EXTENSION OF INDUCTION PERIOD OF SOME VEGETABLE OILS BY USING NATURAL PLANT EXTRACT (NPE) AND REFINED RICE BRAN OIL

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Abstract

To avoid use of the synthetic additives, the natural plant extract (NPE) of rosemary and sage leaves and also refined rice bran oil (as a source of natural antioxidant and high stability oil) were added to sunflower, soybean and blended oils to extend the induction period (oxidative stability) and cause improvement of the quality of these oils.

- The results indicated that, the phenolic compounds in natural plant extract (methanolic extract) of rosemary and sage leaves fractionated and determinated by HPLC, that about 12 phenols were detected, vanillic, coffein, syringic, coumarin, protocatachoic, chrisin, cinnamic, ferrulic, norengenin, caffeic, Galic and chlorogonic.
- Refined rice bran oil (RRBO) was found to be more stable towards oxidation (25.0 hr.) and it contains high amount of total tocopherols (454.22 ppm).
- The addition of NPE of rosemary and sage leaves and RRBO to the investigated oils caused remarkably the induction period (stability) and total tocopherols and reduced the peroxid value, acidity, triene at 232nm and triene at 268nm. of these oils.
- Rosemary extract was superior to that of sage extract increasing oils stability and quality.

Keywords: Rice brane oil, rosemary extract, sage extract, stability, phenolic compounds and total tocopherols.

INTRODUCTION

Lipids in seed oils are important components of foods and have a significant effect on their quality even though they constitute a minor component. They not only contribute to flavor, odor, color and texture, but also confer a feeling of satiety and palatability to foods. However, the major problem in these oils lies in lipid oxidation during storage or food processing, which lead to the rancidity and defective nutrition due to degradation products such as reactive oxygen species, resulting in harmful effects on human health (Jang- Hunk *et al.,* 2008).

Oxidation of lipids not only produces rancid odours , unpleasant flavours and discoloration, but can also decrease the nutritional quality and safety. Use of antioxidant is one of the methods that retard or prevent lipid oxidation, preserve the quality and extend the shelf -life of food products (Helena and Veronika 2006).

Recently, many attempts have been made to prevent the oxidative deterioration of lipids by using natural antioxidants (Chaudiere, 1994).

Rosemary (*Rosmarinus officinals* L.) is a very important medicinal and aromatic plant, which belongs to the lamiaeae family and has been cultivated for along time. Anthropologists and archaeologists have found evidence that rosemary herbs were used as medicinal, culinary and cosmetic virtues in ancient Egypt, Mesopotamia, China and India. It is well known that the activity of rosemary extracts in food industry and medicine due to the presence of some important antioxidant oil and phenolic components, to prevent oxidative degradation of oil and lipid containing foods. Its antioxidant properties not only exploited by the food industry but by the plant protection techniques and therapy (Eva stefanovits-Banyais *et al.,* 2003).

Some components in natural products such as carotenoids, flavonoides, anthocyanins and phenolic compounds are known to function as scavengers in both primary and secondary oxidation process. In particular, it has been reported that potential antioxidants exists in a number of natural plant extracts including grapes, green teas, berries, tomatoes and rosemary (Jang- Hunk Ahn, *et al.,* 2008).

Among plant reported to have antioxidative activity, rosemary (*Rosmorinus officinalis*) in its ground form or as an extract is widely used in many food applications. A number of phenolic compounds that vary in structure, polarity and mutual interactions have been identified to be responsible for the antioxidative properties of rosemary extracts, the main antioxidant effect of rosemary extracts to phenolic diterpenes such as carnosol, carnosoic acid and methyl carnosate, and phenolic acids such as rosmarinic and coffeic acids (Frankel *et al.,* 1996 and Petra Terpin *et al.,* 2009).

Many investigators have studied the free radical scavenging activity to better understand the antioxidant properties of rosemary extracts. Recently the capability of rosemary extracts to scavenge free radicals was investigated by (Nogala- Kalucka *et al.,* 2005)

A power function was found to best represent the time dependence of the content of free radicals scavenged, as well as the content of B- carotene bleached in the presence of rosemary extracts. (Petra Terpinc *et al.,* 2009).

