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EFFECT OF ADDITION DIFFERENT LEVELS OF ROSEMARY, BASIL AND MINT ON THE QUALITY OF FRESH BEEF SAUSAGE DURING REFRIGERATED STORAGE

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ABSTRACT

This study was carried out to evaluate the effect of using rosemary, basil and mint at levels of 100, 200 and 300ppm (according to its content of phenolic compounds calculated as gallic acid) on quality of fresh beef sausage stored at 4±1°C for 5 days. Phenolic compounds in rosemary, basil and mint extracts were identified and determined by HPLC. The total phenolic compounds were 10304, 1559, and 1938 ppm, respectively. Organoleptic evaluation results indicated there are no high significant differences between control and samples treated with rosemary, basil and mint at levels 100 and 200 ppm, so these samples were used to evaluate the physical, chemical and microbial evaluation. WHC, pH, total volatile nitrogen (TVN), thiobarbituric acid (TBA) as well as microbial counts were determined after 0, 1, 3 and 5 days of refrigerated storage as an indicator of spoilage. Addition of rosemary, basil and mint to fresh sausage formula reduced pH, TBA, TVN values and microbial total count compared with control, On the other hand, WHC was increased. Depend on the results obtained in this work, rosemary, basil and mint at levels100 and 200 ppm are recommended to use as antioxidant, antimicrobial, flavoring and coloring agents.

Keywords: Fresh sausage, Rosemary, basil, mint, Phenolic compounds, antimicrobial.

INTRODUCTION

Sausage is one of the oldest known forms of processed meat products and is very popular in many regions. Fresh sausages, e.g. fresh pork sausage, country-style pork sausage, fresh kielbasa (Polish), Korr (Swedish), Italian sausage, bratwurst, bockwusrt, chorizo (fresh) and thuringer (fresh), are some common examples (Romans, *et al*., 1994).

Flavour is an important consideration that may limit the use of some antioxidants in meat and meat products. Spices and herbs have been added in many cuisines to impart flavour, aroma and piquancy to food. Several studies have shown the antimicrobial and antioxidant potential of spices and herbs such as basil, thyme, rosemary, garlic, clove, coriander, ginger, mustard and pepper (Sebranek, *et al*. 2005; Tipsrisukond, *et al*. 1998).

The use of natural preservatives to increase the shelf-life of meat products is a promising technology since many herbs, plants, fruits, and vegetables extracts or powders have antioxidant and antimicrobial properties. Antioxidant effects of oregano essential oil (Viuda-Martos, *et al*., 2010),

Rosemary (Rosmarinus officinalis L.), like other aromatic herbs and spices which has been planted in many areas and used in Mediterranean and other cuisines, is not used to improve or modify only food flavours but also to provide some functionality. For example, its extract has been used widely as an antioxidant in the food industries. Carnosol, carnosic acid and rosmarinic

acid have been identified as major constituents that contribute to the antioxidant activity of rosemary (Aruoma *et al*., 1992). Utilising DPPH and ABTS radical-scavenging assays, and the ferric thiocyanate test (Erkan *et al.*, 2008) pointed out that rosemary extract had a higher phenolic content than had blackseed (Nigella sativa L.) essential oil, thus leading to a higher antioxidant activity. Many reports have indicated that rosemary extracts can retard lipid oxidation and prolong the shelf-life of meat products (Georgantelis, *et al.*, 2007). In addition, rosemary extracts have been shown to have some antimicrobial effect (Angioni *et al*., 2004).

Basil has been used traditionally in Mediterranean and Southeast Asian foods. The most commonly used types in European and American cuisine are local sweet basil, lemon basil, purple ruffle and minter Egyptian basil. Holy basil (Ocimum sanctum Linn), an annual herbaceous plant with slightly hairy, pale green leaves, is used widely as flavouring in Southeast Asian cuisine especially in Thai stirred fries. Holy basil leaves are spicy and have lemony notes (Uhl, 1996. ; Javanmardi, *et al*. (2003) reported that Iranian basil possess valuable antioxidant properties for culinary and possible medicinal use.

