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OXIDATIVE STABILITY OF SAMNA AS AFFECTED BY USING NATURAL ANTIOXIDANTS EXTRACTED FROM MINT LEAVES AND RED PEPPER FRUITS

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ABSTRACT

Natural antioxidants were extracted from mint leaves and red pepper fruits using ethanol 70%. Extracts were examined for yield, phenolic compounds and antioxidant activity using DPPH radical scavenging assay. Results showed that mint leaves extract contains the higher amounts of total phenols than red pepper extracts revealing 2.20 and 1.42 g /100g as Gallic Acid Equivalent, respectively. Extracts showed a varying degree of antioxidant activity which was found to be in parallel with their phenolic content. Mint leaves extract showed higher antioxidant activity than red pepper fruits extract. DPPH radical scavenging activity (inhibition) was 78.64 and 70.40% after a reaction time of one hour for mint leaves and red pepper fruits extracts, respectively. Ethanolic extracts of both dry mint leaves and red pepper fruits were added to Samna at levels of 200 and 400 ppm. Butylated Hydroxyl Anisole (BHA) was also added to Samna at a concentration of 200 ppm for comparison. Samples of all treatments were incubated at 63˚C for 21 days for accelerated oxidation. Determination of peroxide value, thiobarbiuturic acid (TBA) and acid value were taken as indices to evaluate the oxidative stability of Samna under accelerated oxidation conditions. Both extracts enhanced the oxidative stability of Samna. Mint leaves extract was more effective in this respect particulary with higher level of addition (400 ppm).

Key words: Samna, mint leaves, red pepper fruit, antioxidant extract.

INTRODUCTION

Antioxidants play a significant role in retarding lipid oxidation reactions in food products. The deterimental effects of excessive lipid oxidation such as formation of off-flavour and undesirable oxidized chemical compounds (aldehydes, ketones and organic acids) are well known (Saad *et al.,* 2007). Synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA), Butylatedhydroxyl Toluene (BHT), Tert-bytyl Hydroquinone (TBHQ) and Propylgallate (PG) are widely used as food additives, but their application has been reassessed because of possible toxic or carcinogenic components formed during their degradation (Jo *et al.,* 2006; Maisuthisakul *et al.,* 2007).

The toxicological effects of synthetic antioxidants together with consumer preference for natural products have resulted in an increased interest in the search and use of alternative natural and safe sources of food antioxidants. A number of natural antioxidants have been added during processing of some fatty products and have elongated the shelf life and oxidative stability of the stored products *e.g.* essential oils of many herbs (El-Laban, 1998; Shehata, 2005), methanol extracts of certain herbs (El-Abbassy, 2001), tocopherols (Ibrahim, 2003), propolis (Abdel Fattah and Abo Dawood, 2004; Isla *et al.,* 2005; Zaghlool *et al.,* 2009) and ghee residue (Ramadan, 2014).

Large scale screening for natural antioxidants present in numerous plant materials such as oil seeds, cereal crops, vegetables, fruits, leaves, roots and barks is recently the subject of extensive research (El-Shourbagy and El-Zahar, 2014; Soliman *et al.,* 2014; Zaki *et al*., 2014; Atwa *et al.,* 2015 ; El-Hadary *et al*., 2015).

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Mints are herbs rich in phenolic compounds that appeared to be capable of preventing many diseases (Fatiha *et al*., 2012). Mints rank among the most important *Lamiaceae* plants. In addition to essential oil, they contain many valuable phenolic compounds, including flavonoids and phenolic acids that participate in mints' pharmacological properties (Fialova *et al.*, 2015). Mint leaves are refreshing, antiseptic, antioxidant asthmatic, stimulative, diaphoretic, stomachic, and antispasmodic. Singh *et al.* (2015) evaluated the antibacterial and antioxidants activities of pepper mint (*Mentha piperta* L.). They showed that oil and extracts exhibited asignificant antioxidant activity and oil showed about half potency when compared to the standard BHT.

