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Evaluation of Chemical Characterization, Antioxidant Activity and Oxidative Stability of Some Waste Seed Oil

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A B S T R A C T

In this study, fatty acid composition, antioxidant activity, total phenolic compounds (TPC) and oxidative stability of cherry seed (SCO), sweet cherry seed (SCSO), mulberry seed (MSO) and plum seed oil (PSO) were determined. Oleic acid was determined as primary fatty acid (42.9-67.3%), and followed by linoleic acid (23.4-41.8%) for SCO, SCSO and PSO. Linoleic acid was determined as primary fatty acid in MSO. γ-tocopherol was determined the main and highest tocopherol isomers varied from 579.9 to 605 mg/kg oil in SCO, SCSO and PSO, whereas δ-tocopherol was determined main tocopherol isomer with 1354mg/kg oil value in MSO. Plum seed oil (PSO) was the highest antioxidant activity values in both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays. There was no significant differences in lipid hydroperoxide and TBARS (2-thiobarbituric acid-reactive substance) formation among SCO, SCSO and MSO. PSO had the highest induction period (15.1 h), followed by MSO (1.4 h), SCSO (1.5 h), SCO(1.3 h). PSO was oxidatively more stable than the other oil samples. This research shows that these waste seed oils have high antioxidant capacity and tocopherol content, so they could be used in food industry.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 5(1): 48-53, 2017

Bazı Atık Tohum Yağlarının Kimyasal Karakterizasyonunun, Antioksidan Aktivite ve Oksidatif Stabilitelerinin Değerlendirilmesi

M A K A L E B İ L G İ S İ

Araştırma Makalesi
Geliş 13 Temmuz 2016
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Anahtar Kelimeler:
Yan ürün
Atık çekirdek yağları
Antioksidan aktivite
Oksidatif stabilite
Tokoferol

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Ö Z E T

Bu çalışmada, kiraz, vişne, dut ve erik çekirdek yağlarının yağ asidi komposizyonu, antioksidan aktivitesi, toplam fenolik madde ve oksidatif stabilitesi araştırılmıştır. Oleik asit (%42,9-67,3), kiraz, vişne, ve erik çekirdek yağların da en fazla bulunan yağ asidi olarak belirlenmiş olup ve onu linoleik asit izlemiştir (%23,4-41,8). Linoleik asit (%77,6) ise dut çekirdek yağında en fazla bulunan yağ asidi olarak belirlenmişitir. Kiraz, vişne ve erik çekirdek yağlarında, γ-tokoferol, başlıca tokoferol izomeri olarak belirlenmiş olup, değeri 579,9’dan 605 mg/kg yağ’a kadar değişmekte iken, dut çekirdek yağında δ-tokoferol, 1354mg/kg yağ değeri ile başlıca tokoferol izomeri olarak tespit edilmiştir. Erik çekirdeği yağı, 2,2-diphenyl-1-picrylhydrazyl (DPPH) ve 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) testlerinin her ikisinde de en yüksek antioksidan aktivite değerine sahiptir. kiraz, vişne, dut ve erik çekirdek yağları lipid hidroperoksit ve TBARS (2-tiobarbuturik asit-reaktif substant) oluşumları arasında önemli bir fark yoktur. Ayrıca, erik çekirdeği yağı en yüksek indüksiyon periyodu değerine (15,1 saat) sahip olup onu, dut (4,1 saat) kiraz (1,5 saat) ve vişne (1,3 saat) çekirdek yağları izlemektedir. Bu araştırma sonuçları bu atık çekirdek yağlarının yüksek antioksidan kapasite ve tokoferol içeriğine sahip olduğunu ve böylelikle bu yağlardan gıda endüstrisinde faydalanabiliceğini göstermiştir.

