THE EFFECT OF CARDAMOM AND CORIANDER OILS ON THE MICROBIOLOGICAL LOAD AND QUALITY OF MEAT SAUSAGE

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ABSTRACT

The object of the present study is evaluation of possibility of using cardamom and coriander oils mixture (1:4 w/w) as natural antimicrobial in meat sausage industry to increase the shelf-life of sausage. The physical and chemical properties of the sausage was improved by addition of cardamom and coriander oils mixture at the concentrations of 600,900 and 1200 ppm to sausage before storage at -18℃ for 3 months. The moisture, protein content and carbohydrates were decreased about 1.06, 1.02 and 0.90 fold as compared to zero time, respectively. Whereas, ash, pH value and fat content increased up to 6.19, 18.44, and 6.31%, respectively, as compared to zero time. Improvement has been observed after addition of cardamom and coriander oils mixture and, it works to increase the storage period of the sausage and maintain the stability of the desired qualities for consumers. Regarding to microbial load, samples treated with 900 and 1200 ppm of oils mixture revealed significant reduction after 90 day of storage as compared with the control samples. Sample (1200 ppm) had the highest score for overall acceptable among all investigated samples. Addition of cardamom and coriander oils shows growth inhibition to some types of bacteria and fungi.

Key words: Sausage, Chemical composition, Microbiological quality, Sensory characteristics

INTRODUCTION

Food safety is a fundamental concern for both consumers and food producers alike. Despite the high degree ofawareness of food preservation methods there is increasing occurrence of disease outbreaks caused bypathogenic and spoilage microorganisms in foods (Meng& Doyle, 1998). Currently some ready mealmanufacturers add synthetic antimicrobial agents such nitrates, nitrites, benzoates to inhibit the growth of foodspoilage and food pathogenic microorganisms (Gould, 1996). However, the uncontrolled use of chemicalpreservatives over the years has led to emergence of microbial resistance to classic antimicrobial agents whichhas become a major health concern (Kiesslinge*t al.,* 2002). Consumer awareness and concern that syntheticchemical additives may have some toxic or even carcinogenic effects, has increased the demand for high-quality,minimally processed foods with extended shelf-life, preferably free from or with a reduced level of addedchemical antimicrobial agents (Zink, 1997). Recently there has also been an observed trend towards reducing saltlevels in ready meals as a result of the proven association between excessive sodium intake, the development ofhypertension and increased risk of cardiovascular disease (Desmond, 2006). Therefore, there is growing interestin using natural antimicrobial compounds, including extracts of herbs and spices, as salt replacers or alternativesto synthetic compounds for food preservation (Smid&Gorris, 1999). Recently it has become very interesting and highly recommended to use naturalantioxidants as food additives by the food industry (Solomakos *et al.*, 2008; Karre *et al.,* 2013).

Essential oils from aromatic and medicinal plants has been known to possess potential as natural agents for food preservation, including antibacterial, antifungal and antioxidant; in fact, many essential oils have been qualified as natural antioxidants and offered as potential substitutes of synthetic antioxidants in specific sections of food preservation where their use is not in contrast with their aroma (Ruberto and Baratta 2000; Polite0o *et al.,* 2007). Antioxidants are used in the food industry to increase the shelf life of the foods. Antioxidants can also prevent the reaction of free radicals with biomolecules in the human body and reduce cell injury and death, chronic and cardiovascular diseases and etc. (Ayoughi *et al.,* 2011).Natural aromatic plants and spices have been widely used in many food products such as meat and meat products, dairy and bakery products for preserving and for their medicinal value (Reddy *et al.,* 2005; Shahsavari *et al.,* 2008). Most antioxidant activity investigations have been managed on refined oils (Lean and Mohamed, 1999).Cardamom oil is used in food, perfumery, and liquor a pharmaceutical industries as a flavour and a carminative. In medicine, it is used as a powerful aromatic, antiseptic, stimulant, carminative, stomachic, expectorant, anti-spasmodic and diuretic (Baytop (1984) Korikontimath, *et al.,* (1999)).Cardamom has antioxidant properties and can increase levels of glutathione and antioxidant enzymes in the body.