Red pepper (*Capsicum annum* L.) is widely used as a spice and exhibits a wide range of physiological and pharmacological (Choi, 2004; Srinivasan, 2005). Red pepper is the mostly used spice for food throughout the world, especially in Central America, Latin America, Africa and Asia (Chukwu, 2006). Red pepper possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing tissue damage (Govindarajan and Sathyanarayana, 1991). Fruits of pepper (*Capsicum annuum* L.), are highly consumed in fresh form, processed or as spice in various dishes around the world. It has also been found that peppers are a good source of polyphenolic compounds with antioxidant properties (Medina-Juarez *et al.,* 2012).

Samna is commonly used for cooking in Egypt and in most countries of the Middle east where it is consider to be one of the most important dairy products. Oxidative rancidity is the major pathway by which Samna undergoes deterioration. This is referred to as autooxidation because the rate of oxidation increase as the reaction proceeds under usual processing and storage conditions. Development of rancidity decreases both the quality and the shelf life of the product.

Therefore, the present investigation was carried out to acsess information on the antioxidant activity of the ethanolic extracts of mint leaves and red pepper fruits. The effect of these extracts on the oxidative stability of Samna during storage under accelerated oxidation conditions was also evaluated.

MATERIALS AND METHODS

Materials

Mint leaves and red pepper fruits

Mint as leaves and red pepper fruits had been chosen as natural antioxidants. About two kilograms of each material has been collected from local market.

Milk

Fresh buffalo's milk was obtained from Dairy Technology Unit, Food Science Department, Faculty of Agriculture, Zagazig University, Egypt.

Butylated Hydroxyl Anisole (BHA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH)

BHA was obtained from BDH Chemical Ltd, Poole, U.K.

DPPH was purchased from Sigma (St. Louis, MO, USA).

Methods

Extraction of antioxidant compounds

Antioxidant compounds were extracted according to the method described by Adegoke and Gopala Krishna (1998) as follows:

Mint leaves and red pepper fruits were washed and air-dried at room temperature for 2 weeks, then ground to uniform powder. The powder of each dried samples was extracted using ethanol (70%) at ratio of 1: 10 (*W/V*), with constant stirring for 24 hours at room temperature (25 ± 2˚C). The extracts were filtered through Whatmann No. 1 filter paper. The filtered material was re-extracted to maximize the effective material extract. The filtrate were evaporated under vacuum in a rotary evaporator at 45˚C and weighed to determine the extracted yield of each plant material.

Determination of total phenolic contents

The concentration of total phenolic compounds of all extracts was measured by the Folin Ciocalteu colormetric method as described by Skerget *et al*. (2005). The method is based on a

colorimetric oxidation reduction reaction. The absorbance was measured at 650nm using UV-VIS spectrophotometer (Jenway UV-VIS Spectrophotometer). Results are expressed as Galic Acid Equivlant (GAE).

Identification of phenolic and flavonoid compounds by HPLC

The phenolic and flavonoid compounds of the samples were extracted according to the method described by Goupy *et al.* (1999) and Mattila *et al.* (2000) by using HPLC instrument (Hewlett Packard) composed of column C_{18} hypersil BDS with particle size 5 mm. The separation was carried out with a methanol and acetonitrile as a mobile phase, with flow rate of 1ml/min. Quantification was carried out for a calibration based on the standards phenolic acid and flavonoids.

Determination of antioxidant activity of extracts

DPPH radical scavenging activity assay

The free radical scavenging activity of the obtained extracts was measured by bleaching of the purple coloured solution of DPPH according to the method of Hanato *et al*. (1988) and modified method by Gulcin *et al.* (2004).

DPPH scavenging activity (inhibition %) $=A_c - A_s / A_c \times 100$ where A_c is the absorbance of control reaction containing all reagents except the tested extract. As is the absorbance in the presence of the tested extract.

Oxidative stability testes

Peroxide value and acid value

Peroxide and acid values of Samna were determined according to AOAC (2007).

Thiobarbiuturic Acid (TBA)

TBA value of Samna was determined according to Keeny (1971).

Statistical Analysis

Statistical analysis for the obtained data was carried out according to the method described by Clarke and Kempson (1997).

