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INFLUENCE OF SOME NATURAL ANTIOXIDANTS ON SAMNA STABILITY

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ABSTRACT

This study was carried out to determine the antioxidant activity of olive leaves extract (OLE) and mango leaves extract (MLE). Also to evaluate the effect of these natural additives on the oxidative stability of Samna. These additives were used in Samna manufacture at different concentrations (200, 400 and 600 ppm for both OLE and MLE), compared with Samna-fortified with 200 ppm of Butylated Hydroxyl Anisole (BHA) and control Samna (without additives). All treatments were incubated at 63°C for 21 days and analyzed when fresh and after 3, 6, 9, 12, 15, 18 and 21 days of storage for oxidative stability tests (peroxide value, acid value and TBA test). Results showed that OLE and MLE contained high content of phenolic compounds. OLE had the highest content of phenolic compounds OLE gave the highest antioxidant activity, followed by MLE. The Samna containing 400 ppm of OLE or MLE, had the highest oxidative stability (lowest in the peroxide value, acid value or TBA test) than the other treatments. Generally, Samna samples containing natural antioxidants (OLE or MLE) showed lower figures regarding in peroxide value, acid or TBA test compared with Samna containing BHA and control Samna during incubation period. From these results it could be concluded that using OLE at the ratio of 400 ppm in manufacture of Samna as natural antioxidants could be more suitable to improve its oxidative stability during storage.

Key words: Mango leaves, olive leaves, natural antioxidant, Samna.

INTRODUCTION

Oxidative deterioration of milk fat is one of the major factors that limit the storage life of Samna (Mehta, 2006). The onset of rancidity in Samna is mainly due to the oxidation of unsaturated glycerides leading to development of peroxides and/or due to hydrolysis of glycerides resulting in increased levels of free fatty acids (Muir, 1996). Synthetic antioxidants such as butylated hydroxyl anisole (BHA), propyl gallate and tertiary butyl hydroquinone (TBHQ) are often used in Samna to prevent oxidative deterioration (Pawar *et al.*, 2012). However, scientific studies have shown that application of synthetic antioxidants in foods may cause damage to liver and have been responsible for carcinogenesis (Sherwin, 1990). These reasons have directed the attention towards the use of edible plant resources as safer and natural antioxidants; also consumer demand for natural food ingredients has resulted in extensive research on naturally occurring antioxidants. Recently, the use of natural antioxidants in the food industries has increased rapidly (Iqbal *et al.*, 2007).

Olive mill and olive processing residues are attractive sources of natural antioxidants. An important part of these residues is olive tree leaves. Olive leaf extract has been reported to have antioxidant capacity, antimicrobial activity, anti-HIV properties, vasodilator effect, and hypoglycemic effect. (Erbay and Icer, 2010; Lalas *et al.*, 2011; Mujic *et al.*, 2011; Theodora *et al.*, 2013).

Mango (*Mangifera indica* L.) leaves are a rich sources of phenolic compounds with strong antioxidant power, particularly mangiferin, a special xanthone commonly called as "super antioxidant" because of their potent antioxidant capacity, and other phenolic compounds like quercetin, (Masibo and He, 2009). Antioxidant properties of mango leaves extracts can be used

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as natural preservatives in food applications (Morsi *et al.*, 2010).

The present study was planned to measure the antioxidant activity and phenolic compounds of some natural antioxidants (OLE and MLE).Further the addition of these natural antioxidants to Samna was applied to improve its oxidative stability.

MATERIALS AND METHODS

Materials

Plant sources

Different common natural sources namely, mango leaves (Hendy type) and olive leaves (Chemllaly type) had been chosen as natural antioxidants. About four kilograms of each material has been obtained locally.

Milk

Fresh buffalo's milk (6.5%fat) for Samna making was obtained from Dairy Technology Unit, Food Science Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Butylated hydroxyl anisole (BHA)

Butylated hydroxyl anisole (BHA) was obtained from BDH chemical Ltd, Poole, U.K.