Coriander seeds are also used as a flavoring agent in different foods namely pastries, cookies, buns, cakes and breads (Akgul, 1993; Coskuner and Karababa, 2007; Bhuiyan*et al.,* 2009). In addition to the traditional food uses, these have been widely used in the folk medicine system as carminative, spasmolytic, digestive, and galactagogue (Ghani, 2003; Bhuiyan*et al.,* 2009).

This plant is of economic importance since it has been used as a flavoring agent in food products, perfumes, cosmetics and drugs. This culinary and medicinal plant widely distributed and mainly cultivated for the seeds which contain an essential oil (ranges between 0.3% and 1.1%) (Neffati*et al.*, 2011).The object of the present study is evaluation of possibility of using cardamom and coriander oils mixture (1:4 w/w) as natural antimicrobial in meat sausage industry

MATERIALS AND METHODS

Source of samples: Cardamom and coriander oils were purchased from Kato Aromatic Company (Cairo, Egypt).Meats was obtained from local commercial markets.

Preparation of meat sausage: Frozen boneless meats was thawed at 10°C just before sausage manufacture. It was ground through a 0.4-cm grinder plate (Super grinder-MK-G3; Matsushita Electric Industrial, Japan). These corresponding levels of the two oil mixed (cardamom and coriander 1:4 w/w) forms are weight equivalent. The ingredients of sausage sample were as showed in Table (1).

This formula was mixed in an emulsifier (Kenmix Electronic, model FP800, Kenwood Ltd., Britain) for 3 min and the resulting mixture was stuffed tightly into hydrocellulose casings 1.5 cm in diameter (Viskase Corporation, Chicago, USA), which were subsequently divided into food-casing lengths of about 12 cm per unit. The sausage units were vacuum packaged in polyethylene bags, labeled and stored at -18°C for 3 months.

Table (1): Ingredients of Sausage

Ingredients	Weight (g)
Sausage meat	850
Fat	150
Starch	50
Sodium chloride	20
Sodium polyphosphate	15.5
Glucose	10
Monosodium glutamate	5
Black pepper	3
Ascorbic acid	0.40
sodium nitrate	0.1

To the above formula mixture of oils cardamom and coriander (1:4 w/w),Spices mixture oils (600,900 and 1200 ppm) were added

Culture preparation: A loopful of 24 h surface growth on a NA (Nutrient Agar) slope of each bacterial strain (NA) was transferred individually to 5 ml of Brain Heart Infusion (BHI) broth (pH 7.6, Difco). After incubation at 37°C for 24 h, bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and suspended in 0.1% peptone water. Turbidity was adjusted to match that of a 5 McFarland standard (10^7 CFU/ml). Then, a 1:10 dilution of the cell suspension was performed to give an inoculum concentration of 10^7 CFU/ml.

Aerobic plate count: Sausage sample (10 g) was homogenized with 90 ml of sterile peptone water (1 g/l) in a laboratory homogenizer (AM-5 Ace homogenizer, Nihonseiki, Japan) and serial dilutions were prepared, then 0.1 ml of each dilution was spread with a bent sterile glass rod on duplicate plates

of pre-poured and dried standard plate count agar (Nissui Pharmaceutical, Japan). After 48h incubation at 25^0C, colonies were counted and results were expressed as log CFU/g of sausage sample.

Chemical analysis: Moisture content, crude protein (total N x 6.25), fat and ash contents were determined according to A.O.A.C. (2000). Total carbohydrates content was calculated by differences. For determination of the pH, 10 g of sample were homogenized with 50 ml distilled water and pH value was measured by a digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd.,Tokyo, Japan).