Evaluation of oxidative stability of Samna using the extracted natural antioxidants

Samna used in this study, was made from butter which was prepared from pasteurized and unripened buffalo's cream. Churning of the cream to butter was carried out according to Eckles *et al.* (1951). The butter was converted to Samna by boiling off according to the method described by Fahmi (1961). Resultant Samna was divided into 6 equal portions and treated as follows; the first portion was left without additives and served as a control (C) the second portion was treated with BHA as asynthetic antioxidant at a level of 200 ppm (C1). The third and fourth portions were treated with ethanolic extract of mint leaves at concentrations of 200 and 400 ppm, respectively (T1 and T2) while the last two portions were treated with ethanolic extracts of red pepper fruits at levels of 200 and 400 ppm (T3 and T4).

All samples were incubated in an oven at 63 ± 1˚C to accelerate the fat autoxidation. Samples were analyzed every three days until the end of the incubation period (21 days) for peroxide, thiobarbituric acid test (TBA) and acid value. All treatments were triplicated.

RESULTS AND DISCUSSION

Characteristics of Extract

Yield of extracts

The yield of mint leaves and red pepper fruits extracts varied from 9.30-12.42g /100g (Table 1), Ethanolic mint extract had higher yield (12.42g /100g) as compared with the ethanolic extract of red pepper (9.30 g/100 g), The variation in the extraction yields could be attributed to differences in polarity of compounds found in plants such differences have been reported by Prakasha *et al*. (2001) and El-Shourbagy and El-Zahar (2014).

Total phenolic compounds

Ethanolic extracts of mint leaves and red pepper fruits were evaluated for total phenols (Table 1). Data showed that mint ethanolic extract had the highest percentage of total phenols being 2.20g / 100g, while red pepper extract was 1.42g/100g.

Mint and red pepper are good sources of bioactive compounds namely: polyphenols, vitamins (C and A), microelements and dietary fibers. These compounds have a highly antioxidative properties as reported by Abdul Rahim and Ishak (2012) and Curutchet *et al*. (2014). The total phenolic contents could be used as a base for rapid screening for antioxidative activity (Shukla *et al*., 2009).

Table 1. Extracts yield and total phenolic compounds of mint leaves and red pepper fruits (g/100g)

Material	Yield	Total phenolic compounds
Mint	12.42	2.20
Red pepper	9.30	1.42

Identification of phenolic compounds by HPLC

Tables 2 and 3 show the percentage of each phenolic compound in both mint and red pepper extract. There was a great variation among the components identified in the ethanolic extract of each plant material. Phenolic compounds are widely distributed in nature. It is suggested that antioxidant activity is related to their cingulated rings and hydroxyl groups (Mattila *et al*., 2000). Phenolic compounds which were identified in mint extract were Gallic, Quercetin, Caffeine, Catechen, Procyanidin B1, Procyanidin B2, Rutin, Morin, EpiCatechin and Epigallocatechingallate with amounts ranging from 5.2- 414 mg/100g. The obtained results are similar to that reported by Veljkovic *et al.* (2013). Phenolic compounds which were identified in red pepper extract were Quercetin, Capsaicin, Dihydrocapsaicin, Apigenin, Lutoeolin, ferulic acid and sinapic acid with amount ranging from 3.6- 42.8mg/100g. The obtained results are similar to that reported by Malgorzata and Irena (2005).

Ethanolic extract radical scavenging activity (RSA) of both mint and red pepper

The results of ethanolic extract radical scavenging activity (RSA) assays after (zero time, 15, 30 and 60 min) with DPPH and DPPH as a control are shown in Table 4.

The radical scavenging activity of the two extracts showed that mint extract was higher than that of red pepper extract. They were 70.24%, 68.32%, 72.14% and 78.64% for ethanol extract of mint. The corresponding values for the extract of red pepper were 62.44%, 60.80%, 64.36% and 70.40% after 0, 15, 30 and 60 minutes, respectively. Flavonoids and tannins that found in the mint and red pepper are phenolic compounds that act as primary antioxidants or free radical scavengers. The majority of antioxidants function as free radical scavengers are due to their phenolic structure. They act as hydrogen or electron doners. The phenoxy radical formed in the reaction of antioxidant with a fatty acid peroxy radical is stabilized by delocalization of the impaired electrons around the aromatic ring (Sharma, 2002; Ramadan *et al.*, 2003).

