

Application of normal essential oil and loaded-solid lipid nanoparticles as antioxidant

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ABSTRACT

The industry adds antioxidants in the formulation of meat products to prevent or delay meat oxidation reactions. Therefore, this investigation was carried out to study the effect of normal essential oil and loaded-solid lipid nanoparticles as antioxidants and the effect of the abovementioned volatile oils on chemical properties (TBA) and shelf life of frozen beef burger. The antioxidant activity of volatile oils (cinnamon and ginger) normal and nano particle size at different concentrations were assessed. Also, the effect of the above mentioned volatile oils addition with different concentrations on chemical properties and shelf life of frozen beef burger was investigated. Results indicated that, the diphenylpicrylhydrazyl free radical scavenging activity increased by increasing the volatile oil concentrations for all studied treatments. Moreover, the antioxidant activity of volatile oils (cinnamon and ginger) nanoparticles was higher than that of the same concentrations of normal volatile oil. The chemical properties values thiobarbituric acid value (TBA) of beef burger decreased by increasing volatile oils concentrations and essential oils nanoparticles reduced chemical properties (TBA) values more than normal essential oils, beside extending the shelf life of beef burger treatments up to 6 months in comparison with control sample which have a four-month shelf life. In conclusion, the transformation of essential oils into nanoparticle size can affect their antioxidant activity and promote the chemical properties and shelf life of beef burger.

Keywords: Volatile oils; Antioxidant; Cinnamon; Ginger.

INTRODUCTION

Cunha *et al.* (2018) explained that to prevent or delay meat oxidation reactions, the industry adds antioxidants in the formulation of meat products. However, the majority of these ingredients are synthetic mainly butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). Nevertheless, some studies have shown the adverse effects of the synthetic antioxidants on the consumer's health, thereby increasing the demand for natural antioxidants. On the other hand, compounds with antioxidant activity can be naturally found in plants, oils, fruits, nuts, and several studies have shown the efficacy of the substitution of natural antioxidants for the synthetic ones. Nonetheless, the effectiveness of natural antioxidants in meat products depends mainly on the composition of vegetable extracts and their antioxidant activity, application form, food processing, and meat matrix, especially the composition of the lipid and protein fraction.

Moreover, Si *et al.* (2018) reported that lipid oxidation is a major cause of food product deterioration. Consequently, it is a necessary to use food additives to inhibit food oxidation. Ginger extract (GE) possesses antioxidant properties. However, components isolated from ginger have been rarely reported to inhibit

fat oxidation. Herein, antioxidant properties of GE and four pure components derived from it (6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol) were examined and their properties were compared to those of butylated hydroxytoluene. GE and the constituent components exhibited antioxidant properties that might be attributed to their hydroxyl groups and suitable solubilizing side chains.

Ghani *et al.* (2018) reported that the new bioactive film from soluble soybean polysaccharide (SSPS) incorporated with different concentrations of cinnamon essential oil nanoemulsions (CNO) and their functional properties were evaluated. Then CNO-SSPS film was applied to meat refrigerated for 8 days. The use of CNO in film production has reduced thickness, water vapor permeability, water solubility, lightness (L*), redness (a*) and whiteness (WI) and increased antioxidant activity of SSPS-films.

MATERIALS AND METHODS

Meat and fat tissues

Imported frozen beef meat and fat tissues (sheep tail) were purchased from the private sector shop in the local market at Giza, Egypt.

The chosen spices

Cinnamon bark (*Cinnamom cassian*) and Ginger roots (*Zingiber officinale* Roscoe) were obtained from a spices shop at Giza, Egypt. The dried spices were ground into a fine powder and kept frozen at -18°C in polyethylene bags until extraction of volatile oil.

Ingredients used in burger formula

Texturized Soy was obtained from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. It was rehydrated by water (at a ratio of 1:2 w/v) and minced through 3 mm plate twice. Bread crust, egg, salt, fresh onion and spices were purchased from local market at Giza, Egypt. The spices were powdered in a laboratory mill, and then a mixture of the powdered spices was prepared as follows: 8.91% laurel leaf powder; 6.01% cardamom; 6.86% nutmeg; 14.08% cinnamon, 11.22% clove, 9.14% thyme, 29.39% cubeb and 14.39% white pepper.

Extraction of spices volatile oils

Extraction of volatile oil from Cinnamon bark (*cinnamom cassian*) and Ginger roots (*Zingiber officinale* Roscoe) were carried out by Hydro distillation method according to A.O.A.C. (1995).