Rosmary and sage are two plant sources of AO (antioxidants) that have been studied intensively and proven effective for stabilizing frying oils (Irwandi *et al.,* 2000).

Rice bran oil contains the antioxidant, tocotrienols, tocopherols. Savenosterol and also γ- oryzanol (Berger, 2005).

The purpose of this work was to study the effect of addition of natural plant extract (methanolic extract) of rosemary and sage leaves and also addition of refined

bran oil on the oxidative stability, total tocopherol and physical and chemical characteristics of sunflower, soybean and it blended oils.

MATERIALS AND METHODS

Materials

1. Sunflower and soybean oils: Refined Sunflower and soybean oils: Without antioxidant, were obtained from El-Ekhowa Co. for processing oils. El-Sadat City.
2. blended oil: Refined sunflower and soybean oil were mixed with 1: 1 ratio.
3. Refined rice bran oil (RRBO): were obtained from CME group, ACME/ Chicago Board of Trade, NYMEX company.
4. Plant leaves:

The rosemary and sage leaves were collected from rosemary and sage plants of faculty and Agricultural farm (Cairo University).

Methods

1- Preparation of natural plant extract (NPE) of rosemary and sage leaves (Methanolic extract)

The fresh rosemary and sage leaves were washed by tap water to remove any earth, particles then air dried at 40°C over night, and ground to obtained a fine powder. The dried ground leaves were extracted at room, temperature with methanol: water (80: 20%) for 24 hours. Then the extracts were filtered through Whatman paper No.4. The filterate of extracts were concentrated using rotary evaporator below 40°C under vacum and air dried to remove any solvent and ground to obtain a fine powder from NPE of rosemary and sage leaves.

2- Separation and quantification of phenolic compounds

Five grams from dried leaves powder of rosemary and sage plants, were extracted with methanol 80% for 24 hours. Then extracts were filtered through Whatman filter paper No.4. The filterate were placed in the 100 ml measuring flask and methanol 80% was added to the mark and shaked then the separation and determination of phenolic compounds in methanolic extracts according to the method described by (Goupy *et al.,* 1999)

3- Addition of NPF of rosemary and sage leaves and refined rice bran oil (RRBO) to oils (oils investigated)

- Sunflower oil without any addition (control)
- Sunflower oil + 3% RRBO.
- Sunflower oil + 200ppm form NPF of rosemary leaves.
- Sunflower oil + 200ppm form NPF of sage leaves.
- Soybean oils without any addition (control)

- Soybean oil + 3% RRBO
- Soybean oil + 200 ppm from NPE of rosemary leaves.
- Soybean oil + 200 ppm from NPE of sage leaves.
- Blended oil (sun + soy 1: 1) without any addition (control)
- Blended oil + 3% RRBO
- Blended oil + 200 ppm from NPE of rosemary leaves.
- Blended oil + 200 ppm from NPE of sage leaves.

4- Physical and chemical properties of oils

- Refractive index : of the oils was determined at 25°C according to A.O.A.C (2000) by using refractometer (NYRL-3 Poland).
- Acid and peroxid values: were determined according to the methods of the A.O.A.C (1995).
- Absorbency in ultraviolet at 232 and 268 nm: Ultraviolet and visible spectra were conducted using a pye unicum double beam recording spectrophotometer model Sp 1600, as described by Kates (1972). The samples were dissolved in freshly distilled cyclohexan and the absorption were taken at 232 and 268nm.

5- Fatty acid composition

The fatty acids methyl esters were prepared using trans- esterification with cold methanolic solution of potassium hydroxide. The fatty acid methyl esters were identified by GC- capillary column according to the methods of IOOC (2001)

6- Determination of total tocopherols

The total tocopherols content in samples were determined according to the method of Wong *et al.* (1988).