Mint (Mentha spicata) is an extensively used herb in Indian cuisine and also for curing several common ailments (Choudhury, *et al* 2006). showed that mint extract had very well antioxidant potential which was comparable to that synthetic one, butylated hydroxy toluene (BHT), (Kanatt, *et al* , 2007). Mint extract did not show any antibacterial activity, though essential oils of some Mentha species have been reported to have antibacterial activity (Marino, *et al.*, 2001; Moreira, *et al.*, 2005).

Therefore, main objectives of this work were to study the effect of addition various levels of rosemary, basil and mint on the quality of fresh beef sausage during refrigerated storage as a trial to prolong shelf-life of this fresh product using natural preservatives.

MATERIALS AND METHODS

Materials:

Rosemary (Rosmarinus officinalis L.), Holy basil (Ocimum sanctum Linn), Mint (Mentha spicata), minced beef meat, minced beef fat, natural casings and other spices were purchased from local market of Mansoura city, Egypt.

Chemicals and media used for following examinations were purchased from El-Gomhoria Company, Mansoura branch, Egypt.

Methods:

Fresh beef sausage preparation:

Minced meat (66%) was mixed thoroughly with beef fat (15%), ice water (17%) and salt (1.79%) with a mixer for 1.5 min. Afterwards, other spices and seasonings were added including 0.008% sugar, 0.056% black paper, 0.033% nutmeg powder, 0.033% cardamom, 0.04% cubab, and 0.04% cloves, pre-assigned amounts (100, 200 and 300 ppm) of ground rosemary, basil or mint according to its content of phenolic compounds as gallic acid,

then mixed for another 1.5 min. (El-Dashlouty, 1978). The resulting mixtures were stuffed into natural casings. Raw sausages were manually linked, hanged up and stored at 4±1°C for 5 days. All chemical tests were done after 0, 1, 3 and 5 days of storage.

Chemical analysis:

Moisture, ether extracts (fat), protein, crude fiber and ash content in frozen meat were determined using the methods outlined in AOAC (1990). Carbohydrates content was calculated by difference.

Phenolic compounds of rosemary, basil and mint were analyzed at Bio-technology Lab., Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. Analysis was performed with a high pressure liquid chromatography HPLC "HP1050" equipped with a 4.6 mm x 150 mm ODS C18 column with UV detector and the injection volume was 5µl. Isocratic mobile phase was 40 methanol: 60 distilled water. The wave length in the UV detector was 230 nm, total run time for the separation was approximately 15 min. at a flow rate of 0.60 ml/min. according to the proposed method of Waskmundzka *et al*. (2007).

pH value was measured as described by Lima dos Santos *et al*. (1981) using a digital pH meter (HANNA instruments pH 211 Microprocessor pH meter).

Total volatile nitrogen (TVN) was determined according to the method mentioned by Pearson (1968). Results were expressed as mg nitrogen per 100g sample.

Thiobarbituric acid value (TBA) was determined as described by Tarladgis *et al*. (1960). TBA value was expressed as mg of malonaldehyde/ kg fat using the following equation:

TBA=7.8 x OD

where: OD = optical density at 538 nm.

Water holding capacity:

Water holding capacity (WHC) was determined as described by Soloviev (1966).

Microbial evaluation:

Total bacterial count was determined using the media described by Difco (1971). Mould and yeasts counts were determined using malt extract agar medium (Pitt., 1979). Lipolytic and Proteolytic bacteria were enumerated as described by Chalmers (1962).

Organoleptic evaluation:

Sensory evaluation of texture, taste, aroma and color were conducted by 10 panelists at the Food Industries Department, Faculty of Agriculture, Mansoura University, Egypt. Hedonic scale (0-1 very poor, 2-3 poor, 4-5 fair, 6-7 good and 8-9 very good) according to Molander (1960).

Statistical analysis

Data of chemical analysis and sensory evaluation of fresh beef sausage were subjected to analysis of variance (ANOVA) using SPSS program, version 17, 2d008, (SPSS ,2008)

RESULTS AND DISCUSSION

Organoleptic evaluation of cooked beef sausage:

Organoleptic evaluation of cooked beef sausage samples are given in Table (1). Statistical analysis was performed to determine the effect of adding rosemary, basil and mint on sensory attributes. It was found that there were no high significant differences between the control and samples R1 (100ppm), R2 (200ppm) for rosemary, B1 (100ppm) for basil as well as M1 (100 ppm) for mint. However, high significant differences were obtained between control and treated samples with level 300ppm for all herbs. These differences in color, taste, aroma, texture and overall acceptability could be seen easily when the sausage were treated with all herbs at level 300ppm, so these samples were discarded from the research. Depend on the results obtained in this work, rosemary, basil and mint at level (100-200 ppm) are recommended to use as, flavoring and coloring agent.