Introduction

Recently, there is growing interest to utilization in waste of foods (Shaker, 2006). After food production from fruist, vegetables or oilseeds, there is being high amounts of waste materials such as seeds, stones, peels (Djilas et al., 2009). Generally, some waste materials are used as animal feeds or fertilizers (Schieber et al., 2001), but most of them are kept as waste. It has been reported that some waste material contain valuable nutrients, such as antioxidant, polyunsaturated fatty acids, vitamins (Ramadan and Moersel, 2006). It is known that antioxidant compounds have positive effects on human health; for example, decreasing the heart and coroner diseases risks and helping for prevent the cancer (Williams and Elliot, 1997). Moreover, recently consumers prefer natural antioxidants rather than synthetic antioxidants in their meals, due to health benefits (Jayaprakasha et al., 2001; Moure et al., 2001; Schieber et al., 2001). However, it has been reported that some seeds are good source of antioxidant (Emad, 2006), but there are a few by-products used in food or cosmetic industry, due to antioxidant compounds, such as grape seed and olive waste extracts (Peschel et al., 2006).

According the FAO (2016), annual production of Cherry (*Prunus cerasus*), sweet cherry (*Prunus avium*), plum (*Prunus cerasifera*) and White mulberry (*Morus alba L.*) are 194989, 417905, 240806, 50000 tons, respectively in Turkey. Cherry, sweet cherry, plum and mulberry are consumed both fresh and use for production of juice, jelly, jam or molasses (Ajayi et al., 2006; Schieber et al., 2001). After these productions, huge amount of waste materials such as peel, stone, seed, stalk are being occur. Generally, cherry stalks are used in cosmetic industry, but the others are not used.

In the current study, fatty acid compositions, tocopherol isomers, oxidative stability and antioxidant capacity of cherry (CSO), sweet cherry (SCSO), plum (PSO) and mulberry (MSO) seed oil were determined. The results of this study are important for utilization of these waste products in the food and the others industries due to their antioxidative properties.

Materials and Methods

Materials

All chemicals and reagents for analysis were analytical grade and were purchased from Sigma Chemical Co.(St. Luis, MO, USA), Aldrich Chemical Co. (Steineheim, Germany), or Merck (Darmstad, Germany). A fatty acid methyl ester (FAME) mixture (37 component FAME mix) was purchased from Supelco (Bellefonte, PA). A tocopherol standard (50 mg of α, β, γ, and δ-tocopherol mixture) was from Calbiochem (La Jolla, CA).

Sample Preparation

Cherry, sweet cherry and plum, mulberry were purchased from the local farmers. Outer shells of the seeds are removed manually from kernel of cherry, sweet cherry and plum. Then, the seeds were cleaned and dried, they were broken into pieces smaller than 1 mm by using a grinder and then sieved. The oils were extracted by using the laboratory type oil-press (Cesalsan, Giresun). The oils were kept in glass-containers having -20°C nitrogen atmosphere.

Total Oil Content

The total oil content of the samples was determined in soxhlet apparatus by AOAC standard methods (1990). The seeds were ground by using a coffee miller. Then the oils from seeds (20 g) was extracted using 200 mL of hexane for 8 h. The solvent was removed using a rotary evaporator (Buchi, Switzerland) in 40 ^{0}C. The total oil content was determined in triplicate and the oil content was expressed in percentage.

Fatty Acid Analysis

Fatty acid compositions of the oils were determined by gas chromatography (GC). Briefly, 50 mg of oil was methylated with 3 mL 8% HCl in methanol at 95°C for 1h. The fatty acid methyl esters (FAMEs) were extracted with 2 mL of hexane and dried over sodium sulfate (Jennings et al., 1999). 1 µL of the FAMEs was analyzed with Shimadzu GC-17A (Shimadzu Company, Japan) using a SP-2560 capillary column (100m x0.25 mm i.d., x 0,2 µm; Cat No. 2-4056) with a flame ionization detector (FID), and an AOC-20i automatic injector. The oven temperature was programmed as follows: 120°C for 5 min, increased to 240°C at 4°C/ min, and kept at 240°C for 25 min. The injector and detector temperatures were each kept at 260°C. The carrier gas was helium, the flow rate was 30 mL/ min, and the split ratio was 1/50. FAMEs identification was based on the retention times as compared with those of the standard FAME mixture. Results were expressed as percentage of the peak area. Fatty acid analysis was performed in triplicate for each sample, and the average values were reported as percentage.