7- The stability of oils

The oxidative stabilities of oils were estimated by using a 679 Rancimat (Metrohn Herisou Co., Switzerland) at 100°C with an air flow rate of 20 lit/hr according to the method described by Mendez *et al.,* (1997).

RESULTS AND DISCUSSION

Phenolic compounds of methanolic extracts of rosemary and sage leaves.

HPLC technique had been utilized for the identification and quantification of the phenolic compounds in methanolic extracts of rosemary and sage leaves. The data are shown in Table (1) Regarding phenolic components it could be noticed that about tweleve phenolic compounds were detected in both rosemary and sage extract. From the results in this table it emerges that the rosemary extract which recorded higher values for vanillic, protocatchoic, ferrulic, caffeic and chlorogenic components and have the lowest values for Gallic and chrisin components. At the same time Sage

extract have the highest values for caumarin, protocatchoic and ferrulic components and have the lowest values for syringic chrisin and cinnamic component.

Physical and chemical characteristic of refined rice bran oil (RRBO)

The physical and chemical properties of refined rice bran oil were determined. The obtained results are shown in Table (2).

Regarding these data, it could be observed that the RI, FFA, PV, diene at 232nm. triene at 268nm., stability at 100°C and total tocopherols were 1.4723, 0.6%, 0.15 mequ/ kg, 2.736, 1.736 nm., 25.0 chr. and 454.22ppm respectively. The low peroxide value indicated that RRBO contained a considerable amount of natural antioxidant (total tocopherol) which results in self protection during oxidation stage. Therefore, the stability of this oil recorded a higher value 25.0 hr. Also from the obtained results in the same table, it could be noticed that the predominant unsaturated fatty acid of refined rice bran oil was oleic acid 43.3% followed by linoleic acid 30.82% but it contained the lower amount of lenolenic acid 0.83%. On the other hand, the major saturated fatty acids of this oil was palmitic acid 20.3%. These results mentioned that the refined rice bran oil contained a higher amount of monounsaturated and essential fatty acids ($C_{18:1}$ and $C_{18:2}$) which increased the nutritional properties of investigated oil.

Effect of addition of NPE and RRBO on the physical and chemical properties of oils

Data in Tables (3, 4 and 5) show the change in RI, FFA, PV, K232nm. and K268 nm. of sunflower, soybean and its blended oils in absence and presence the NPF of rosemary and sage leaves and refined rice bran oil. Refractive index (RI), the addition of NPE rosemary and sage leaves to previous samples caused decrease in RI values in all samples compared with RI values of control. This decrease in RI values related to the phenolic compound of NPE exhibited the highest decrease in the oils refractive index phenomenon. Also, the addition of refined rice bran oil caused decrease in RI values. Acidity (FFA), the addition of NPE of rosemary and sage leaves to the studied oils caused a depression in acidity of these oils compared with FFA of the control samples. These results indicated that the phenolic compounds in NPE decreased the oils hydrolytic rancidity but no clear change in these values as a result of presence the refined rice bran oil.

Peroxide value: Data in these tables illustrated that the presence of NPE of rosemary and sage leaves in the investigated oils which reduce the peroxid values compared to the control oil. These results indicated that the capability of NPE of rosemary and sage leaves to scavenging free radical in this respect, Nogala- Kalucka *et al.,* (2005) and Helena and Veronika (2006). Also the addition of RRBO caused

decrease in the peroxide values of oils under study. This decrease may be due to the rice bran oils which contain the antioxidants (tocotrienols, tocophorol and γ-oryzanal) Berger, (2005).

- Absorbency in ultraviolet at 232 and 268nm. Regarding to results in same tables, it could be observed that the K232nm. and K268nm. of the investigated oils were parallel with the results of peroxide values.