Cinnamon and ginger essential oil loaded solid lipid nanoparticles (EO-SLNs) preparation

Solid lipid nanoparticles (SLNs) were prepared using ultrasonic solvent emulsification technique according to Asnawi *et al.* (2008).

Determination of essential oil loading efficiency

The encapsulation efficiency (EE) of cinnamon and ginger volatile oils were determined as described by Tiyaboonchai *et al.* (2007) and Nayak *et al.* (2010).

Transmission electron microscopy (TEM)

On the other hand, Wang *et al.* (2009) reported that the yield of cinnamon bark essential oil ranged from 0.65 to 2.50% in freshly ground cinnamon bark.

Structural characterization and the morphology of oil loaded SLNs were observed with JEOL JEM-2100 transmission electron microscopy (TEM).

Preparation of beef burger

Beef burger was prepared as described by Mikkelson (1993).

Analytical Methods

Thiobarbituric acid value (TBA)

The TBA as an indicator of secondary lipid oxidation was determined as the method described by Tarledgis *et al.* (1960).

Statistical analysis

Obtained data were subjected to analysis of variance (ANOVA). Means were compared by using Duncans test at the 5% level of probability as reported by Snedecor and Cochran (1995).

RESULTS AND DISCUSSION

Separation and identification of spice volatile oils

Volatile oil content of investigated spices

Volatile oils were extracted from the two investigated spices by steam distillation and their percentages were determined. From the obtained data in Table (1) it could be observed that, the volatile oils percentages in the investigated spices were 1.30 and 1.20 % for cinnamon bark and ginger rhizome respectively. These results are in agreement with those obtained by (Sharma *et al.*, 2016) who reported that the yield of ginger root essential oil ranged from 1 to 2 % in freshly ground ginger root.

Table 1. Essential oils content in cinnamon bark and ginger rhizome.

Spices	Volatile oil % (v/w)
Cinnamon bark	1.30
Ginger rhizome	1.20

Cinnamon and Ginger essential oils loaded with solid lipid nanoparticles and its characterization

Oil encapsulation efficiency (EE) is a critical factor for nanoparticles. A good nano carrier should have high oil encapsulation efficiency.

The results show the encapsulation efficiency in the stearic acid as coating nanoparticles. It was positively correlated to the amount of Cinnamon and Ginger essential oils, that it is increased with the increasing oil concentration to stearic acid. Data showed that the encapsulation efficiency of Cinnamon and Ginger oils nanoparticles significantly increased at the concentration 25%. The morphology and characterization of Cinnamon and Ginger oils loaded solid lipid nanoparticles at different concentrations to stearic acid as coating material were visualized using transmission electron microscopy (TEM).

Figures (1 and 2) show the particles appearing round, spherical in shape, a good dispersion and narrow size distribution, when Cinnamon and Ginger oils used at 25% conc. the particle size seems to be larger (ranging 50-70 nm) (Figure, 1), These results are in agreement with Yang *et al.*, (2009), they recorded that the oil-loading efficiency could reach 80% at the optimal ratio of garlic essential oil to 10% of polyethylene glycol (PEG) and proportion for other nano systems. Gomah and Nenaah (2014) detected that the sizes polydispersity index (PDI) and loading efficiency for eight

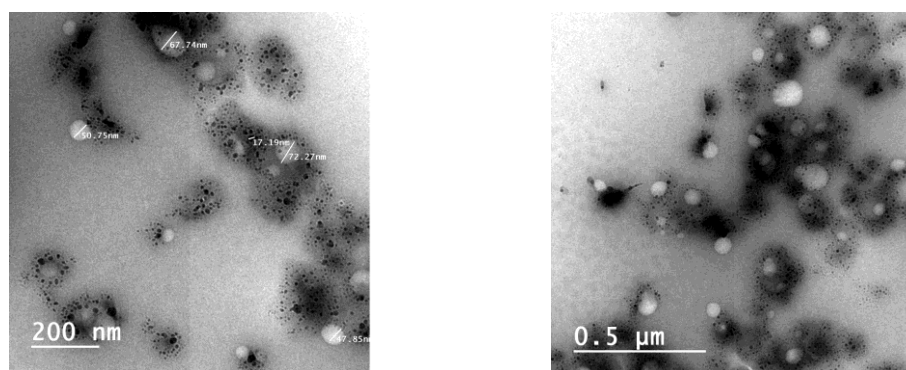


Figure 1 (a and b). TEM micrograph of cinnamon volatile loaded-solid lipid nanoparticles (EO-SLNs) 25% conc.