Methods

Extraction of antioxidant compounds

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

The leaves of mango or olive were washed with tap water and air-dried at room temperature for 2 weeks then ground to a uniform powder. The powder of each dried samples was extracted using ethanol (70%) at ratio of 1 : 10, with constant stirring for 24 hrs., 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 was evaporated under vacuum in a rotary evaporator at 45 °C and weighed to determine the extracted yield of each plant material.

Manufacture of Samna

The butter, used for preparing Samna in the present study, was made from pasteurized and unripened buffalo's cream. Churning of the cream to butter was carried out according to Parmarpankaj *et al.* (2013). The butter was converted to Samna by boiling off according to the method described by Fahmi (1961). Samna was divided into equal ten portions as follow:

Ethanolic mango and olive leaves extracts were added to Samna at concentrations of 200,400 and 600 ppm (Treatments from T1 to T6). Also, BHA was added at a concentration of 200 ppm(C1).Samna without any addatives was serve as control (C). All samples were incubated at 63 ± 1°C/21 days to accelerate the fat autooxidation according to Rossell (1989). Samples were analyzed when fresh and every three days until the end of the incubation period (21 days) for peroxide and acid values and thiobarbituric acid (TBA) test. All experiments were triplicated.

Determination of total phenolic content

The concentration of total phenols in all extracts was measured by a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer), based on a colorimetric oxidation/ reduction reaction, as described by Škerget *et al.* (2005). The used oxidizing reagent was Folin-Ciocalteu reagent (AOAC, 2007).

Identification of phenolic compounds by HPLC

The phenolic and flavonoid compounds of the samples were identified according to the method described by Goupy *et al.* (1999) and Mattila *et al.* (2000) by using HPLC instrument (Hewlett Packard) composed of column C18 hypersil BDS with particle size 1 mm. The separation was carried out with methanol and acetonitrile as a mobile phase. The rate of flow was 1ml/min. Quantification was carried out for a calibration based on the standards phenolic and flavonoid compounds.

Determination of Antioxidant Activity

Radical scavenging activity (Scavenging DPPH)

The electron donation ability of the obtained extracts was measured by bleaching of the purple coloured solution of the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al.* (1988) and modified method by Gulcin *et al.* (2004).

Oxidative Stability Testes

Peroxide value

Peroxide value of Samna was determined according to AOAC (2007).

Acid value

Acid value of Samna was determined according to AOAC (2007).

Thiobarbiuturic test (TBA)

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

Statistical Analysis

All data were statistically analyzed using the general linear models procedure of the statistical analysis system SAS (1998). Significances of differences were defined at $p < 0.05$.All experiments as well as related analysis results were repeated three times and all obtained data are expressed as an average.

RESULTS AND DISCUSSION

Yield of Leaves Extracts

The yield of OLE and MLE varied from 13.34-16.33 g/100g (Table 1). Ethanolic OLE had higher yield (16.33g/100g) than the ethanolic MLE (13.34g/ 100g).The variation in the extraction yield may be attributed to the content of total phenol compounds and the polarity of compounds in plants. Such differences have been reported by Jaya prakasha *et al.* (2001).

Total Phenolic Compounds

Ethanolic OLE and MLE were determined for total phenols (Table 1). The data showed that ethanolic OLE had the highest percentage of total phenols with 2.628g/ 100g, while MLE was the next with 2.342g/100g.These results agree with that reported by Mohan *et al.* (2013). Therefore, leaves of mango and olive are a good source of bioactive compounds which have high antioxidative properties.

Identification of Phenolic Compounds by HPLC

Table 2 shows the percentages of phenolic compound in OLE and MLE. There were great variations among the components identified in the methanol extract of each plant. Phenolic compounds are widely distributed in nature. It is suggested that thier antioxidant activity is related to their cingulated rings and hydroxyl groups (Mattila *et al.*, 2000). Phenolic compounds identified in OLE ranged from 1.87 to 176.22 mg/100g. The obtained results are similar to those reported by Herrero *et al.* (2011). Phenolic compounds identified in MLE ranged from 0.03 to 2028.80 mg/100g.