Oxidative stability (induction period) of oils

The Rancimat method is a commonly used procedure in food industry to examine the oxidative stability of edible oils and to predict their shelf life. Results in Table (6) shows the changes occurring in oxidative stability of sunflower, soybean and its blended oils as a result of addition of natural plant extract (NPE) of rosemary and sage leaves and refined rice bran oil. From these results, the presence of NPE of rosemary leaves extended the induction period of the previous oils to 14.0, 16.1 and 15.6 hr. respectively. Followed by refined rice bran oil to 13.9, 15.5 and 14.2 hr. respectively and then NPE of sage leaves to 13.5, 15.4 and 12.9 hr respectively compared with control samples 10.9, 13.5 and 11.9 hr respectively. This may be due to the presence of some important antioxidant and phenolic components in methanolic extract of rosemary and sage leaves which it prevents the oxidative degradation of oil (under investigation), and also due to the presence the phenolic diterpenes such as carnosoic acid and methyl canosate, and phenolic acids such as reosmarinic and caffeic acids, Franket *et al.*,(1996) and Petra Terpin *et al.* (2009). Also the rice bran contains a higher amount of antioxidant, tocotrienols, tocopherols, δ-aveno sterols and γ-oryzonal, Berger (2005).

These results agree well with that, reported by Helene and Veronika (2006) who mentioned that the induction period of camelina oil treated with rosemary extract was extended by 60% as compared to untreated oil.

Total tocopherol of oils

Total tocopherols are considered as antioxidant which can quench free radicals. Quenching of O_2 by tocopherol homologues decreases in the order α >β- > δ-> γ-> tocopherol, Goupy et al. (1999). Data in table (7) illustrated that the addition of NPE of rosemary and sage leaves and refined rice bran oil to sunflower oil, soy bean oil and its blended oils caused increased in total tocopherols from 95.5, 205.05 and 151.15ppm respectively in control oils to 135.58, 258.0 and 183.23ppm in presence the NPE of rosemary leaves to 133.85, 230.31 and 179.97 ppm in presence the NEP of sage leaves and to 120.62, 248.11 and 168.30ppm in presence of the refined rice bran oils. This may be related to the refined rice bran oil which contains a

higher amount of total tocopherols (454.22 ppm) under study, also rosemary and sage are two plant sources of antioxidant as mentioned by Irwandi *et al.* (2000).

Table 1. Phenolic compounds of methanolic extract of rosemary and sage leaves

Phenolic compounds(ppm.)	Methanolic extract	
	Rosemary leaves	Sage leaves
Vanillic	5500	420
Caffien	180	100
Syringic	190	60
Caumarin	600	2500
Protocatachoic	6120	3900
Chrisin	17	20
Cinnamic	110	20
Ferrulic	3900	2700
Narengenin	260	90
Caffeic	1660	440
Gallic	70	110
Chlorogenic	4720	260

Table 2. Physical and chemical characteristics of refined rice bran oil (RRBO).

Physical and chemical characteristics	(RRBO)
Refractive index at 25°C	1.4723
Free fatty acid (FFA%)	06
Peroxide value (mequ/Kg oil)	0.15
Diene at 232 nm.	2.763
Triene at 268nm.	1.736
Stability at 100°C(hr).	25.0
Total tocopherels (ppm.)	454.22
Fatty acids composition (%): -	
$C_{14:0}$	0.39
$C_{16:0}$	20.30
$C_{18:0}$	1.96
$C_{18:1}$	43.31
$C_{18:2}$	30.82
$C_{18:3}$	0.83
$C_{20:0}$	1.22
$C_{20:1}$	1.17

Table 3. Effect of addition of the NPE and RRBO on the physical and chemical properties of sunflower oil.

Physical and chemical properties	Control (without)	Addition of NPE (200ppm)		Addition of RRBO (3%)
		Rosemary leaves	Sage leaves	
Refractive index at 25°C	1.4739	1.4732	1.4732	1.4737
Free fatty acid (FFA %)	0.5	0.34	0.46	0.56
Peroxide value (mequ/kg oil)	0.24	0.13	0.19	0.14
Diene at 232nm.	2.627	2.617	2.610	2.606
Triene at 268nm.	1.593	1.581	1.580	1.583

Table 4. Effect of addition of the NPE and RRBO on the physical and chemical properties of soybean oil.