Tocopherol Analysis

Tocopherols were analyzed by a HPLC system (Shimadzu Prominence, Kyoto, Japan) and was determined as described by Turan et al. (2007). The normal phase column in the system was an Inertsil ODS-3 column (250 mm x 4.6 mm, 5 µm) and column temperature was maintained at 30°C. Separation of tocopherols was based on isocratic elution with n-hexane/isopropanol (96:4) at flow rate of 1 mL/min. The eluate was monitored at 292 nm by using a photodiode-array detector (SPD-M20A). The compounds were identified by comparing their retention times and the UV spectra with the authentic standards. Tocopherols were quantified based on the peak areas compared with the external standards. Tocopherol analysis was performed in triplicate for single samples of each variety, and the average values were expessed as mg/kg in oil.

Antioxidant tests

DPPH assay: The radical scavenging power was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Bondet et al., 1997). Initially, DPPH solution was dissolved in a small volume ethyl acetate and then diluted with ethyl acetate by adjusting the absorbance to

0.700±0.02 at 520 nm. In a test tube 20 mg oil sample was weighed, then 80 µL ethyl acetate and 2.9 ml DPPH$^{•}$ free radical solution were added. Next, the sample was agitated with a vortex mixer for 20 seconds. After 30 min of incubation in darkness, absorbance was measured at 520 nm against ethyl acetate. Trolox was used as a standard and the results were expressed as µg trolox equivalent/100 g oil.

ABTS assay: The radical scavenging power was determined by 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) method *(*Re et al., 1999). ABTS radical cation ($ABTS^{+}$) stock solution was prepared by reacting 7.0 mM ABTS stock solution with 2.45 mM (final concentration) potassium persulfate in dark for 16 h. The solution was diluted with ethanol by adjusting the absorbance to 0.700 ± 0.020 at 765 nm. 100 µL diluted oil samples in ethanol and 2.9 mL diluted $ABTS^{+}$ solution were added. The solution was agitated with a vortex mixer for 20 seconds. The absorbance was measured after 6 min at 765 nm. The results were expressed as µg trolox equiv /100g oil.

Total phenolic compounds (TPC): Total phenols were determined by Folin-Ciocaltue method according Gutfinger (1981) with minor modifications. Two hundred microlitres of methanolic extracts were added to 2.5 mL Folin-Ciocalteu reagent (diluted 1x10). After 3 min, 2 mL of sodium carbonate (%7.5) were added. After 2 h of incubation at room temperature, the absorbance 765 nm was measured. Gallic acid (GA) was used as a standard for determining the phenol content by Folin-Ciocalteu method. The results were expressed as µg gallic acids equiv (GAE) /g oil.

Oven Test

Ten grams of oils were weighed in glass petri plates (15 mm height and 80 mm diameter) and placed in a forced-draft air oven set at 60±1 ^{0}C. All oils (0.5 g) were removed from the oven at regular interval and flushed with nitrogen, covered with parafilm and kept –20 ^{0}C for the oxidative stability test.

Oxidative Stability

Peroxide value and TBARS (2-thiobarbituric acid-reactive substance) were measured as primary and secondary odidation products, respectively. The peroxide value (PV) was determined according the ferric thiocyanate method (Jayaprakasha et al., 2001). 0.1 g oil sample was weighed and then 9.7 ml ethanol added. 0.1 mL NH_4SCN and 0.1 mL $FeCl_2$ were added to the above mentioned solution and kept in room temperature for 5 min. Absorbance of the sample was measured at 500 nm. The results were expressed in mequiv/kg oil.

TBARS (2-thiobarbituric acid-reactive substance) was determined as described by Abuzaytoun and Shahidi (2006). 0.05–0.20 g oil sample was weighed into 25 mL volumetric flasks, dissolved in a small volume of 1-butanol and made up to the mark with the same solvent. A 5.0- mL portion of this mixture was transferred into a dry test tube, and then a fresh 2-TBA reagent (5 mL of a solution of 200 mg 2- TBA in 100 mL 1-butanol) was added to it. The contents were mixed and heated in a water bath at 95°C for 2 h. The absorbance of the resultant colored complex was measured at 532 nm. We used as standard 1,1,3,3-tetramethoxypropane because it is a precursor of malonaldehyde (MA). The results were expressed in mmol MA equiv/g oil.