Table (1): Organoleptic evaluation of cooked beef sausage treatments as affected by refrigerated storage and natural additives:

Sausage treatments		Sensory Characteristics				
Herbs & Spices	Control + ppm*	Colour (9)	Taste (9)	Aroma (9)	Texture (9)	Overall acceptability (9)
Control		$8.25^{a}\pm0.083$	$8.08^{a}\pm0.044$	$7.87^{a}\pm0.209$	$8.18^{a}\pm0.063$	$8.35^{a}\pm0.087$
R1	100	$7.97^{ab}\pm0.105$	$7.21^{b}\pm0.193$	$7.53^{ab}\pm0.126$	$7.23^{bc}\pm0.289$	$7.44^{ab}\pm0.122$
R2	200	$7.04^{bc}\pm0.076$	$6.99^{c}\pm0.096$	$7.01^{bc}\pm0.287$	$7.11^{bc}\pm0.102$	$7.12^{bc}\pm0.102$
R3	300	$5.93^{e}\pm0.088$	$5.79^{d}\pm0.195$	$5.93s^{d}\pm0.105$	$6.58^{cd}\pm0.122$	$5.96^{d}\pm0.060$
B1	100	$7.41^{bc}\pm0.114$	$7.34^{bc}\pm0.088$	$7.13^{bc}\pm0.089$	$7.26^{bc}\pm0.108$	$7.36^{bc}\pm0.091$
B2	200	$7.19^{bc}\pm0.066$	$7.05^{bc}\pm0.072$	$6.93^{de}\pm0.084$	$7.08^{bc}\pm0.122$	$7.29^{bc}\pm0.129$
B3	300	$5.83^{e}\pm0.089$	$5.91^{d}\pm0.050$	$5.57^{d}\pm0.054$	$5.93^{d}\pm0.065$	$6.01^{d}\pm0.072$
M1	100	$7.87^{ab}\pm0.072$	$7.10^{bc}\pm0.104$	$7.25^{bc}\pm0.323$	$7.24^{bc}\pm0.085$	$7.95^{ab}\pm0.097$
M2	200	$7.13^{bc}\pm0.077$	$6.99^{cd}\pm0.091$	$7.02^{bc}\pm0.090$	$6.97^{cd}\pm0.030$	$6.96^{cd}\pm0.056$
M3	300	$6.03^{cd}\pm0.082$	$5.43^{e}\pm0.054$	$5.87^{d}\pm0.058$	$5.22^{e}\pm0.025$	$5.42^{e}\pm0.051$

R1= (Rosemary 100 ppm)
R2= (Rosemary 200 ppm)
R3= (Rosemary 300 ppm)
B1= (Basil 100 ppm)
B2= (Basil 200 ppm)
B3= (Basil 300 ppm)
M1= (Mint 100 ppm)
M2= (Mint 200 ppm)
M3= (Mint 300 ppm)
Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

Phenolic compounds and total phenolic content in herbs:

Data in Table (2) showed the content of different phenolic compounds of investigated herbs (mg/100g).The obtained results indicated that phenolic compounds ranged from 0.39 to 252.86 mg/100g in rosemary, 0.34 to 74.61 mg/100g in basil and 0.782 to 64.18 mg/100g in mint. Additionally, this results showed the total phenolic content in rosemary, basil and mint were 10304, 1559 and 19381 mg/100g (as gallic acid) respectively. These obtained results were in accordance with those given by (EL- Gammal and Sabrene,

2012 and Biswas.A.K.*et.al*, 2012), who reported that the total phenolic contents of mint leaf extracts ranged from 2500 to 3500 mg/100g depending on the extraction methods.

Table (2): Phenolic compounds content (mg/100g) in Rosemary, Holy basil and Mint).