Sensory evaluation: Sensory evaluation was carried out on sausage samples immediately. The sausage products were evaluated for color, flavor,taste and overall acceptability according to the procedures of American Meat Science Association(AMSA 1995).

Statistical analysis: A one-way ANOVA followed by Duncan's multiple range test (DMRT) were performed using IBM ® statistics software SPSS 11.00 (SPSS Inc., Chicago, IL, USA) to analyze and compare the data. Results were presented as mean ± SD and P- values ≤ 0.05 were regarded as statistical significance

RESULT AND DICUSSION

In this study, the improvement in quality and shelf life increase was determined, when oils herb usedagainst food borne in sausage product. For control sample, the moisture content decreased from 49.14 to 45.00%, protein was decreased from 25.56 to 24.11%, carbohydrates decreased from 3.91 to

3.11%, ash and fats were increased from 3.14 to 6.19 and 18.25 % to 21.59 % after 3 months storage. The protein decrease may be due to protein hydrolysis by natural meat enzymes and bacterial enzymes that are produced as well as the loss of water soluble protein with separated drip(BadpaandSaghir 2014). The ash percent for all sausage samples increased gradually with increasing time of storage. Regarding ash results indicated that sausage had high substitute of herbs (sample control without oil mixture) contain high percentage of ash.These results are similar to that obtained by Gibriel*et al.* (2007) and Madkour*et al.* (2000).

Results in Table (2) indicated that the moisture, cure protein and carbohydrate were decreased by increasing storage period from zero to 90 days. At zero time the moisture, crude protein and carbohydrate were ranged from 49.11 to 49.59%, 25.21% to 25.52% and 5.10 to 5.32%, and decreased after 90 days storage period at -18°C which ranged from 45.83% to 46.03, 24.15% to 24.39% and 4.51 to 4.81% in all treatments withoils mixture. Whereas, ash (4.79 to 4.72%), fat (20.72 to 20.33%) and pH values (6.33 to 6.66) were increased with increasing storage period for 90 days in all concentrations ofoils(600,900 and 1200 ppm/kg).The highest moisture, crude protein and carbohydrate wereachieved at 1200 ppm/ kg ofoil mixture of cardamom and coriander at zero time while gave the highest value at pH (6.95) after 90 days of storage period. Whereas the highest amount of ash and fat were recorded at 600 ppm ofoil mixture after 90 days of storage.These results are in agreement with those reported by Nuzhat*et al.* (2002) Bahlol and Abd El-Aleem (2004) and Abd El-Aleem and Mohamed (2005).

Sensory evaluation of prepared sausage using different concentration of oils mixture:

Colour and taste measurement are a critical objective quality parameter that can be used for quality index measurements of quality of the meat products as well as quality changes as a result of processing, storage and other factors (Busatta*et al.,* 2008). Odor, overall acceptability and appearance are probably the most important attributes that influence the sensoryproperties for consumers. The consumer panel data of this treatments were tabulated in Table (3) and indicated that significant differences ($P<0.05$)

Table (2): Chemical composition of sausage produsedwith cardamom and coriandermixture for 90days storage period at - 18°C.

