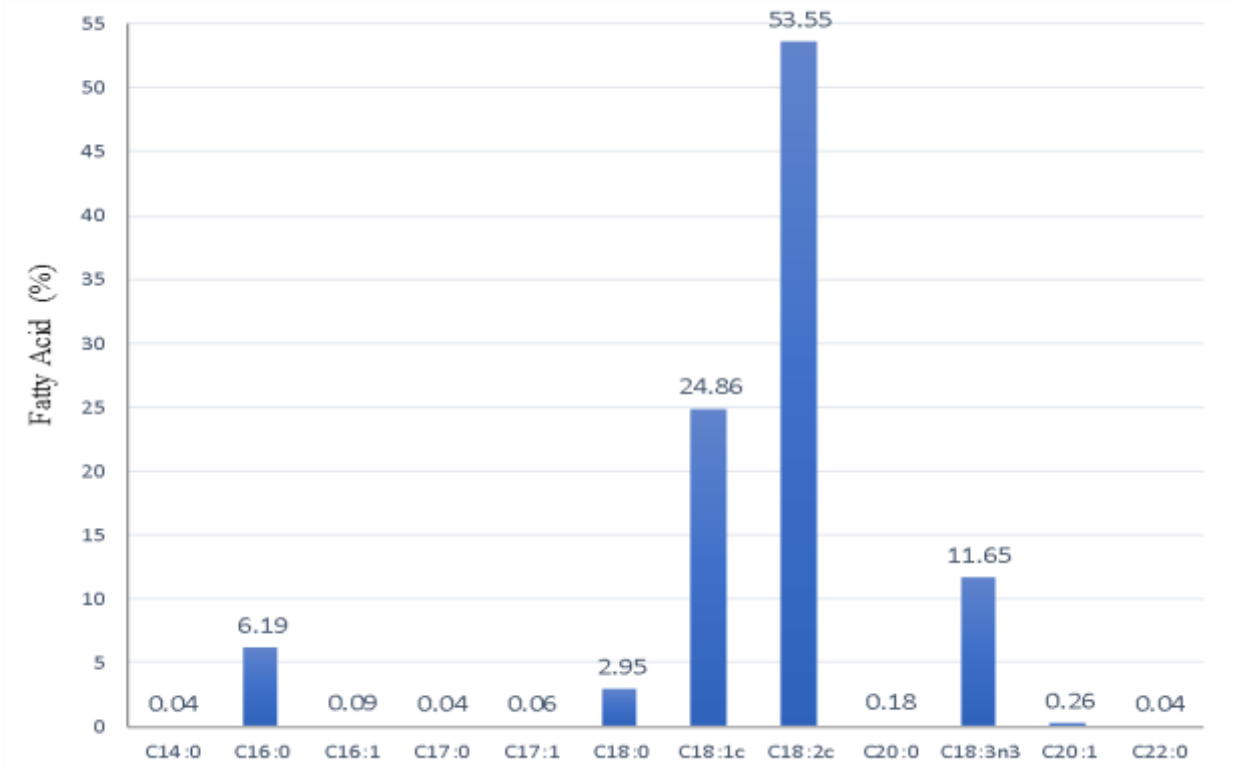

Fig1. Fatty acid composition of walnut oil

In this study, the fatty acid content of Iranian walnut kernel virgin oil included:C14:0(04/0 %)، C16:0 (19/6 %)، C16:1 (09/0 %)، C17:0 (04/0 %)، C17:1(06/0 %)، C18:0(95/2 %)، C18:1c (86/24 %)، C18:2c (55/53 %)، C20:0 (18/0 %)، C18:3n3(65/11 %)، C20:1 (0.26%) andC22:0(0.04%) was determined.

Gaoet al. (2021)determined the fatty acid content of walnut kernel oil extracted by cold pressing. In their study, these researchersC16:0 (56/6 %)، C18:0(24/3 %)، C18:1c (64/14 %)، C18:2c (05/67 %)، C18:3n3(31.8%) andC20:1 (0.22%) was determined[46].

More than 60% of walnut oil is composed of polyunsaturated fatty acids..[47] The fatty acid profile of virgin walnut kernel oil contains high levels of essential linoleic acid (50-65%), which is higher than other oil seeds and nuts..[48,49,50,51,52]

3.5-Antioxidative functionUltrasonically extracted germinated rice paddy extractInVirgin Iranian walnut kernel oil extracted by cold pressing method

3-5-1-Peroxide index(PV)

Peroxide index change curve of virgin walnut kernel oilAs a function of the type and concentration of antioxidant (natural: rice bran extract and synthetic:BHT) and incubation time (5 days at room temperature)theC90) Under accelerated oxidation conditionsIn Figure 2 is presented.