Phenolic compounds	Concentration (mg/100g)		
	Mint	Basil	Rosemary
gallic acid	64.18	74.61	167.41
Catechol	4.76	7.23	48.79
Chlorogenic	2.78	4.65	13.52
Catechien	48.77	31.08	252.86
Vanillic	-	0.60	-
Syringic	4.77	3.95	-
P.OH. Benzoic	1.773	0.98	16.23
Coumaric	0.782	1.49	40.28
Sallicylic	22.91	9.19	81.69
Caffeine	2.53	2.96	13.41
Cinnamic	1.76	1.08	2.59
Caffiec	3.12	-	22.59
Chrisin	-	0.34	0.39
Total as gallic acid	1938	1559	10304

Chemical composition of frozen minced meat:

The chemical composition of frozen meat determined , in order to estimate ratios of other components in sausage formula ,especially fat tissues, water……etc. in able (3). Chemical analysis cleared that crude protein 22.67%, crude fat 3.98 %, ash 0.87 and carbohydrates 0.52 % (WB). These results were within the permissible limits as recommended by EOS (2005a).

Table (3): Chemical composition of frozen minced meat:

Components	Crude minced meat (Wet basis)	*EOS (2005a)*
Moisture(g/100g)	71.96	70% or less
Crude Protein (g/100g)	22.67	Not less than 18%
Crude fat (g/100g)	3.98	20% or less
Ash (g/100g)	0.87	-
Carbohydrates%	0.52	
pH	5.38	5.6 -6.2
TVN(g/100g)	13.19	Not exceed 20
TBA of minced meat (mg MDA/kg sample)	0.201	Not exceed 0.9
TBA of added fat (mg MDA/kg sample)	0.169	

TVN=Total volatile nitrogen value. EOS: Egyptian Organization for Standardization
MDA = malonaldehyde

However, the moisture content (71.96 %) is higher than EOS. These results were in agreement with Ghoneim (2012), who showed that the frozen meat contain 72.66, 21.6, 3.28, 0.96% for moisture, crude protein, crude fat and ash, respectively. Total volatile nitrogen (TVN) was13.19 mg/100g,

malonaldehyde contents measured by the TBA procedure were 0.201 mg malonaldehyde/ kg sample for minced meat and pH value of frozen meat was 5.38, these results were in agreement with registered limits by EOS (2005a), which mentioned that TBA value of minced meat samples do not be more than 0.9 mg malonaldehyde/ kg sample, TVN value not exceed 20 and pH value ranged from 5.6 to 6.2.

Effect of adding of different levels of Rosemary, basil and mint on pH value of fresh sausage:

Results in Table (4) show the change in pH values of fresh sausages during refrigerated storage. During the first 3 days, the pH values were ranged from 5.16 to 5.72 and no significant differences between control and all treated samples with different levels of herbs were observed. Thereafter, the pH values significantly increased at the fifth day refrigerated storage. These results showed that pH values in all treated sausage samples were lower than control during storage period. These results are nearly similar to Liu *et al*. (2009), who found that the pH values of the sausage remained stable and were approximately 6.4 to 6.6 during the first 10 days of refrigerated storage. Biswas, *et al*. (2012) reported that there were no significant differences in pH values between control and treated samples at the beginning of storage. On the third day, the pH value of all samples were significantly increased. This could be attributed to the greater numbers of aerobic bacterial multiplication in the control than the treated samples. This is may be due to increase the value of total bacterial count and decomposition of protein during storage period.

Table (4): Effect of adding different levels of Rosemary, basil and mint on pH value for fresh beef sausage during refrigerated storage:

Sausage treatments		Cold storage (Time/day)			
Herbs & Spices	Control + ppm*	Zero	1	3	5
Control		5.25^{ab}±0.012	5.40^{a}±0.012	5.72^{a}±0.202	6.41^{b}±0.018
R1	100	5.27^{a}±0.014	5.31^{b}±0.015	5.58^{b}±0.015	6.12^{e}±0.012
R2	200	5.23^{abc}±0.035	5.29^{b}±0.009	5.57^{b}±0.018	6.19^{d}±0.006
B1	100	5.29^{a}±0.013	5.32^{b}±015	5.59^{b}±0.008	6.27^{c}±0.015
B2	200	5.22^{abc} ±0.039	5.30^{b} ±0.012	5.51^{c}±0.012	6.09^{e}±0.023
M1	100	5.18^{bc}±0.018	5.32^{b}±0.014	5.70^{a}±0.006	6.80^{a}±0.006
M2	200	5.16^{c}±0.018	5.24^{c}±0.014	5.61^{b}±0.012	6.41^{b}±0.012