Physical and chemical properties	Control (without)	Addition of NPE (200ppm)		Addition of RRBO (3%)
		Rosemary leaves	Sage leaves	
Refractive index at 25°C	1.4736	1.4729	1.4730	1.4732
Free fatty acid (FFA %)	0.62	0.33	0.36	0.65
Peroxide value (mequ/kg oil)	0.63	0.16	0.39	0.40
Diene at 232nm.	2.614	2.549	2.548	2.586
Triene at 268nm.	1.432	0.931	1.140	1.409

Table 5. Effect of addition of the NPE and RRBO on the physical and chemical properties of blended oil

Physical and chemical properties	Control (without)	Addition of NPE (200ppm)		Addition of RRBO (3%)
		Rosemary leaves	Sage leaves	
Refractive index at 25°C	1.4737	1.4731	1.4731	1.4735
Free fatty acid (FFA %)	0.58	0.30	0.32	0.60
Peroxide value (mequ/kg oil)	0.52	0.38	0.45	0.29
Diene at 232nm.	2.640	2.599	2.606	2.607
Triene at 268nm.	1.444	1.230	1.345	1.384

Table 6. Effect of addition of the NPE and RRBO on the stability of oils (hrs.)

Oils	Control (without)	Addition of NPE (200ppm)		Addition of RRBO (3%)
		Rosemary leaves	Sage leaves	
Sunflower oil	10.9	14.0	13.5	13.9
Soybean oil	13.5	16.1	15.4	15.5
Blended oil	11.9	15.6	12.9	14.2

Table 7. Effect of addition of the NPE and RRBO on the total tocopherols of oils (ppm.)

Oils	Control (without)	Addition of NPE (200ppm)		Addition of RRBO (3%)
		Rosemary leaves	Sage leaves	
Sunflower oil	95.5	135.58	133.85	120.62
Soybean oil	205.05	258.00	230.31	248.11
Blended oil	151.15	183.23	179.97	168.31

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إطالة فترة ثبات بعض الزيوت النباتية باستخدام مستخلص النبات الطبيعى وزيت رجيع الكون المكرر

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لتجنب استخدام الإضافات الصناعية فقد تم إضافة مستخلص النبات الطبيعى لأوراق حصى البان والمرمرية وزيت رجيع الكون المكرر (كمصدر غنى بمضادات الأكسدة الطبيعية وكزيت عالى الثبات) لزيوت عباد الشمس والصويا ومخلوطهم لإطالة فترة الثبات وتحسين خواص الجودة لهذه الزيوت وقد أوضحت النتائج الآتى:

- تفريد المركبات الفنيولية بواسطة جهاز الـ HPLC لمستخلص النبات الطبيعى (المستخلص الميثانولى) لأوراق حصى البان والمرمرية كانت حوالى ١٢ مركب هم Caffein، Vanillic، Syringic، Caumarin، Protocatachoic، Chrisin، Cinnamic، Ferrulic، Narengenin، caffeic، Gallic، Chlorogenic.
- زيت رجيع الكون المكرر أكثر ثبات تجاه الأكسدة (٢٥ ساعة على ١٠٠°م) وأيضاً احتواء الزيت على نسبة عالية من التوكوفيرولات الكلية (٤٥٤,٢٢ جزء فى المليون)
- أدى إضافة مستخلص النبات الطبيعى لأوراق حصى البان والمرمرية وأيضاً زيت رجيع الكون المكرر للزيوت موضع الدراسة إلى زيادة الثبات للزيوت والتوكوفيرولات الكلية بدرجة ملحوظة ونقص رقم البيروكسيد والحموضة والقياس فى المنطقة الفوق بنفسجية على طول موجى ٢٣٢، ٢٦٨ نانوميتر لهذه الزيوت.
- تفوق مستخلص حصى البان على مستخلص المرمرية وذلك من خلال زيادة ثبات الزيوت وخواص الجودة.