Also, the induction periods of the oil samples were determined by the Metrohm Rancimat apparatus model 743 (Metrohm, Switzerland) (Kowalski et al., 2004). 4.0 g of each oil sample was weighed in the reaction vessel glassware. The conductimery cells were filled with deionized water up to 90 mL. Samples were heated at 110°C and air was passed through the heated oil at the rate of 20 L/h. The induction period was determined automatically by the device and expressed in hours.

Statistical Analyses

Experimental data were evaluated by using analysis of variance (ANOVA) and the significant differences amongst the means of the three replicates ($P<0.05$) were determined by Duncan’s multiple range test, using the ‘‘SPSS 9.0 for Windows”.

Results and Discussion

Total Oil Content

The oil contents of these seeds were determined ranged from 30.0 % to 38.1%. (Table 1). There was no significant differences between cherry seed and plum seed oil content. In a previous study, the oil content of cherry seed oil (CSO) and plum seed oil (PSO), were determined were 38.8% and 40.6%, respectively. In the current study, the oil content of CSO and PSO were determined 36.1% and 38.1% , respectively. These results were complied with the literature data (Zlatanov and Janakieva, 1998). It has been reported that apricot seeds contain approximately 40% oil (Turan et al., 2007). The oil content value of the seeds was similar the apricot seed oil content. With the ≥30.0% oil content, these oil may be used in food industry.

Fatty Acid Compositions

Fatty acid composition of the samples was shown in Table 1. Mulberry seed oil (MSO) had the highest linoleic acid content, while it had the lowest oleic acid content (Table 1). PSO had the highest oleic acid content among in all samples. There was no significant differences between CSO and sweet cherry seed (SCSO) oil of oleic acid ontent (45.8 and 42.9% , respectively). Palmitic acid content were determined ranged from 6.1 to 9.5% and stearic acid value were determined ≥4.1% and linolenic acid value was lower than 1 % in all samples. Zlatanov and Janakieva (1998) were determined 9.2% palmitic, 3.9% stearic, 70.5% oleic, 15.7% linoleic acid in PSO. Also, they reported that 9.4% palmitic acid, 2.0% stearic acid, 46.9% oleic acid, 41.7% linoleic acid was found in SCSO. Our results are similar with their results. The oleic acid content of MSO was higher than apricot and pomegranate seed oil and the oleic acid content of PSO was similar with the apricot seed oil (Turan et al., 2007, Schubert et al., 1999).

The current study, total saturated fatty acids (SFA) were found ranged from 9.3 to 13.9%, total monounsaturated fatty acid (MUFA) were found ranged

from 8.1 to 67.3% and total polyunsaturated fatty acid (PUFA) were found ranged from 23.4 to 78.0% (Table 1). Total unsaturated fatty acids value (monounsaturated and polyunsaturated fatty acid) was higher than saturated fatty acids (SFA) value in all the samples. Unsaturated fatty acids, such as oleic and linoleic acid, are very important for human diet. Because, they help to prevent cancer and heart diseases risk (Parker et al., 2003).

Tocopherol Content

Tocopherol isomers are shown in Table 2. α-tocopherol contents of CSO, SCSO, MSO and PSO were determined 74.7, 110.5, 33.2 and 92.1 mg/kg oil, respectively. β-tocopherol wasn't determined in CSO, whereas SCSO, MSO, PSO contained 16.7, 47.9, and 7.1, mg/kg oil, respectively ($P<0.05$). However, γ-tocopherol value of CSO, SCSO and PSO was higher than the other tocopherol isomers and its value ranged from 465.3 to 614.5 mg/kg oil, but δ-tocopherol of MSO was greater than (1324.3 mg/kg oil) the other isomers. α- and γ-tocopherol value of the all samples was higher apricot seed oil (Durmaz et.al., 2009) Tocopherols are important antioxidant compounds for oils. The oxidative stability of the oils is mostly based on these compounds (Yu et al., 2002a,). Antioxidants are used as a lipid stabilizator in the food industry (Schmidt and Pokorny, 2005). Therefore, the by-products may be added in food products to preventing oxidation and improving quality.