R1= (Rosemary 100 ppm) R2= (Rosemary 200 ppm) B1= (Basil 100 ppm)
B2= (Basil 200 ppm) M1=(Mint 100ppm) M2=(Mint 200ppm)
Control + ppm*, means that Rosemary, Basil and Mint were added to the control according to its content of phenolic compounds as gallic acid.
Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

Total volatile nitrogen values (mg/100g) of fresh beef sausage:

Data illustrated in Table (5) showed that the change in total volatile nitrogen (TVN) values (mg/100g) of fresh beef sausage samples during refrigerated storage. The TVN was increased as time of storage increased. During the first 3 days, the TVN was ranged from 12.54 to 19.69 mg/ 100 g sample. These results were in accordance with EOS (2005a) which stated

that the TVN not exceed 20 mg/100g sample. But after 5 days the TVN values significantly increased to 24.32 in control and from 20.25 to 23.60 mg /100g in treated samples. These results were higher than EOS (2005a), for this reason, the storage period of sausage was stopped after 5 day. The more added rosemary, basil and mint to sausage samples, the lower observed TVN values. These lower TVN values were probably because these herbs contained some antimicrobial and antioxidant compounds. These results were in agreement with Liu, *et al* (2009), who reported that the TVN values and the microbial counts were increased significantly during storage. In addition, samples that had more level of rosemary or Chinese mahogany, had significantly lower TVN values.

Table (5): Total volatile nitrogen values (mg/100g) of fresh beef sausage treatments as affected by adding natural additives during refrigerated storage :

Sausage treatments		Cold storage (Time/day)			
Herbs & Spices	Control + ppm*	Zero	1	3	5
Control		13.23^{a}±0.018	16.52^{a}±0.023	19.69^{a}±0.018	24.32^{a}±0.018
R1	100	13.01^{c}±0.009	15.13^{d}±0.014	18.60^{d}±0.012	21.87^{c}±0.014
R2	200	12.74^{d}±0.012	14.36^{f}±0.012	16.30^{g}±0.012	20.89^{e}±0.012
B1	100	13.1^{b}±0.006	16.01^{b}±0.020	19.03^{b}±0.014	23.60^{b}±0.012
B2	200	13.01^{c}±0.012	15.37^{c}±0.014	18.68^{c}±0.009	21.32^{d}±0.012
M1	100	12.98^{c}±0.014	14.68^{e}±0.014	17.62^{e}±0.018	20.25^{e}±0.012
M2	200	12.54^{e}±0.023	14.29^{g}±0.014	17.03^{f}±0.014	20.73^{e}±0.014

R1= (Rosemary 100 ppm) R2= (Rosemary 200 ppm) B1= (Basil 100 ppm)
B2= (Basil 200 ppm) M1=(Mint 100ppm) M2=(Mint 200ppm)
Control + ppm*, means that Rosemary, Basil and Mint were added to the control according to its content of phenolic compounds as gallic acid.
Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

Thiobarbituric acid value (TBA, mg malonaldehyde/kg sample) of fresh beef sausage:

The results in Table (6) show the effect of adding rosemary, basil and mint on the TBA values during refrigerated storage of fresh sausage samples. The TBA values significantly increased during the first 3 days, indicating that lipid oxidation had occurred during this period. This increased of TBA values in fresh sausage samples during storage has also been reported by other researchers. In this study, TBA values increased up to a maximum point at the third day followed by gradually decrease and this tendency agreed with a study reported by Liu *et al*. (2009). who explained that during storage , malonaldehyde which is an intermediate by-product during lipid oxidation, is further oxidized to other organic acids and alcohols that can not react with TBA agent. This might be the reason why the TBA values increased then decreased. TBA values of the samples that treated by rosemary, basil and mint were lower than control. This is may be due to antioxidant compounds content in these herbs . These results were in agreement with those reported by Kanatt, *et al*. (2008), who reported that the mint extracts were found to have very good antioxidant activity, which were comparable to that of the

synthetic antioxidant, BHT. Many reports have indicated that rosemary extracts can retard lipid oxidation and prolong the shelf life of meat products (Georgantelis, *et al*. 2007). In this study, TBA values ranged from 0.234 to 0.431 mg malonaldehyde/ kg in control, while increasing of TBA values in all treated samples depend on levels of herbs. Furthermore, these results showed that the TBA values significantly decreased for all samples after 5 days of refrigerated storage and this nearly similar to Liu *et al*. (2009).