Radical Scavenging Activity (RSA) of Plants Ethanolic Extract

The tests expressing antioxidant potency can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions. However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant propertied in a relatively short time, as compared to other methods (Schwarz *et al.*, 2000).The results of ethanolic extract radical scavenging activity (RSA) assays with DPPH and DPPH as a control are shown in Table 3.The radical scavenging activity of the two studied materials showed high values. It was (92.45%) for MLE and (93.62%) for OLE. Flavonoids and tannins that found in the two plants are phenolic compounds that act as primary antioxidants or free radical scavengers. 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 assayed as the colour changes from deep violet to high yellow. The degree of redaction 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

The first step in oxidation is the abstraction of hydrogen atom from a fatty acid and oxygen involvement gives a peroxy radical. Generally, the antioxidants suppress the hydrogen atom abstraction from the fatty acids which leads to the decrease of hydroperoxide formation. It is well known that phenolic compounds act as hydrogen or electron donors to the reaction mixture and therefore, the formation of

Table 1. Total phenolic compounds and yield of mango and olive leaves extract

Plant material	Yield of leaves extract (g/100g)	Total phenolic compounds (g/100g)
Mango leaves extract	13.34	2.342
Olive leaves extract	16.33	2.628

Table 2. Phenolic compounds in methanol extracts of various plant materials as determined by HPLC (mg/100g)

Items	Mango leaves extract	Olive leaves extract
Gallic	ND	1.87
Pyrogallol	2028.80	ND
Protocatechuic	185.58	3.89
Vanillic	76.89	ND
Chlorogenic	480.31	ND
Catechol	102.83	20.73
Caffien	22.31	4.21
Catechin	ND	25.72
Ferulic	ND	24.10
Cinnamic	ND	2.87
Chrysin	0.03	ND
Benzoic	ND	176.22

ND= Not detected

Table 3. DPPH radical scavenging activity of ethanolic extract of olive and mango leaves extract as measured by changes at 515 nm

Test	Samples	
DPPH scavenging activity	**Mango leaves extract (%)**	**Olive leaves extract (%)**
	92.45	93.62

hydroperoxides is decreased. The slow formation of conjugated dines and consequently the secondary products by extracts and their major compounds indicated that these materials may be acted as hydrogen donors to peroxy radicals. Thus, retarding the autooxidation of linoleic acid by chain radical termination (Parmarpankaj *et al.,* 2013; Marwa, 2014).

Peroxide Value (PV)

Data illustrated in Table 4 showed that the peroxide values for all treatments of Samna were significantly ($p<0.05$) increased during incubation period and there were significant differences in peroxide values between all Samna treatments. Control sample showed the highest PV values during the incubation period. All ethanolic extracts (OLE and MLE) show an ability to delay peroxides formation. So it could be noticed that these additives prolonged the induction period to be from 12-15 days for OLE and MLE treated Samna, while control Samna was 6-9 days. The obtained results confirm the previous investigations on the presence of natural antioxidant mango leaves (Irda *et al.,* 2013), olive leaves (Theodora *et al.,* 2013). These results are in agreement with Dpuravankara *et al.* (2000), El-Abbassy (2001), Parmarpankaj *et al.* (2013) and Marwa (2014).

The results obtained reveal that the effect of these additives as natural antioxidants, in retarding of fat-autooxidation in Samna were in the order OLE (400 ppm) and MLE (400 ppm).

Thiobarbituric (TBA) Test

It is well known that TBA values are taken as an index to evaluate the advance of oxidation changes occurred in oils and fats. The addition of OLE and MLE to Samna retarded the oxidative changes during accelerated storage at 63±1°C (Table 5). 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. However, the control Samna samples showed higher TBA values throughout the accelerated incubation period at 63 ± 1°C.

The ethanolic olive leaves extract treated Samna samples at different concentrations showed lower TBA values compared with ethanolic mango leaves extract, throughout the accelerated incubation period at 63 ± 1°C for 21 days. There were no significant differences between treatments up to 3 days of the incubation period. While there were significant differences ($p<0.05$) after 6 days up to the end of incubation period.