The DPPH test provides information on the reactivity of the tested compounds with a stable free radical. DPPH gives a strong absorption band at 515nm in visible region. When the add electron becomes paired off in the presence of a free radical scavenger, the absorption reduced and the DPPH solution is decolourized as the colour changes from deep violet to high yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The obtained results are similar to that reported by Ramadan *et al.* (2003).

Oxidative Stability of Samna

Peroxide value, thiobarbituricacid (TBA) and acid value were taken as indices for the oxidative stability of Samna during accelerated oxidation conditions.

Peroxide value

The changes in peroxide values of Samna with added mint and red pepper extracts are presented in (Table 5). Results showed that peroxide values increased in all treatments during incubation period at 63 ± 1˚C. The highest increases in peroxide values were observed in samples without additives (C). Treating Samna with mint or red pepper extract significantly decreased the rate of peroxide development during incubation. Mint extract was found to be more effective for lowering the peroxide values compared with red pepper extracts. The highest concentration of both extracts being 400 ppm was more effective in this respect. The antioxidant activity of both mint and red pepper extracts were comparative to that of BHA. The obtained results confirm the

Table 2. Identification of phenolic compounds in ethanol extracts of mint leaves as determined by HPLC (mg/100 g)

Item	Mint extract
Gallic	14.0
Quercetin	21.2
Caffien	5.2
Catechin	9.2
Procyanidin B1	32.0
Procyanidin B2	35.0
Rutin	414.0
Morin	12.2
EpiCatechin	42.0
Epigallocatechingallate	64.0

Table 3. Identification of phenolic compounds in ethanolic extract of red pepper fruits as determined by HPLC (mg/100 g)

Item	Red pepper extract
Quercetin	28.6
Capsaicin	42.8
Dihydrocapsaicin	23.0
Apigenin	3.6
Lutoeolin	18.8
Ferulic acid	32.6
Sinapic acid	38.4

Table 4. Scavenging effect at different incubation times of ethanolic extracts of mint leaves and red pepper fruits on DPPH radical scavenging as measured by changes in absorbance at 515 nm

DPPH	Material	
	Mint leaves extract (%)	**Red pepper fruits extract (%)**
Zero time	**70.24**	**62.44**
15 min	**68.32**	**60.80**
30 min	**72.14**	**64.36**
60 min	**78.64**	**70.40**

Table 5. The peroxide value (PV) of Samna containing natural antioxidant during incubation at 63 ± 1˚C for 21 days (meq/Kg)

Sample	Fresh	Accelerated incubation period at 63 ± 1˚C (day)						
	0.0	3	6	9	12	15	18	21
C	0.87^{a}	1.92 ^{a}	2.32^{a}	3.56^{a}	4.64^{a}	5.58^{a}	7.82^{a}	10.76^{a}
C_1	0.84^{a}	0.99^{d}	1.38^{e}	1.44^{e}	2.15^{c}	2.80^{c}	3.64^{e}	5.70^{d}
T_1	0.86^{a}	1.15^{c}	1.60^{c}	1.72^{d}	2.26^{b}	2.98^{c}	3.86^{d}	5.86^{c}
T_2	0.82^{ab}	0.95^{e}	1.24^{f}	1.33^{f}	1.98^{d}	2.76^{d}	3 .50^{f}	5.52^{e}
T_3	0.88^{a}	1.72^{b}	1.77^{b}	2.02^{b}	2.90^{ab}	3.62^{b}	4.56^{b}	6.32^{b}
T_4	0.85^{ab}	1.13^{c}	1.54^{d}	1.88^{c}	2.82^{ab}	3.40^{b}	3.90^{c}	6.12^{bc}

* Means having the same letter (s) within the same column are not significantly different.

C: Control without antioxidants C_1: Samna containing 200 ppm BHA (positive control)

T_1: Samna containing 200 ppm mint leaves extract. T_2: Samna containing 400 ppm mint leaves extract.