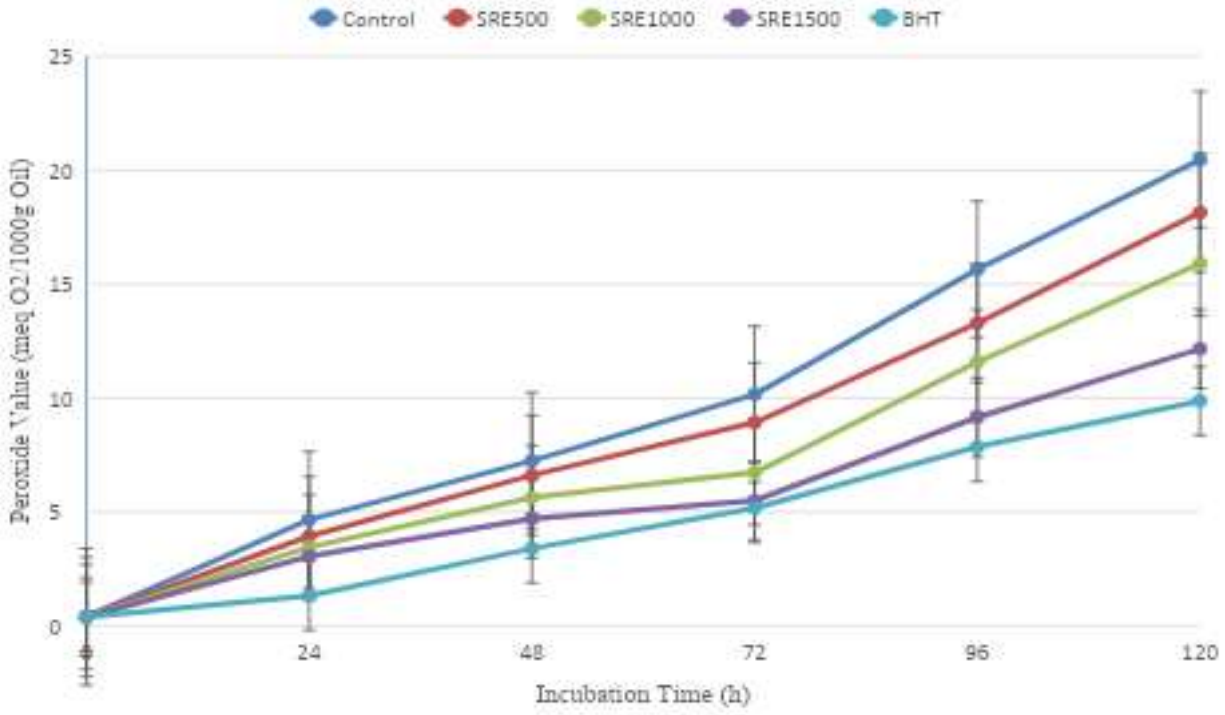

Fig 2. Change in peroxide Value (PV) of walnut oil without antioxidants (control), and with the addition of Iranian germinated paddy rice extracts (SRE) and BHT,analyzed during a 120-hour storage period at 90 theC temperature

According toCODEX-STAN 210–1999The acceptable limit of peroxide value for vegetable oils extracted by cold pressing is related tomegO2/1000g oilIt is 15.[2]

The results of the present study showed thatType of antioxidant, extract concentration and oven timeSignificant impact onTest resultsAndisPeroxide samples contain(، 1/، p<). Over timeGreenhouseFrom zero to 5 days (equivalent to 120 hours), the peroxide index of all samples increased. However, the increase in peroxide index was maximum in the sample of virgin walnut kernel oil without antioxidants (control sample) and decreased over time.Greenhouse production also maintained its maximum value.The peroxide index of all samples at zero oven time was determined to be 0.393, while the control sample, walnut oil samples containingppm 500،ppm 1000 andppm 1500 extractsSprouted rice paddy andBHT (ppm 200) During 120 hours of baking, there was an increasing trend in the peroxide index to the extent ofmegO2/1000g oil 47/20،megO2/1000g oil 14/18، megO2/1000g oil 9/15،megO2/1000g oil12/16 and megO2/1000g oilThey showed 87.9.(، 1/، p<).