Antioxidant Activity and Total Phenolic Content

The DPPH and ABTS radical scavenging capacities and total phenolic content are shown in Table 3. DPPH and ABTS radical scavenging test are commonly used to determined antioxidant capacity (Lu et al., 2002a). DPPH-scavenging capacities of CSO, SCSO, MSO and PSO were found 57.4, 60.5, 63.3 and 63.3 mg trolox/100 g oil, respectively (Table 3). ABTS scavenging capasities of CSO, SCSO, MSO and PSO were found 38.7, 40.9, 44.4 and 43.7 mg trolox/100 g oil, respectively. There was no statiscally differences among DPPH- and ABTS-scavenging capacity of MSO, SCSO and PSO.

It is known that phenolic compounds are contribute to over all antioxidant capacity of oil (Abuzaytoun and Shahidi, 2006). Also, these compounds have a positive effect on preventing cancer (Lu et al., 2002a). Total phenolic content of CSO, SCSO, MSO and PSO were 18.5, 19.9, 19.8 and 29.7 μg gallic acid equiv/g oil. PSO had the higher TPC value than the others and there was no significant differences among the TPC of CSO, SCSO and MSO. Because of higher total phenolic and tocopherol content, these oils could be used in human diet.

Table 1 Fatty acid composition of the seed oils

Fatty acid (%)	Oil type			
	CSO	SCSO	PSO	MSO
Palmitic (C16:0)	6.9± 0.03^{b}	9.7 ±0.03^{b}	6.1± 0.1^{c}	9.48 ± 0.1^{b}
Stearic (C18:0)	2.6±0.03^{c}	3.5±0.07^{c}	2.1 ±0.2^{d}	4.1 ±0.4^{c}
Oleic (C18:1)	45.8±0.08^{a}	42.9±0.10^{a}	67.3 ±0.7^{a}	8.6 ±0.7^{b}
Linoleic(C18:2)	41.8±0.1^{a}	40.7±0.41^{a}	23.4 ± 0.5^{b}	77.6 ± 0.2^{a}
ΣSFA	11.4 ± 0.1	15.3±0.01^{a}	9.3 ± 0.5	13.9 ± 0.1
ΣMUFA	49.1±0.1^{b}	43.4±0.02^{c}	67.3 ±0.7	8.1 ±0.7
ΣPUFA	42.4 ± 0.1^{a}	41.3±0.01^{a}	23.4 ± 0.5	78.0 ± 0.5
Total oil Content (%)	36.1± 0.1	32.1± 0.36^{c}	38.0 ±0.4	30.0±0.4

Each value is the mean ± SD of triplicate determinations. Means with different letters in the cloumn are significantly different ($P<0.05$).

Table 2 Tocopherol content and isomers of the oils

Tocopherol isomers (mg/kg oil)	Oil type			
	CSO	SCSO	MSO	PSO
α-tocopherol	74.7±0.5^{b}	110.5 ±1.01^{b}	33.2±1.55^{d}	92.1±2.0^{b}
β-tocopherol	nd	16.7±0.35^{d}	47.9±0.23^{c}	7.1±0.7^{d}
γ-tocopherol	579.9±5.3^{a}	465.3±2.00^{a}	169.8±0.17^{b}	614.5±13.6^{a}
δ-tocopherol	8.7±0.8^{c}	36.5±0.17^{c}	1354.25±17.9^{a}	24.4±0.1^{c}
Total tocopherol	663.4±2.17	629.0±0.88	1604.9±0.9	738.1±3.9

Each value is the mean ± SD of triplicate determinations. Means with different letters in the cloumn are significantly different ($P<0.05$). nd: not detected.