T_3: Samna containing 200 ppm red pepper fruits extract. T_4: Samna containing 400 ppm red pepper extract.

previous investigations on the presence of natural antioxidant in mint (Veljkovic *et al.*, 2013), red pepper (Malgorzata and Irena, 2005).The trend of the obtained results is in agreement with El-Abbassy (2001), Pankaj *et al.* (2013), El-Shourbagy and El-Zahar (2014) and Atwa *et al*. (2015).

TBA value

Addition of ethanolic mint and red pepper extracts to Samna retarded the oxidative changes during accelerated storage at 63 ± 1˚C (Table 6). This means that the formation of malonaldehyde, which affect the formation of pink colour intensity from the reaction of TBA material with malonaldehyde took place at a relatively lower rate in treated Samna samples. The ethanolic mint extract treated Samna samples at different concentrations 200 and 400 ppm showed lower TBA values followed by ethanolic red pepper extract at ratio of 200 and 400 ppm. The highest concentration of each extract (400ppm) was more effective in retarding the development of TBA values. The trend of the results agree with El-Abbassy (2001), Pankaj *et al.* (2013), El-Shourbagy and El-Zahar (2014) and Atwa *et al.* (2015).

Acid value

The acid values of Samna of the different treatments are shown in Table 7. From these result it could be noticed that there were gradual increase in the acid value in all treatments during incubation at 63˚C. The highest acid values were observed in control Samna samples without additives (C).

Acid values of Samna treated with ethanolic mint and red pepper extracts were lower than that of control. This observation was more noticeable with the highest concentration of added extracts (400 ppm).

Moreover, mint extract was more effective in this respect. The acid values of Samna treated with natural antioxidants were comparative to that treated with BHA (C1). The general trend of the obtained results is in agreement with Hussein *et al*. (2000) and Pankaj *et al*. (2013).

In conclusion, ethanolic extracts of mint and red pepper showed considerable antioxidant activity. Mint extract was more effective in this respect. Incorporation of Samna with each extract at level of 400 ppm improved its oxidative stability as indicated from the development of peroxide, TBA and acid values during incubation under accelerated oxidation condition.

Table 6. The TBA value of Samna containing antioxidant during incubation at 63 ± 1˚C for 21 days (O.D at 532 nm)

Sample	Fresh	Accelerated incubation period at 63 ± 1˚C (day)						
	0.0	3	6	9	12	15	18	21
C	0.011^{a}	0.018^{a}	0.036^{a}	0.062^{a}	0.068^{ab}	0.073^{a}	0.086^{a}	0.10^{a}
C_1	0.011^{a}	0.018^{a}	0.022^{ab}	0.040^{c}	0.064^{b}	0.066^{b}	0.074^{bc}	0.082^{e}
T_1	0.010^{a}	0.018^{a}	0.026^{a}	0.042^{b}	0.063^{a}	0.066^{b}	0.078^{ab}	0.085^{d}
T_2	0.008^{a}	0.014^{a}	0.022^{ab}	0.040^{bc}	0.058^{ab}	0.064^{c}	0.074^{ab}	0.082^{e}
T_3	0.010^{a}	0.018^{a}	0.028^{a}	0.044^{bc}	0.066^{ab}	0.068^{b}	0.084^{b}	0.088^{b}
T_4	0.010	0.018^{a}	0.024^{ab}	0.042^{c}	0.064^{b}	0.067^{b}	0.082^{bc}	0.086^{c}

* Means having the same letter (s) within the same column are not significantly different.

C: Control without antioxidants C_1: Samna containing 200 ppm BHA (positive control)

T_1: Samna containing 200 ppm mint leaves extract. T_2: Samna containing 400 ppm mint leaves extract.

T_3: Samna containing 200 ppm red pepper fruits extract. T_4: Samna containing 400 ppm red pepper extract.