Antioxidants are primarily used to delay the accumulation of oxidation products and thus improve the oxidative stability of lipids. The primary products of lipid oxidation are hydroperoxides, commonly referred to as

peroxides. IndexPeroxide is the milliequivalent of gram peroxide or active oxygen present in one kilogram of an oil or fat sample. The peroxide value is an indication of the initial stage of oxidative changes. The peroxide value is a measure of hydroperoxides. Hydroperoxides are identified as primary oxidation products and can be decomposed into volatile and non-volatile secondary products. [53]. The increase in phenolic compounds and the delay in oxidation indicate the important role of phenolic compounds during seed germination, and the enhancement of the nutraceutical potential of seeds is provided by germination..[54]According to the results of the present study, the addition of germinated rice bran extract, which is rich in antioxidant compounds, can prevent the onset of spontaneous oxidation in virgin walnut kernel oil.

Farhooshet al. (2011) showed that changes in peroxide value during 10 days of incubation atC° 50 in sunflower oil increased and then decreased, so the peroxide test alone cannot assess the severity of oil spoilage because these compounds are unstable to heat and are converted to carbonyl compounds such as aldehydes and ketones upon decomposition. .[55]

Gharachorloo et al. (2013) Extract capabilityMade from white beans(*Common bean*L.), broad bean(*Vetch sativa*), lentils(*Culinary lenses*)And peas (*Chickpea*) Sprouted barley was investigated in delaying the oxidation of sheep tallow.[56] .

3-5-2-Para-anisidine index(*p*-OF)

Variation curve of para-anisidine index of virgin walnut kernel oilAs a function of the type and concentration of antioxidant (natural: rice bran extract and synthetic:BHT) and incubation time (5 days at room temperature) theC90) Under accelerated oxidation conditionsIn Figure 3 is presented.

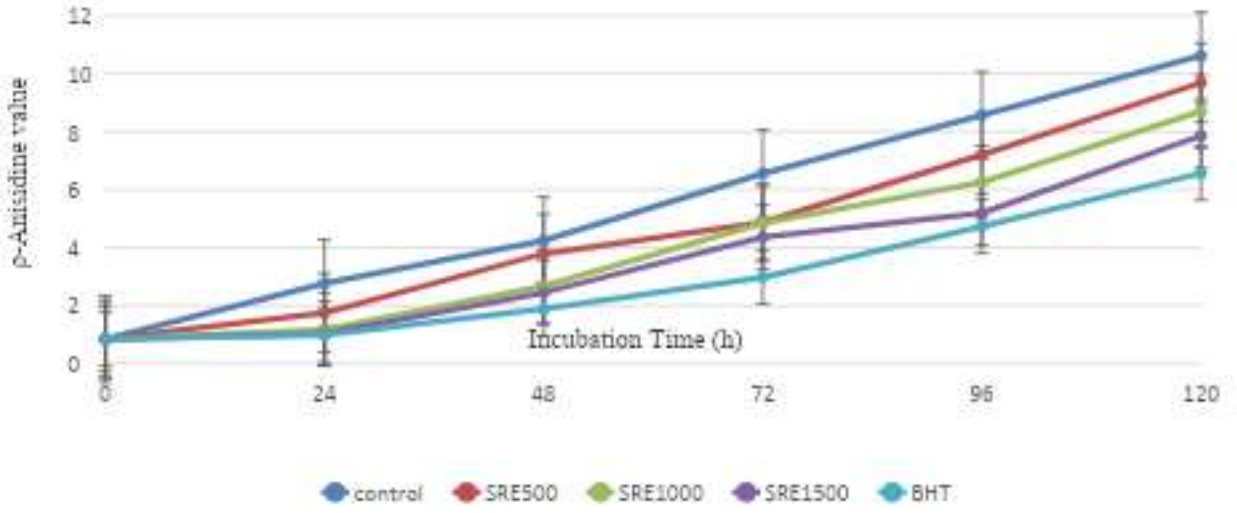

Fig 3. Change in *p*-anisidine Value (*p*-AV) of walnut oil without antioxidants (control), and with the addition of Iranian germinated paddy rice extracts (SRE) and BHT,analyzed during a 120-hour storage period at 90 theC temperature.