Storage time Days	pH values	Control					
		Moisture (%)	Crude protein (%)	Fat (%)	Ash (%)	Carbo hydra te (%)	SEM±
0	6.2	49.14	25.56	18.25	3.14	3.91	0.16
7	6.09	49.12	25.45	18.34	3.25	3.84	0.13
14	6.14	47.52	25.23	19.12	4.33	3.80	0.05
21	6.22	47.02	24.91	19.51	4.09	3.65	0.05
28	6.35	46.38	24.63	20.25	4.21	3.61	0.08
60	6.56	45.56	24.21	21.28	5.77	3.18	0.15
90	6.76	45.00	24.11	21.59	6.19	3.11	0.02
Concentration of Coriander and Cardamom oil at (600 ppm) Kg (v/w)%							
0	6.06	49.11	25.21	18.47	2.11	5.10	0.04
7	6.11	49..00	25.11	18.67	2.20	5.02	0.03
14	6.13	48.61	25.00	19.04	2.43	4.92	0.11
21	6.23	48.40	24.89	19.51	2.65	4.85	0.01
28	6.25	48.10	24.75	19.55	3.51	4.79	0.04
60	6.29	46.20	24.31	20.52	4.37	4.60	0.09
90	6.33	45.83	24.15	20.72	4.79	4.51	0.05
Concentration of Coriander and Cardamom oil at (900 ppm) kg (v/w) %rat							
0	6.08	49.52	25.50	18.44	1.30	5.24	0.05
7	6.20	49.70	25.30	18.19	1.70	5.11	0.07
14	6.23	49.01	25.01	18.44	2.53	5.01	0.06
21	6.29	48.42	24.80	19.22	2.59	4.97	0.07
28	6.33	48.22	24.73	19.35	2.80	4.90	0.05
60	6.57	47.31	23.46	20.09	4.39	4.75	0..08
90	6.66	46.00	24.32	20.33	4.72	4.63	0.06
Concentration of Coriander and Cardamom oil (1200 ppm) kg (v/w) %							
0	6.31	49.59	25.52	18.44	1.13	5.32	0.05
7	6.42	49.45	25.11	18.70	1.53	5.21	0.08
14	6.46	48.51	25.00	18.54	2.76	5.19	0.02
21	6.60	48.03	25.00	19.01	2.89	5.07	0.04
28	6.75	46.65	24.95	20.05	3.32	5.03	0.05
60	6.82	46.33	23.75	20.11	4.85	4.96	0.03
90	6.95	46.03	24.39	20.12	4.65	4.81	0.06

Each value is the average of three Replicates.

in all sensory characteristics were observed between sausage samples with different mixture oils concentrations and control sample.

The highest score of colour (8.47), taste (8.72), odor (9.43), appearances (8.21) and overall acceptability (8.02) were recorded by samples containing 1200 ppm of oils mixture. Whereas the sausage samples with 600 ppm of mixture oils gave the lowest scores of the colour, taste, appearance and overall acceptability being 7.32, 5.23, 7.78 and 6.26 respectively. This result was agreements with Caceres *et al*., (2004)

Table (3): Sensory evaluation of prepared sausage contained different concentration of cardamom and corianderoils mixture (ppm).

Storage time (Days)	Control	Concentration ppm of ois mixture		
		600	900	1200
Colour	8.01a	7.32b	7.79a	8.47a
Taste	7.36a	5.23c	6.50a	8.72a
Odor	9.00a	8.25a	9.12a	9.43a
Overall acceptability	7.12b	6.26c	7.25b	8.02a
Appearance	8.02a	7.78a	8.00a	8.21a

a-c: Means with different superscript within the same row differ significantly atp < 0.05.

The microbiological charactertics of sausage prepared using different concentration of oil mixture of oils: Cardamom and coriander oils mixture (1:4) ww concentration of 600,900 and 1200 ppm were applied as natural preservatives during the processing of beef sausage. The effects of these preservatives on microbiological characteristic of prepared sausage were studied during 90 days storage period at -18°C and compared with control.

The microbiological characteristics were determined as the counts of total bacteria, yeast and molds.

Results of microbial microbiotaof prepared sausage contained different concentrations of cardamom and coriander oils mixture (1:4) ww during 90 days of storage at -18°C were presented in Table (4). The results indicated that the total bacterial count of prepared sausage containing 1200 ppm of oils mixture was lower than the treatments contained 600,900 ppm and control, which decreased from 3.30 to 1.88log cfu /g after 90 days, whereas the highest count of totalbacteria countwas recorded in the control samples after 90 days (5.10 log cfu /g). In addition, *Staphylococci,* total *coliform*, *yeast* and *molts* not detected after 21 days of storage in samples count 1200ppm. Whereas, the control samples and samples contained 600 ppm oilsmixtures gave the highest counts (total bacteriacount (5.10 and 4.62), *Staphylococci*(1.62 and 1.22),total*coliform* (1.64 and 1.44) and*yeast* and *molts*(1.62 and 1.38), respectively, after 90 days of storage. Similar finding were performed by Jay (2005).