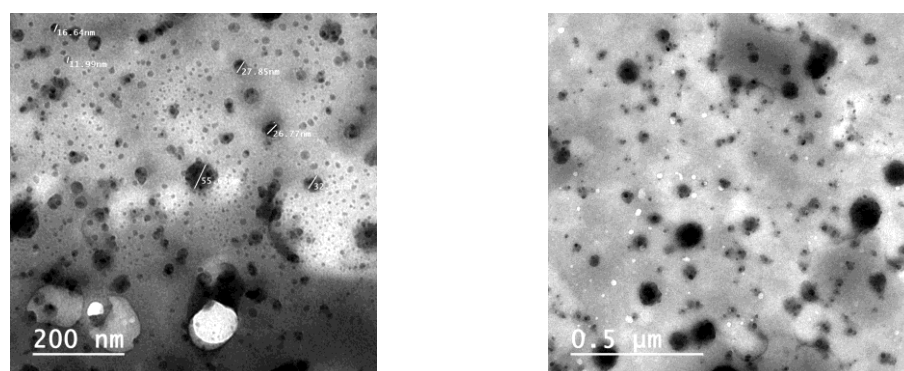


Figure 2 (a and b). TEM micrograph of ginger volatile oil free and loaded-solid lipid nanoparticles (EO-SLNs) 25% conc.

essential oil-nanoparticles, the 10% ratio EO-PEG showed the best relationship between a low PDI (which measure the size of distribution of nanoparticles) and a high loading efficiency.

Antioxidant activity of volatile oils

Diphenylpicrylhydrazyl (DPPH) radical scavenging activity

Data given in Table (2) showed the radical scavenging activity of two different volatile oils (cinnamon and ginger) free and loaded-solid

lipid nanoparticles (EO-SLNs) used in this study compared with BHT as a synthetic antioxidant. From the obtained results, it could be evidenced that, the free radical scavenging activity of all volatile oils is lower than that of BHT. The highest DPPH free radical scavenging activity (% inhibition 95.12 %) was recorded for cinnamon volatile oil nano followed by ginger volatile oil nano (90.25%). On the other hand, the lowest scavenging activity (72.12%) was observed for ginger volatile oil free. These results are in line with those obtained by Wang

et al. (2008), who found that, cinnamon volatile oil had the highest DPPH radical scavenging activity when compared with clove, ginger, cardamom and coriander volatile oils. On the other hand, antioxidants are believed to intercept the free radical chain of oxidations, and to contribute hydrogen from the phenolic hydroxyl groups themselves, thereby forming stable free radicals which do not initiate or propagate further oxidation of lipids (Si *et al.*, 2018).

Table 2. DPPH radical scavenging effect of three different volatile oils and their nano particle compared with BHT.

Volatile oils	DPPH free radical scavenging (%)
Cinnamon	83.25
Ginger	72.10
Cinnamon nanoparticle	95.12
Ginger nanoparticle	90.25
BHT	97.28

These results explain that the volatile oils have effective activity as a hydrogen donor and as a primary antioxidant by reacting with the lipid radical. This may be responsible for the main cause of suppression of autoxidation, in DPPH assay.

Chemical properties of beef burger

Thiobarbituric acid (T.B.A) values

TBA test is a sensitive test for the decomposition products of highly unsaturated fatty acids which do not appear in peroxide value determination (Mohamed, 2005).

Table 3. Thiobarbituric acid (mg malonaldehyde/kg) of different beef burger treatments as affected by cinnamon essential oil (normal and nanoparticle) and their percentages during frozen storage at – 18 °C up to 6 months.