The peroxide index alone does not indicate the oxidation of the oil, because this number is an indicator of the presence of primary oxidation products and does not indicate the production of secondary oxidation products. Therefore, it seems necessary to perform a test such as determining the para-anisidine index, which is an indicator of the extent of oxidation development and the production of secondary products of this reaction.[57]. This index is one of the parameters used to evaluate oils for unsaturated aldehydes or secondary oxidation products. The results of this test confirm well the results of the peroxide index. This shows that large amounts of peroxides formed in the early stages have decomposed and converted to aldehydes during the final days of the experiment. The value of the para-anisidine index of the oil is a measure of the presence of significant amounts of secondary oxidation products. An increase in this index indicates the expansion of the spontaneous oxidation reaction and an increase in

secondary products resulting from the decomposition of hydroperoxides and carbonyl compounds.

The results of the present study showed thatType of antioxidant, extract concentration and oven timeSignificant impact onTest resultsPara-anisidineIt has oil samples. ($p<0.01$). Over timeGreenhouseVirgin walnut kernel oil from zero to 5 days (equivalent to 120 hours), index levelPara-anisidineAll samples showed an increasing trend. The rate of increase in the indexPara-anisidineIn the sample of virgin walnut kernel oil lacking antioxidants (control sample), the maximum was observed and decreased over time.Greenhouse production also maintained its maximum value.AndisAnisidineAll samples were determined to be 0.84 at zero oven time, while the control sample, walnut oil samples containingppm 500،ppm 1000 andppm 1500 extractsSprouted rice paddy andBHT (ppm 200) During the 120 hours of baking, the index increased in order ofAnisidineTo the extent 62/10، 69/9, 70/8, 86/7 and 57/6They showed(،1/، p<). Significant difference in para-anisidine index values between control and antioxidant samplesBHTAnd all extract concentrations were observed, which means a decrease in the rate of peroxide decomposition during the oxidation process in the treated samples. Therefore, by adding germinated rice bran extract to virgin walnut kernel oil, the formation of primary oxidation products as well as the formation of secondary oxidation products can be prevented.

3-5-3-Totox Index

Change curveAndisTotox virgin walnut kernel oilAs a function of the type and concentration of antioxidant (natural: rice bran extract and synthetic:BHT) and incubation time (5 days at room temperature)theC90) Under accelerated oxidation conditionsIt is presented in Figure 4.

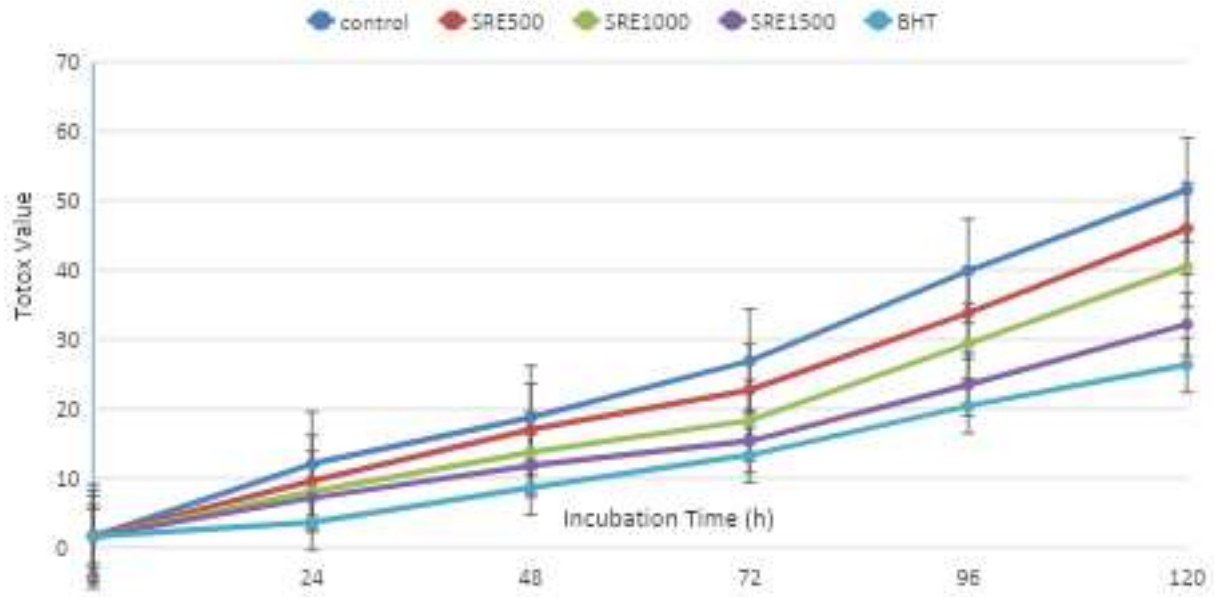

Fig 4. Change in Totox Value (TV) of walnut oil without antioxidants (control), and with the addition of Iranian germinated paddy rice extracts (SRE) and BHT,analyzed during a 120-hour storage period at 90 ^{the}C temperature.