Table 3 Oxidative stabilitiy values, antioxidant activity and total phenolic content of the oils

Parametres	Oil type			
	CSO	SCSO	MSO	PSO
DPPH	57.4±0.2^{b}	60.5±0.14^{a}	63.3±0.2^{a}	63.3±0.40^{a}
ABTS	38.7±0.1^{b}	40.9±0.88^{a}	44.4±0.3^{a}	43.6±0.03^{a}
TPC	18.5±1.0^{b}	19.9±1.08^{b}	19.9±1.08^{b}	29.7±1.75^{a}

Each value is the mean ± SD of triplicate determinations. Means with different letters in the rows are significantly different ($P<0.05$). DPPH and ABTS values are expressed as mg trolox/100g oil. TPC value is expressed as μg gallic acid equiv/g oil.

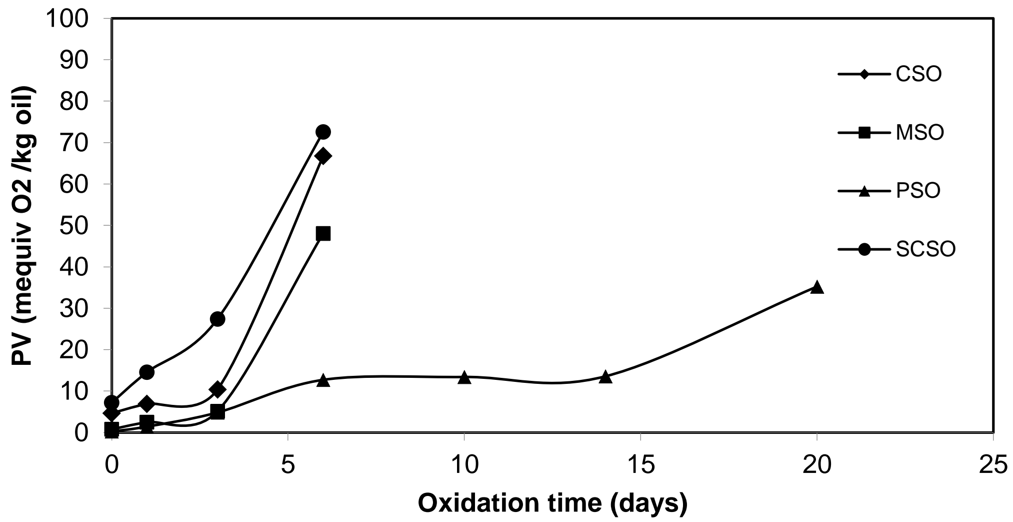

Figure 1 Lipid hydroperoxides formation of the oils at 60°C

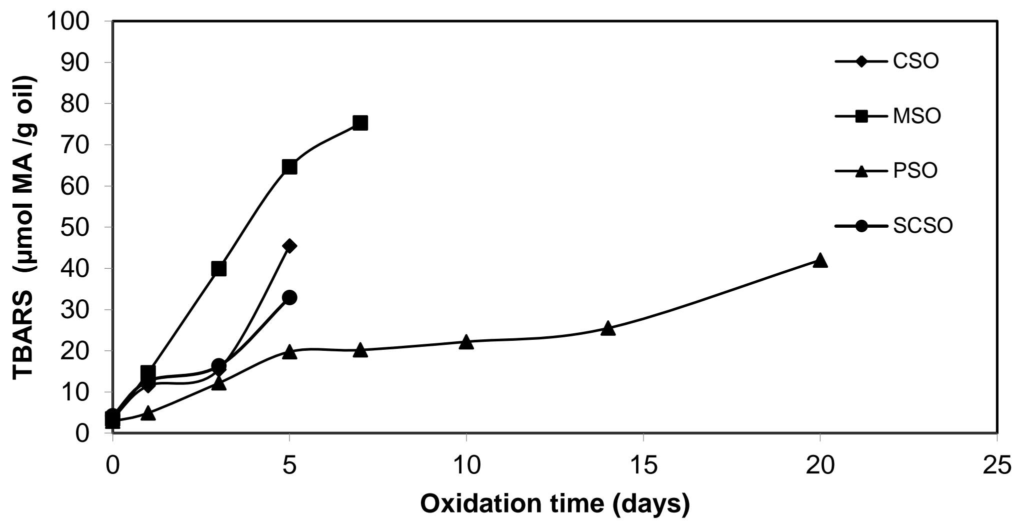

Figure 2 TBARS formation of the oils at 60°C

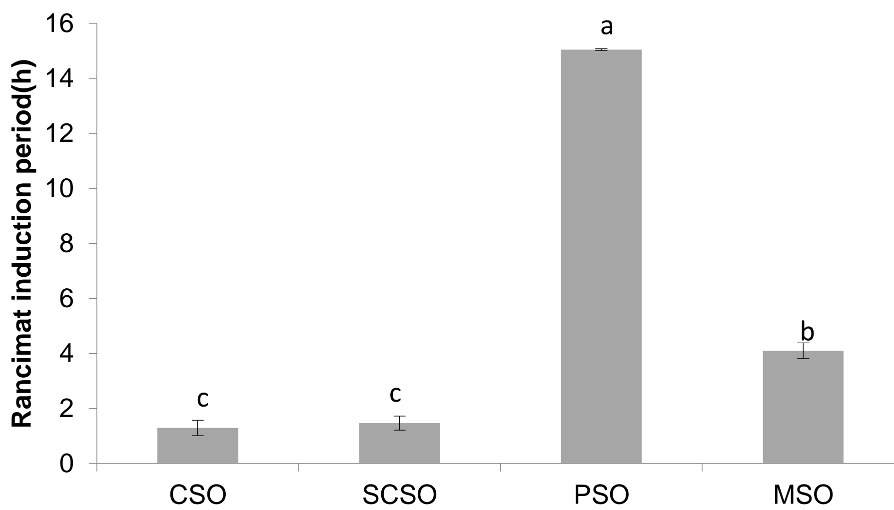

Figure 3 Rancimat induction period of the oils

Oxidative Stability Test

The lipid hydroperoxide value (PV) and TBARS (2-thiobarbituric acid-reactive substance) are commonly used to estimate the level of oxidative stability of the oils (Anjum et al., 2006; Abuzaytoun and Shahidi, 2006). The PV of non-oxidized oils ranged from 0.22 to 4.67 meq O_2/kg oil (data not shown). These values were below the acceptable level according the Codex Alimentarius Commission (El-Adawy and Taha, 2001). The oil samples were incubated at 60°C and extent of lipid hyroperoxide and TBARS (2-thiobarbituric acid-reactive substance) was monitored (Figure1 and 2). The PV of CSO, SCSO and MSO continued increasing for 5 day storage. The PV formation of PSO was relatively stable until 14th day and then it dramaticalty increased after 14 day storage.