Antifungal activity of cardamom and coriander oils:

Results in Table (5) showed the antifungal activity of cardamom and coriander oilsmixture against some fungal strains (*Aspergillusfllavus*, *Aspergillusniger* and *saccharomyces cerevisiae*).The inhibitory effect of these oils was detected using agar diffusion method.Results indicated that the antifungal activity of all tested oils mixture were varied from one to another and reached the maximum effect at 1200 ppm concentration. The highest inhibition zone diameter was 11.50 mm and recorded by *Saccharomyces cerevisiae* at 1200 ppm. On contrary, the lowest inhibition zone diameter was

attained by cardamom oil (4.01mmfollowed by coriander oil (4.25mm) at 600 ppm concentration against the growth of *Aspergillusflavus*. These results in agreement Yano *et al* (2006) reported that the inhibitory effects of cardamom and coriander oils significant increased with increasing the concentration of oil.

Antibacterial activity of cardamom and coriander oils: As seen in Table (6), cardamom and coriander oils markedly inhibition growth of most bacteria tested including *Staphylococcus aureus, salmonella typhimurium, Bacillus subtilis* and *Escherichia coli*. It could be noticed that the inhibitory effects differed with regard to the type of bacteria. Cardamom and coriander oils generally showed stronger antibacterial effects of for *Staphylococcus aureus*, *Bacillus subtilis a*nd *E. coli* than *salmonella typhimurium* in the presence of 1200 ppm oils concentration, where the inhibition zones diameter of cardamom oil (1200 ppm) were 11.00, 15.30, 10.30 and 7.50 mm, respectively and coriander oil (1200 ppm) produced inhibition zones 10.90, 12.40, 11.10, and 8.3 mm for the some tested bacteria strains, respectively. These results were in agreement withJoydeep*et al* (2014).

Table (4): Microbial count (log_{10}cfu/g) of prepared sausage content different concentrations of cardamom and coriander oils mixture (1:4) w/w during 90 days storage period (-18°C)

Treatment	Storage period (days)	Microbial count (log cfu/g)			
		Total bacterial count	*Staphylococci* count	Total *Coliform*	*Yeast* and *mold*
Control	0	3.44a	2.94a	2.64a	2.25a
	7	3.92a	1.40a	1.88a	1.92a
	14	4.08a	1.40a	1.88a	1.88a
	21	4.22a	1.42a	1.80a	1.84a
	28	4.40a	1.48a	1.78a	1.78a
	60	4.62c	1.52a	1.70c	1.70a
	90	5.10c	1.60a	1.64c	1.62a
600 ppm	0	3.42a	2.92c	2.44a	2.22b
	7	3.82a	1.40c	1.82b	1.84b
	14	4.06a	1.36c	1.74c	1.74b
	21	4.16a	1.30c	1.66c	1.66b
	28	4.28a	1.24b	1.52c	1.50c
	60	4.40a	1.20c	1.48a	1.44c
	90	4.62b	1.22c	1.44b	1.38c
900 ppm	0	3.38a	2.90c	2.40b	2.20b
	7	3.44a	1.40c	1.62b	1.62b
	14	3.50a	1.28d	1.40c	1.48b
	21	3.62a	1.16d	1.28c	1.30b
	28	3.72a	1.08c	1.14c	1.12b
	60	3.80a	≤ 1	≤ 1	≤ 1
	90	3.88a	≤ 1	≤ 1	≤ 1
1200 ppm	0	3.30a	2.84c	2.32b	2.15b
	7	3.22a	1.20e	1.44b	1.32b
	14	3.04a	1.08e	1.12c	≤ 1
	21	2.86a	≤ 1	≤ 1	≤ 1
	28	2.48a	≤ 1	≤ 1	≤ 1
	60	2.22a	≤ 1	≤ 1	≤ 1
	90	1.88a	≤ 1	≤ 1	≤ 1