This index, by considering the peroxide index and the anisidine index simultaneously, provides a more complete picture of the oxidation status of oil samples. An increase in the TOTOX index indicates an increase in the amounts of primary and secondary lipid oxidation products.HenceA decrease in the Totox index means better oil quality. In the present study,The effect of the independent variables of antioxidant type, extract concentration, and oven time on the trend of changes in the Totox index of the samples was

significant.(‧1/‧ p<). Within 24, 48, 72, 96 and 120 hours Oven-baking, significant difference in the toxin index of the control sample with antioxidantBHTAll extract concentrations were observed to indicate a simultaneous slowing of the rate of formation of primary and secondary lipid oxidation products.Over timeGreenhouseFrom 0 to 5 days (equivalent to 120 hours), the Totox index of all samples increased. However, the increase in this index was maximum in the sample of virgin walnut kernel oil without antioxidants (control sample) and decreased over time.Greenhouse production also maintained its maximum value.The Totox index of all samples at zero oven time was determined to be 1.63, while the control sample, walnut oil samples containingppm 500،ppm 1000 andppm 1500 extractsSprouted rice paddy andBHT (ppm 200) During 120 hours of baking, the Totox index showed an increasing trend to 51.56, 45.97, 40.50, 32.18, and 26.32, respectively.(‧1/‧ p<).

Since the TOTOX index is a measure of total oxidation and includes primary and secondary oxidation products,Adding germinated rice bran extract to virgin walnut kernel oil can prevent the formation of primary oxidation products as well as the formation of secondary oxidation products.

3-5-4-Acid Index

Change curveAcid indexVirgin walnut kernel oilAs a function of the type and concentration of antioxidant (natural: rice bran extract and synthetic:BHT) and incubation time (5 days at room temperature)^{the}C90) Under accelerated oxidation conditionsIt is presented in Figure 5.

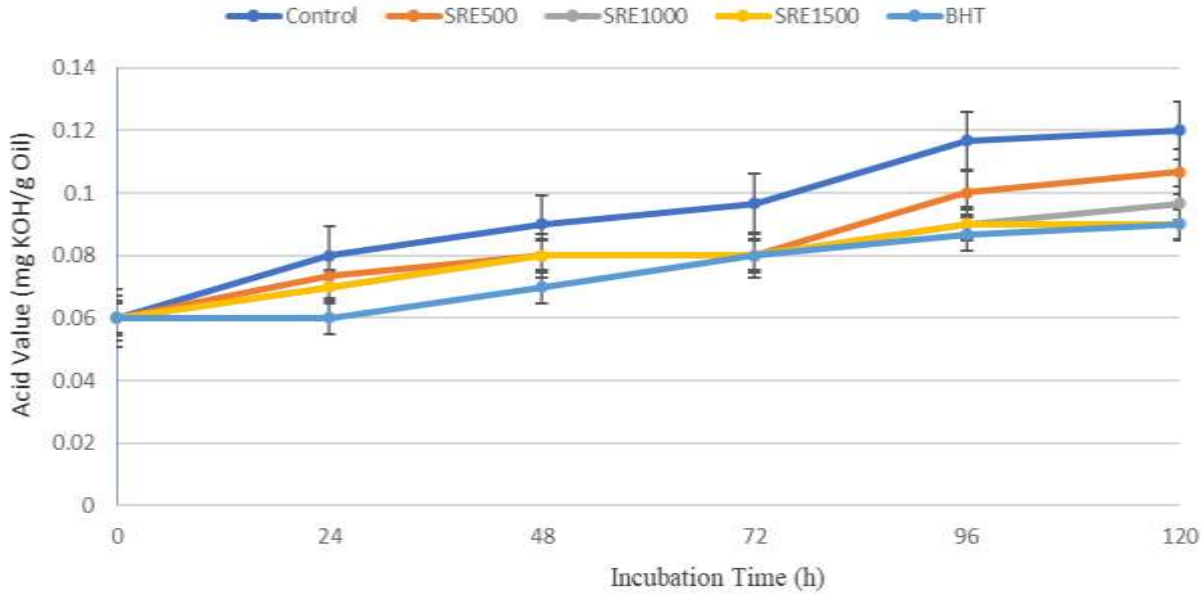

Fig 5. Change in Acid Value (AV) of walnut oil without antioxidants (control), and with the addition of Iranian germinated paddy rice extracts (SRE) and BHT,analyzed during a 120-hour storage period at 90 ^{the}C temperature.