Storage period (months)	Treatments							LSD
	Control	Cinnamon essential oil (%)			Cinnamon nano-particulate (%)			
		0.05	0.1	0.15	0.05	0.1	0.15	
Zero time	0.274 ^{Ad}	0.280 ^{Ad}	0.271 ^{Ad}	0.278 ^{Ad}	0.279 ^{Ad}	0.275 ^{Ae}	0.277 ^{Ad}	0.182 ^{ns}
1	0.479 ^{Ad}	0.381 ^{Ac}	0.377 ^{Ac}	0.365 ^{Ad}	0.367 ^{Ad}	0.335 ^{Ab}	0.314 ^{Ac}	0.171 ^{ns}
2	0.652 ^{Ac}	0.599 ^{ABb}	0.597 ^{ABc}	0.572 ^{Bc}	0.482 ^{Ac}	0.445 ^{ABd}	0.402 ^{Ac}	0.165 [*]
3	0.866 ^{Ab}	0.687 ^{ABab}	0.659 ^{Bbc}	0.611 ^{Cbc}	0.600 ^{Bbc}	0.560 ^{ABd}	0.511 ^{ABb}	0.153 [*]
4	1.672 ^{Aa}	0.791 ^{Bab}	0.780 ^{BCb}	0.722 ^{Db}	0.684 ^{BCab}	0.629 ^{BCc}	0.597 ^{ABb}	0.972 [*]
5	-----	0.899 ^{Ba}	0.843 ^{Ca}	0.811 ^{Dab}	0.780 ^{Ca}	0.712 ^{BCcb}	0.681 ^{Bb}	0.091 [*]
6	-----	-----	0.951 ^{Ca}	0.913 ^{Ba}	0.843 ^{Aa}	0.794 ^{Ca}	0.740 ^{Ba}	0.009 [*]
LSD	0.168 [*]	1.35 [*]	0.149 [*]	0.154 [*]	0.145 [*]	0.145 [*]	0.132 [*]	

Where: Mean values in the same row (as a capital letter) or column (as a small letter) with the same letter are not significantly differences at 0.05 level;LSD: Least significant differences; Ns: Non-significant differences; * significant differences.

Data given in Table (3 and 4) showed the changes of thiobarbituric acid values (mg malonaldehyde/kg sample) of different beef burger treatments as affected by two type of volatile oils (cinnamon and ginger) normal and loaded-solid lipid nanoparticles (EO-SLNs) and their percentages (0.05, 0.1 and 0.15%) during frozen storage period at -18 °C up to 6 months. From these results, it could be noticed that, there were no significant differences ($P>0.05$) in thiobarbituric acid (TBA) between different beef burger treatments immediately after processing and after one month of frozen storage. Also, non-significant differences ($P>0.05$) were recorded between control sample and beef burger treatments prepared with ginger volatile oil free at 0.05% and after two months of frozen storage. On the contrary significant differences ($P<0.05$) were observed between control and beef burger prepared with ginger volatile oil free at 0.15%. Meanwhile significant differences ($P>0.05$) were recorded between control sample and beef burger treatments prepared with cinnamon volatile oil normal and after zero time of frozen storage at any concentration. At zero time of frozen

storage, thiobarbituric acid values of different beef burger treatments ranged between 0.271 and 0.280 mg malonaldehyde/kg sample. These results are in line with those obtained by Hassan (2010) who found that TBA values of beef burger samples ranged between 0.498 to 0.785 mg malonaldehyde/Kg samples.

These values increased significantly by increasing storage time to range between 0.597 and 1.657 mg malonaldehyde/kg sample at 4th month of frozen storage. The highest thiobarbituric acid value 1.672 mg malonaldehyde/kg sample was recorded for control sample followed by beef burger prepared with 0.05% ginger volatile oil with significant differences between them. Meanwhile the lowest values (0.597 mg malonaldehyde/kg sample) was recorded for beef burgers treatments prepared with 0.15% cinnamon volatile oil nano followed by beef burger prepared with 0.15% ginger volatile oil nano with significant differences. During frozen storage at -18 °C up to 6 months, thiobarbituric acid values of all beef burger treatments either control sample or

Table 4. Thiobarbituric acid (mg malonaldehyde/kg) of different beef burger treatments as affected by ginger essential oil (normal and nanoparticle) and their percentages during frozen storage at -18 °C up to 6 months.

Storage period (months)	Treatments							LSD
	Control	Ginger essential oil (%)			Ginger nano-particle (%)			
		0.05	0.1	0.15	0.05	0.1	0.15	
Zero time	0.274 ^{Ad}	0.274 ^{Ad}	0.276 ^{Ae}	0.273 ^{Af}	0.277 ^{Ae}	0.276 ^{Ae}	0.277 ^{Ad}	0.182 ^{ns}
1	0.479 ^{Ad}	0.422 ^{Bd}	0.411 ^{Bd}	0.392 ^{Af}	0.377 ^{Ae}	0.363 ^{Ad}	0.372 ^{Accd}	0.171 ^{ns}
2	0.652 ^{Ac}	0.625 ^{BCc}	0.605 ^{Cc}	0.599 ^{Be}	0.502 ^{Bd}	0.480 ^{ABd}	0.468 ^{ABc}	0.165*
3	0.866 ^{Ab}	0.716 ^{Cb}	0.693 ^{Cc}	0.642 ^{Bd}	0.610 ^{Bc}	0.594 ^{BCc}	0.560 ^{Bb}	0.153*
4	1.672 ^{Aa}	0.845 ^{Dab}	0.801 ^{Db}	0.769 ^{Cc}	0.711 ^{Cab}	0.699 ^{Dc}	0.632 ^{BCb}	0.972*
5	-----	0.974 ^{Aa}	0.932 ^{Ea}	0.859 ^{Db}	0.811 ^{Cb}	0.771 ^{Db}	0.703 ^{CDab}	0.091*
6	-----	-----	-----	0.985 ^{Ea}	0.894 ^{Da}	0.869 ^{Aa}	0.813 ^{Aa}	0.009*
LSD	0.168*	1.65*	0.172*	0.131*	0.147*	0.151*	0.127*	