The means with similar letters in the same column are not significantly different ($p > 0.05$)

Table (5): Antifungal activities of cardamom and coriander oils:

Organisms	Blank	Inhibition zone diameter (mm)					
		Cardamom oil ppm			Coriander oil ppm		
		600	900	1200	600	900	1200
Aspergillusflavus	-	4.01a	7.00b	925b	4.25a	6.58b	8.44b
Aspergillusniger	-	6.00a	8.40b	10.00c	6.12a	8.30b	9.54b
Saccharomyces cerevisiae	-	7.20a	10.10b	11.50c	7.35a	9.35b	10.90c

The means with similar letters in the same column are not significantly different($p>0.05$)

Table (6): Antibacterial activities of cardamom and coriander oils

Bacterail strains	Blank	Inhibition zone diameter (mm)					
		Cardamom oil ppm			Coriander oil ppm		
		600	900	1200	600	900	12··
Staphylococcus. aureus	-	3.50a	7.55b	11.00b	3.30a	8.20b	10.90b
Bacillus subtilis	-	6.00a	9.40b	15.30b	5.90a	9.90b	12.40b
salmonella typhimurium	-	3.70a	5.10b	7.50b	3.55a	5.70b	8.30b
E.coli	-	6.00a	10.10b	10.30b	7.10a	9.90b	11.10b

The means with similar letters in the same column are not significantly different ($p>0.05$)

CONCLUSION

From the obtained results, it could be concluded that addition of oils mixture to sausage at concentration of 600.900 and 1200 ppm not only minimize lipid oxidation but also improved the sensory characteristics and enhanced the wholesomeness of the product during 90 day of storageat -18°C. Furthermore, during storage at -18°Csausage prepared using 1200ppm oils mixture extended the shelf life for 90 day. Cardamom and coriander oils were

found to have important antimicrobial activity against the test strains. In this regard the use of Cardamom and Corianderoils volatile compounds as natural preservatives in food products and it may be an alternative to the use of chemical additives.

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تأثير مستخلصات زيت الكزبرة والحبهان على الحمل الميكروبى وجودة السجق

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المستخلص

تهدف هذه الدراسة إلى تاثير إضافة زيت الكزبرة والحبهان بدلا من إضافتهم كأعشاب فى تحسين الخواص الطبيعية والكميائية والحمل الميكروبى للسجق وقد لوحظ عند إضافة مخلوط الزيوت السابق ذكرها بنسبة ٦٠٠ و ٩٠٠ و١٢٠٠ جزء فى المليونزيادة فى نسبة الرطوبة من ٤٥,٠٠ إلى ٤٦,٠٣% والكربوهيدرات من ٣,١١إلى ٤,٨١% والبروتين من ٢٤,١١إلى ٢٤,٣٩% وتقليل نسبة الرماد من ٦,١٩إلى ٤,٦٥ % والدهون من ٢١,٥٩إلى ٢٠,١٢% وذلك عند مقارنة لعينات الكنترول وتركيز 1200 ppm فى نهاية فترة التخزين عند ٩٠ يوم وتقليل الحمل الميكروبى عند هذا التركيز إلى أقل حمل ميكروبى وهو أفضل تركيز من الناحية الكميائية والبيولوجية والحسية ويعمل على زيادة الفترة التخزينية للسجق والحفاظ على ثبات الصفات المرغوبة لدى المستهلكين.

عند إضافة زيت الكزبرة والحبهان إلى بعض أنواع البكتريا والفطريات لقياس مدى تثبيطها لوحظ تثبط لهذة الميكروبات والفطريات