Determining the acid index is one of the methods for diagnosing spoilage and aging of edible oils and fats. The acid index of an oil usually increases gradually and with a low slope due to the breakdown of triglycerides and the release of fatty acids. Oils undergo qualitative changes over time due to factors such as heat, humidity, and pollution, and the aforementioned index reflects these changes.In the present study,The effect of the independent variables of antioxidant type, extract concentration, and oven time on the trend of changes in the acid index of the samples was significant.(‧1/‧ p<). Within 24, 48, 72, 96 and 120 hours Oven-baking, significant difference in acid index of control sample with antioxidantBHTand all concentrations of the extract were observed to indicate a

slowingBreakdown of triglycerides and release of fatty acidsIt is.Over timeGreenhouseFrom zero to 5 days (equivalent to 120 hours), the acid value of all samples increased. However, the increase in the index was maximum in the sample of virgin walnut kernel oil without antioxidants (control sample) and decreased over time.Greenhouse production also maintained its maximum value.The acid index of all samples at zero oven time was determined to be 0.06, while the control sample, walnut oil samples containingppm 500،ppm 1000 andppm 1500 extractsSprouted rice paddy andBHT (ppm 200) During 120 hours of baking, the acid index showed an increasing trend to 0.12, 0.106, 0.97, 0.09, and 0.09, respectively.(،1/، p<).

3-5-5-Oxidative stability index (OSI)

Results of comparison of average impactType and concentration of antioxidants (natural: rice bran extracts and synthetic:BHT) Zinc Oxidation Stability IndexVirgin kernel oilWalnut It is presented in Figure 6. As can be seen,Type and concentration of antioxidantsMeaningful impact(01/0>p)Zinc Oxidation Stability IndexVirgin kernel oilWalnut According to the results, the highest oxidative stability index was(After the antioxidantSyntheticBHT) Related toVirgin walnut kernel oilContains germinated rice paddy extract with a concentration of (ppm1500).