Table 7. The Acid values of Samna containing antioxidant during incubation at 63 ± 1˚C for 21 days (mg KOH/g oil)

Sample	Fresh	Accelerated incubation period at 63 ± 1˚C (day)						
	0.0	3	6	9	12	15	18	21
C	0.12^{a}	0.17^{a}	0.27^{a}	0.38^{a}	0.46^{a}	0.62^{a}	1.16^{a}	1.30^{a}
C_1	0.10^{a}	0.15^{ab}	0.19^{b}	0.24^{bc}	0.32^{c}	0.39^{c}	0.58^{c}	0.66^{cd}
T_1	0.12^{a}	0.17^{a}	0.22^{ab}	0.29^{b}	0.38^{b}	0.44^{bc}	0.64^{bc}	0.76^{c}
T_2	0.10^{a}	0.15^{ab}	0.20^{b}	0.25^{bc}	0.36^{b}	0.40^{c}	0.60^{c}	0.74^{c}
T_3	0.12^{a}	0.17^{a}	0.23^{ab}	0.34^{ab}	0.42^{ab}	0.52^{b}	0.70^{b}	0.84^{b}
T_4	0.12^{a}	0.16^{ab}	0.21^{ab}	0.30^{ab}	0.40^{ab}	0.48^{bc}	0.68^{b}	0.80^{b}

* Means having the same letter (s) within the same column are not significantly different.

C: Control without antioxidants C_1: Samna containing 200 ppm BHA (positive control)

T_1: Samna containing 200 ppm mint leaves extract. T_2: Samna containing 400 ppm mint leaves extract.

T_3: Samna containing 200 ppm red pepper fruits extract. T_4: Samna containing 400 ppm red pepper extract.

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تأثيـــر اســـتخـدام مضـــــادات الأكســـــــدة الطبيعيـــة علـــى ثبـــات الســـمن ضـد الأكســدة

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أجريت هذه الدراسة لتقدير النشاط المضاد للأكسدة لمستخلص أوراق النعناع وثمار الفلفل الأحمر، وكذلك لتقييم تأثير هذه الإضافات الطبيعية على درجة ثبات السمن ضد الأكسدة، حيث تم استخدام هذه الإضافات فى صناعة السمن بنسب مختلفة (٢٠٠ ، ٤٠٠ جزء فى المليون) لكلاً من مستخلص ورق النعناع ومستخلص ثمار الفلفل الأحمر بالإضافة إلى عينة سمن مضاف إليها ٢٠٠ جزء فى المليون من مركب مضاد أكسدة صناعي BHA وعينة سمن للمقارنة (بدون إضافات)، تم تحضين كل المعاملات فى الحضان على درجة حرارة ٦٣ درجة مئوية لمدة ٢١ يوم، وتم تحليل العينات لكل المعاملات وهى طازجة وبعد ٣، ٦، ٩، ١٢، ١٥، ١٨ و٢١يوم من التحضين من حيث اختبارات الثبات ضد الأكسدة (رقم البيروكسيد، رقم الحموضة ورقم TBA)، وقد أوضحت النتائج احتواء هذه المواد على نسبة عالية من المواد الفينولية وكانت النسبة الأعلى لمستخلص ورق النعناع ثم مستخلص ثمار الفلفل الأحمر، كذلك أعطى مستخلص ورق النعناع أعلى نشاط مضاد للأكسدة تلاه مستخلص ثمرة الفلفل الأحمر، كما أوضحت النتائج أن عينات السمن المحتوية على ٤٠٠ جزء فى المليون من مستخلصى النعناع والفلفل الأحمر كانت الأعلى من حيث الثبات ضد الأكسدة (الأكثر انخفاضا فى أرقام البيروكسيد، الحموضة وTBA) عن باقى المعاملات، وعموما فإن عينات السمن المحتوية على مضادات الأكسدة الطبيعية (مستخلص ورق النعناع وثمار الفلفل الأحمر) كانت أقل المعاملات انخفاضا فى قيم البيروكسيد والحموضة وTBA مقارنة بعينة السمن المحتوية على مركب BHA وعينة سمن المقارنة (بدون إضافات) خلال فترة التحضين، ومن خلال نتائج هذا البحث توصى الدراسة بإضافة المستخلص الإيثانولى لأوراق النعناع بمعدل ٤٠٠ جزء فى المليون سمن كمضادات أكسدة طبيعية كبديلا للمواد الصناعية وذلك لتحسين ثبات السمن ضد الأكسدة خلال التخزين.

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