It seems that there is a relationship between the antioxidant efficiency and the chemical composition of oils. Ethanolic olive and mango leaves extract contain phenolic compounds which can be used as antioxidants or oxidation inhibitors. These structural requirements were supported by the powerful antioxidants activity of the well-known BHA and BHT (Hussein *et al.*, 2000).

Acid Value

Table 6 shows clearly that there were no significant differences between Samna treatments up to 6 days of the incubation period. While there were significant differences ($p<0.05$) after 9 days up to the end of incubation period.

The acid value remained without noticeable changes within the first 6 days of incubation period at 63 ± 1°C for all Samna treatments. Considerable increases of acid value were observed until the end of incubation period of Samna at 63 ± 1°C, for all samples including the control one.

Olive leaves extracts showed the lower increase in acid value of Samna compared with other natural extracts and synthetic antioxidants' referring to the high effect of olive OLE in delaying Samna hydrolysis. OLE was the best followed by the MLE. Comparing with the control, OLE and MLE were favorable as an antioxidant agent. These results agreed with Hussein *et al.* (2000) and Parmarpankaj *et al.* (2013).

Conclusion

Various extracts of OLE and MLE showed varying degrees of antioxidant activity. It is notable that OLE exhibited strong antioxidant capacity, followed by MLE. Therefore, these extracts could be used as preservative ingredients to improve Samna oxidative stability.

Table 4. Peroxide value (PV) of Samna containing antioxidants during incubation at 63 ± 1°C for 21 days (meq/Kg)

Samples	Storage period (day)							
	Fresh	3	6	9	12	15	18	21
C	0.83 [a]	1.88[a]	2.12 [a]	3.40 [a]	4.22 [a]	5.10 [a]	7.50 [a]	10.12[a]
C1	0.81[b]	0.96[b]	1.18 [b]	1.30 [b]	1.95 [b]	2.60 [b]	3.14 [b]	5.40 [b]
T1	0.80[b]	0.95[b]	1.10 [c]	1.22 [c]	1.86 [c]	2.48 [c]	3.06 [c]	5.36 [c]
T2	0.80[b]	0.93[c]	1.04 [d]	1.13 [e]	1.77 [f]	2.36 [e]	3.00 [d]	5.32 [d]
T3	0.78[cd]	0.89[de]	0.97 [f]	1.02 [f]	1.70 [g]	2.32 [f]	2.96[ef]	5.30 [d]
T4	0.80[b]	0.93[c]	1.04 [d]	1.18 [d]	1.82 [d]	2.44 [d]	2.98 [e]	5.32 [d]
T5	0.76[c]	0.90[d]	1.00 [e]	1.02 [f]	1.80[ef]	2.42[de]	2.93 [f]	5.30 [d]
T6	0.76[c]	0.90[d]	0.98 [f]	1.00 [g]	1.80[ef]	2.40[de]	2.88 [g]	5.12 [e]

Means with the same letter are not significantly different

C: control without antioxidants

C_1: Samna treated with 200ppm BHA (positive control)

T_1: Samna treated with 200ppm mango leaves extract.

T_2: Samna treated with 400pp mango leaves extract.

T_3: Samna treated with 600ppm mango leaves extract.

T_4: Samna treated with 200ppm olive leaves extract.

T_5: Samna treated with 400ppm olive leaves extract.

T_6: Samna treated with 600ppm olive leaves extract.

Table 5. Thiobarbiuturic (TBA) test of Samna containing antioxidants during incubation at 63 ± 1°C for 21 days (O.D 532 nm).

Samples	Storage period (day)							
	Fresh	3	6	9	12	15	18	21
C	0.008[a]	0.008[a]	0.018[a]	0.042[a]	0.048[a]	0.053[a]	0.076[a]	0.090[a]
C1	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.024[b]	0.026[b]	0.034[b]	0.042[b]
T1	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.023[b]	0.026[b]	0.034[b]	0.040[c]
T2	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.023[b]	0.024[c]	0.032[c]	0.040[c]
T3	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.023[b]	0.024[c]	0.032[c]	0.040[c]
T4	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.024[b]	0.026[c]	0.034[b]	0.042[b]
T5	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.022[b]	0.024[c]	0.034[b]	0.040[c]
T6	0.008[a]	0.008[a]	0.008[b]	0.02[b]	0.022[b]	0.024[c]	0.032[c]	0.040[c]

Means with the same letter are not significantly different.