TBARS test is based on measurements color intensity of the reaction between TBA (2-thiobarbituric acid) and secondary oxidation products of polyunsaturated fatty acids (Abuzaytoun and Shahidi, 2006). For, TBARS values of SCO, SCSO and MSO continued increasing during 5 day storage. TBARS value of PSO increased during the storage time and there was large increasing after day 14 storage.

The Rancimat method is a powerful and fast technique for estimating the oxidative stability of oils (Farhoosh and Moosavi, 2007). This technique also was used to determined oxidative stability of the samples. The Rancimat induction periods of oils are shown in Figure 3. According to the results, PSO had the highest induction period (15.1 h), followed by, MSO (4.1), SCSO (1.5 h), CSO (1.3 h)*(*$P<0.05$*)*. There was no statiscally difference in Rancimat induction period of SCO and SCSO.

The results of three oxidation tests showed that PSO was oxidatively more stable than the other oil samples. It could be higher TPC and lower PUFA composition. Because, it was reported that phenolic and unsaturated degree of oils have important impact on its oxidative stability (Bozan and Temelli, 2008).

Conclusion

Cherry, sweet cherry, mulberry and plum seed are by-products and the waste oilseed contain important amount oleic and linoleic fatty acid and have high antioxidant capacity and tocopherol content. So, the oils may be used as natural antioxidative additive to improve the quality, stability of food products. Further research is needed to investigate the other chemical compounds in the oils and try to use in food products.

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