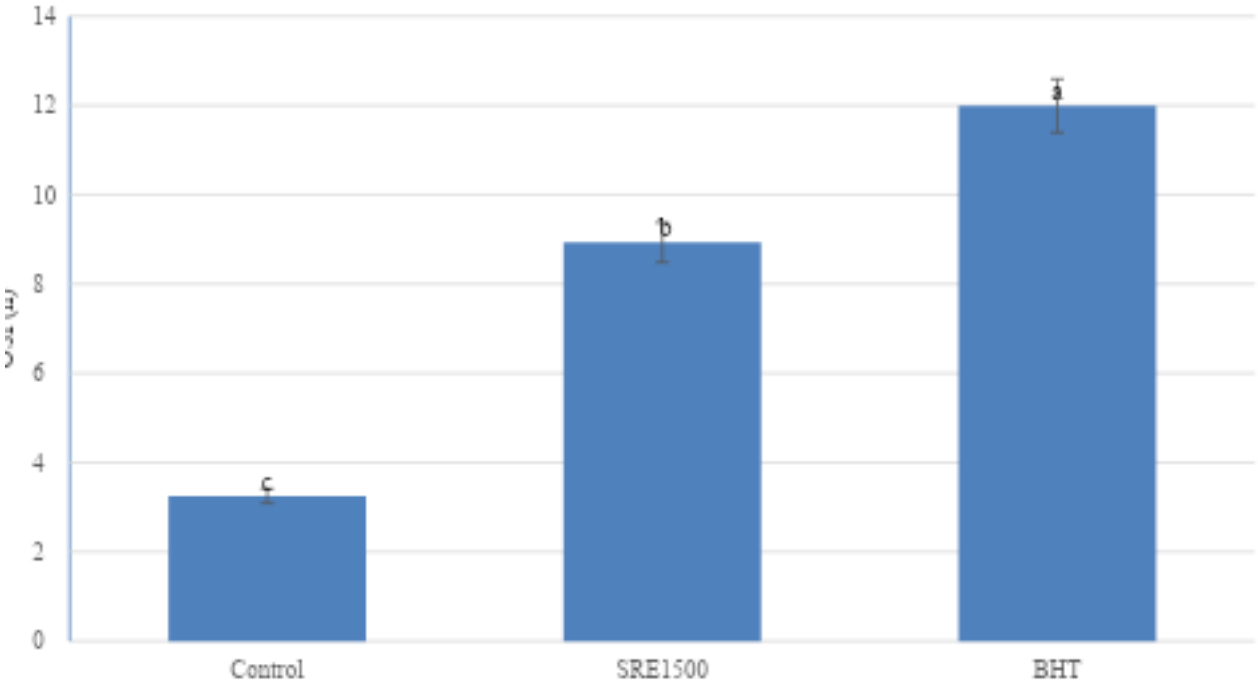

Fig 6. Change in oxidative stability index of of walnut oil without antioxidants (control), and with the addition of Iranian germinated paddy rice extract (1500 ppm) and BHT (200 ppm),analyzed at 110 ^{the}C temperature.
Different letters are signibecantly different (p>0.01).

According to the results of this study, the most influential extract level on oxidation parameters was the concentrationppm1500It was accompanied by a sample. Witness (ppm0) and the sample contains BHT (ppm200) ،For evaluation Oxidative stability index Selected. Oxidative stability index Control sample, oil sample containingppm1500 extracts andBHT (ppm200) The hours were determined as 3/25, 8/93, and 11/99, respectively (p<0/01). The better performance of synthetic antioxidants in the Rancimet test can be attributed to their higher resistance to thermal decomposition or loss due to volatilization, which is referred to as

retaining the property.[2]It is mentioned, attributed.Compared to the control sample, the antioxidantSynthetic BHT And then the extractSprouted rice paddyThey showed a significant effect in increasing the oxidative stability index and consequently the induction period of virgin walnut kernel oil.(‧1/‧ p<).

Oxidative stability is the time required to reach a point where oxidation indicators such as the amount of hydroperoxide or carbonyl compounds suddenly increase, causing undesirable aroma and flavor in the oil. The Rancimet method measures the secondary products of oil oxidation and can be used to predict the oxidative stability of oils. A stream of air is directed from the oxidation reaction site to a cell containing distilled water and the electrical conductivity of the water is measured. The increase in the electrical conductivity of the water is taken as an indicator of the progress of oxidation. The time from the moment of initiation to the point of sudden increase in electrical conductivity is called the stability time or induction period.[3]The oil stability index indicates the oxidation induction period, that is, the length of time the oil resists oxidation.[37]. Various factors are effective in the oxidative stability of oils, such as: fatty acid composition, triacylglycerol composition, the presence of antioxidant compounds such as tocopherols and carotenoids, the presence of prooxidant compounds such as heavy metals, chlorophyll, and compounds resulting from decomposition and oxidation. In the Rancimet method, secondary products resulting from the oxidation of oils and fats, including: aldehydes, ketones, and alcohols, are evaluated. The ability of an oil to resist oxidation is expressed as the oxidative stability of the oil..[58]

The Gurset al. (2019) showed that oxidation damages the nutritional composition of walnut oil and changes the color, viscosity, and stability of walnut oil, as well as affecting the sensory quality and shelf life of the oil.[59].

Gharachorlooet al. (2013) Changes in phenolic compounds and antioxidant activity of extracts of 4 types of legumes called white beans(*Common bean*L.) , broad bean(*Vetch sativa*), lentils(*Culinary lenses*)And peas(*Chickpea*)studied before and after germination. Three solvents, acetone, hexane and methanol, were used to extract the extract. These researchers added different concentrations of the extracts (0.02 and 0.1% w/w) to the talc and evaluated the antioxidant activity of the extracts by monitoring the peroxide index and induction period. The results indicated a significant increase in the phenolic content of the germinated seeds in all samples. Also, a significant difference was observed between the induction period of the talc treated with the germinated extract. Their results showed the greatest increase in oxidative stability in the talc treated with 0.1% concentration of germinated pea extract. The induction period of sheep talc at C°90 was 4.52 hours, which increased in all treatments with the addition of germinated seed extract. The increase in induction period was observed more at 0.1% extract concentration. As a result, the induction period is related to secondary oxidation products and the peroxide index is considered to be one of the primary oxidation products.[56].