Table 6. Acid value of Samna containing antioxidants during incubation at 63 ± 1°C for 21 days (mg KOH/g oil)

Samples	Storage period (day)							
	Fresh	3	6	9	12	15	18	21
C	0.09[a]	0.12[a]	0.25[a]	0.36[a]	0.44[a]	0.58[a]	0.96[a]	1.00[a]
C1	0.09[a]	0.09[a]	0.09[b]	0.20[b]	0.28[c]	0.33[b]	0.52[b]	0.56[b]
T1	0.09[a]	0.09[a]	0.09[b]	0.20[b]	0.3[b]	0.31[c]	0.50[cd]	0.56[b]
T2	0.09[a]	0.09[a]	0.09[b]	0.18[c]	0.28[cd]	0.33[b]	0.50[cd]	0.54[bc]
T3	0.09[a]	0.09[a]	0.09[b]	0.18[c]	0.28[cd]	0.32[bc]	0.50[cd]	0.54[bc]
T4	0.09[a]	0.09[a]	0.09[b]	0.20[b]	0.28[cd]	0.33[b]	0.48[c]	0.56[b]
T5	0.09[a]	0.09[a]	0.09[b]	0.18[c]	0.26[d]	0.32[bc]	0.48[c]	0.52[c]
T6	0.09[a]	0.09[a]	0.09[b]	0.18[c]	0.26[d]	0.32[bc]	0.48[c]	0.52[c]

Means with the same letter are not significantly different.

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تأثيـــر بعـــض مضـــــادات الأكســـــدة الطبيعيـــة علـــــى ثبـــــات الســـــمن

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أجريت هذه الدراسة لتقدير النشاط المضاد للأكسدة لمستخلصي أوراق المانجو وأوراق الزيتون، وكذلك لتقييم تأثير هذه الإضافات الطبيعية على درجة ثبات السمن ضد الأكسدة، حيث تم استخدام هذه الإضافات فى صناعة السمن بنسب مختلفة كالتالى (٢٠٠، ٤٠٠ و٦٠٠ جزء فى المليون) لكلا من مستخلص ورق الزيتون ومستخلص ورق المانجو، بالإضافة إلى عينة سمن مضاف إليها ٢٠٠ جزء فى المليون من مركب BHA وعينة سمن كنترول (بدون إضافات)، تم وضع كل المعاملات فى الحضان على درجة حرارة ٦٣ درجة مئوية لمدة ٢١ يوم، وتم تحليل المعاملات وهى طازجة وبعد ٣، ٦، ٩، ١٢، ١٥، ١٨ و٢١يوم من التخزين 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 لصالح مستخلص ورق الزيتون ثم مستخلص ورق المانجو، كذلك أعطى مستخلص ورق الزيتون أعلى نشاط مضاد للأكسدة تلاه مستخلص ورق المانجو، كما أوضحت النتائج أن عينات السمن المحتوية على ٤٠٠ جزء فى المليون من مستخلصى الزيتون والمانجو كانت الأعلى من حيث الثبات ضد الأكسدة (الأكثر انخفاضا فى أرقام البيروكسيد، الحموضة وTBA) عن باقى المعاملات، وعموما فان عينات السمن المحتوية على مضادات الأكسدة الطبيعية (مستخلص ورق الزيتون والمانجو) كانت أقل المعاملات انخفاضا فى قيم البيروكسيد والحموضة وTBA مقارنة بعينة السمن المحتوية على مركب BHA وعينة السمن الكنترول خلال فترة التحضين، ومن خلال نتائج هذا البحث نوصى بإضافة المستخلص الكحولى لأوراق الزيتون بمعدل ٤٠٠ جزء فى المليون فى صناعة السمن كمضادات أكسدة طبيعية كبديلا للمواد الصناعية وذلك لتحسين ثبات السمن ضد الأكسدة خلال التخزين.

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