Where: Mean values in the same row (as a capital letter) or column (as a small letter) with the same letter are not significantly differences at 0.05 level; LSD: Least significant differences; Ns: Non-significant differences; * significant differences.

Beef burgers prepared with volatile oils free and nano significantly increased as the time of frozen storage increased. This increase in TBA values during frozen storage could be indicated continues oxidation of lipids and consequently the production of oxidative by products. These results are in agreement with those obtained by Osheba (2013) who mentioned that TBA values of meat and chicken burger samples increased as the storage period increased. Furthermore, TBA values of beef burger treatments were decreased significantly by increasing volatile oil percentages. These results are in agreement with EL-Harery (1997) reported that TBA values of beef sausages decreased by increasing cardamom volatile oil concentrations. Control sample was unacceptable for human consumption after four months of storage as their TBA value reached (1.672 mg malonaldehyde/kg sample), and according to Egyptian Standard specification (2005) rejected frozen beef burger samples which have more than 0.9 mg malonaldehyde/kg sample).are rejected Also, from the same Table, it could be observed that, TBA values are significantly affected by the type of volatile oils and their percentages normal and nano Cinnamon volatile oil free and nano had a higher effect on the reduction of TBA values than ginger volatile oil free and nano. This may be attributed to the stronger antioxidant of cinnamon components as reported by Shen and Kamdem, (2015) who said that cinnamon might be considered as potential antioxidant compared with ginger.

CONCLUSION

From this study it could be concluded that, the transformation of essential oils into nanoparticle size can affect their antioxidant activity and may promote the chemical properties and shelf life of beef burger.

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استخدام الزيوت العطرية في صورتها العادية والمتناهية الصغر كمضادات أكسدة

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الملخص العربي

يتم إضافة مضادات الأكسدة عند تصنيع منتجات اللحوم لمنع أو تأخير تفاعلات الأكسدة التي تحدث في اللحوم، لذلك أجري هذا البحث لدراسة تأثير الزيوت العطرية في صورتها العادية والمتناهية الصغر كمضادات أكسدة على الخصائص الكيميائية وفترة الصلاحية لبرجر اللحم المجمد. تم تقييم النشاط المضاد للأكسدة لزيت القرفة والزنجبيل الطيار بتركيزات مختلفة سواء في صورته العادية أو المتناهية في الصغر كما تم دراسة تأثير إضافة الزيوت العادية والمتناهية الصغر السابقة الذكر بتركيزاتها المختلفة على الخواص الكيميائية (حمض الثيوباريتيوريك) وفترة الصلاحية لبرجر اللحم المجمد أشارت النتائج إلى أن التأثير المضاد للأكسدة باستخدام طريقة الداى بيكوبل داي فينيل هيدرازين (القدرة على الإمساك بالشوارد أ والشقوق الحرة) يزيد بزيادة تركيز الزيوت العطرية وخصوصاً في صورتها المتناهية في الصغر، كما أن إضافة الزيوت الطيارة تعمل على اختزال قيم الخواص الكيميائية (حمض الثيوباريتيوريك) لبرجر اللحم وخصوصاً في صورتها المتناهية في الصغر، كما تعمل على زيادة فترة الصلاحية لمدة ستة أشهر بدلاً من أربعة أشهر للكمترول. ونستنتج من هذا أن تحويل الزيوت العطرية من الصورة العادية إلى الصورة متناهية الصغر من الممكن أن تؤثر على نشاطها كمضادات للأكسدة ويعزز الخصائص الكيميائية وفترة الصلاحية لبرجر اللحم.

الكلمات المفتاحية: الزيوت طيارة، مضادة للأكسدة، قرفة، زنجبيل.