4- Conclusion

In this study, the extractSprouted rice paddyBy the modern, efficient and green ultrasound methodand ethanol/water solvent (70:30 v/v)was extracted and its antioxidant effect was evaluated in Iranian walnut kernel oil extracted by cold pressing.IncreaseOxidative stabilityExtractSprouted rice paddyWith synthetic antioxidants BHT In monitoring changes in acid, peroxide, para-anisidine and totox indices,ExamplesVirgin walnut kernel oil، Synthetic antioxidant BHT (ppm200) And

2-Carry Through Property

31- Induction priod

subsequentlyExtractSprouted rice paddy (ppm1500) HighestEffectAntioxidantThey showed. Also synthetic antioxidantsBHT And then the extractSprouted rice paddy (ppm1500) Compared to the control sample, in the Rancimet test, it had a significant effect on increasing the oxidative stability index and consequently the induction period.Iranian walnut kernel virgin oilAccording to the results, the antioxidant extractSprouted rice paddyextracted using ultrasound waves as a source of antioxidant compounds in edible oils.

Conflict of interest

There are no items to declare.

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Authors' Contributions

All authors have participated in the design, analysis, writing, and approval of the final version.

5-Resources

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ارزیابی پایداری اکسیداتیو روغن بکر مغزگردوی ایرانی (*Juglans regia* L.) غنی شده با عصاره شلتوک برنج ایرانی جوانه زده استخراج شده به کمک روش اولتراسوند

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چکیده

هدف از این پژوهش، ارزیابی آثار حفاظتی عصاره شلتوک برنج جوانه زده استخراج شده توسط امواج اولتراسوند در پایداری اکسیداتیو روغن بکر مغز گردوی ایرانی بود. عصاره شلتوک برنج جوانه زده در یک حمام اولتراسوند در دمای ۵۰°C، زمان ۳۰ دقیقه و فرکانس ۴۰ کیلو هرتز با حلال safe اتانول/آب (۷۰:۳۰ حجمی/ حجمی) با نسبت ماده جامد به حلال ۱:۲۰ (وزنی/ حجمی) استخراج شد. راندمان استخراج(۲/۹۱٪)، مهار رادیکال آزاد DPPH° (۷۷/۵۲٪) و میزان ترکیبات فنولیک کل (۹۰۹/۲mg GAE/1000gDW) عصارهٔ شلتوک برنج جوانه‌زده تعیین شد. آثار حفاظتی عصاره شلتوک برنج جوانه زده در پایداری روغن بکر مغز گردو در غلظت های مختلف (۱۵۰۰ ppm, ۱۰۰۰ ppm, ۵۰۰ppm, ۰ ppm) از طریق پایش اندیس پراکسید، اندیس پارا-آنیزیدین، اندیس توتوکس و اندیس اسیدی طی آون گذاری در دمای ۹۰ °C به مدت ۵ روز (۱۲۰ساعت) ارزیابی شده و با آنتی اکسیدان سنتزی BHT (۲۰۰ppm) مقایسه شد. در آزمایشات پایداری اکسیداتیو روغن بکر مغزگردو غنی شده با عصاره آنتی اکسیدانی، افزایش غلظت عصاره باعث عملکرد بهتر آن ها در تأخیر فساد اکسیداتیو شد. بر اساس نتایج، BHT و عصاره شلتوک برنج جوانه زده (۱۵۰۰ppm) بیشترین اثرات محافظتی را نشان دادند ($p < 0.01$). در آزمون رنسیمت شاخص های پایداری اکسیداتیو نمونه های شاهد و روغن بکر مغزگردو ایرانی حاوی ۱۵۰۰ ppm عصاره شلتوک برنج جوانه زده وBHT (۲۰۰ppm) به ترتیب ۳/۲۵، ۸/۹۳ و ۱۲ ساعت تعیین شد ($p < 0.01$). بنابراین آنتی اکسیدان BHTو عصاره شلتوک برنج جوانه زده (۱۵۰۰ppm) در مقایسه با نمونه شاهد، تأثیر معنی داری بر افزایش شاخص پایداری اکسیداتیو روغن بکر مغزگردو ایرانی نشان داد. بنابراین شلتوک برنج جوانه زده می تواند به عنوان منبع ترکیبات آنتی اکسیدانی در روغن های خوراکی مورد استفاده قرار گیرد.