Table (6): Thiobarbituric acid value (TBA, mg malonaldehyde/kg sample) of fresh sausage samples as affected by adding natural additives during refrigerated storage:

Sausage treatments		Cold storage (Time/day)			
Herbs & Spices	Control + ppm*	Zero	1	3	5
Control		$0.234^{a}\pm0.0006$	$0.286^{a}\pm0.0006$	$0.431^{a}\pm0.0006$	$0.355^{a}\pm0.0009$
R1	100	$0.226^{b}\pm0.0006$	$0.269^{b}\pm0.0009$	$0.396^{b}\pm0.0009$	$0.265^{e}\pm0.0009$
R2	200	$0.224^{cd}\pm0.0006$	$0.245^{d}\pm0.0006$	$0.297^{g}\pm0.0012$	$0.164^{g}\pm0.0006$
B1	100	$0.232^{a}\pm0.0003$	$0.253^{c}\pm0.0006$	$0.376^{c}\pm0.0006$	$0.343^{b}\pm0.0012$
B2	200	$0.225^{bc}\pm0.0006$	$0.241^{e}\pm0.0009$	$0.304^{f}\pm0.0009$	$0.286^{c}\pm0.0006$
M1	100	$0.234^{a}\pm0.0006$	$0.246^{d}\pm0.0006$	$0.366^{d}\pm0.0012$	$0.275^{d}\pm0.0009$
M2	200	$0.223^{d}\pm0.0006$	$0.229^{f}\pm0.0006$	$0.345^{e}\pm0.0006$	$0.249^{f}\pm0.0009$

R1= (Rosemary 100 ppm) R2= (Rosemary 200 ppm) B1= (Basil 100 ppm)
B2= (Basil 200 ppm) M1=(Mint 100ppm) M2=(Mint 200ppm)
Control + ppm*, means that Rosemary, Basil and Mint were added to the control according to its content of phenolic compounds as gallic acid.
Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

Water holding capacity (WHC %) of fresh beef sausage:

Data in Table (7) show the effect of adding different levels of rosemary, basil and mint on water holding capacity (WHC) of fresh beef sausage. During storage period, the WHC was significantly increased. These results showed that the WHC in all treated sausage samples were higher than control. This is may be due to two reasons, firstly is the high content of crude fiber in these herbs which were 32%, 20.2% and 17.1% in rosemary, basil and mint, respectively. Secondly is the low temperature degree used during storage period. These results in agreement with Zhang *et al*. (1995), who reported that the salt in the meat products increased WHC, and an increase in temperature resulted in lower WHC at the same force. Joo *et.al*. (1995) found that an increase in WHC was observed after post-mortem is believed to be caused by a decrease in the total water content of the meat.

Table (7): Water holding capacity (WHC %) of fresh sausage as affected by adding natural additives during refrigerated storage :

Sausage treatments		Cold storage (Time/day)			
Herbs & Spices	Control + ppm*	Zero	1	3	5
Control		48.46^{g}±0.068	51.75^{g}±0.095	57.13^{g}±0.017	62.83^{g}±0.012
R1	100	57.22^{b}±0.015	61.19^{b}±0.015	62.47^{b}±0.018	65.62^{b}±0.012
R2	200	60.57^{a}±0.018	61.98^{a}±0.012	62.53^{a}±0.012	66.66^{a}±0.006
B1	100	53.25^{d}±0.009	57.57^{d}±0.015	60.50^{d}±0.012	64.65^{d}±0.012
B2	200	55.30^{c}±0.009	58.95^{c}±0.006	61.47^{c}±0.018	65.12^{c}±0.015
M1	100	48.59^{f}±0.009	54.91^{f}±0.012	58.55^{f}±0.017	63.83^{f}±0.015
M2	200	50.69^{e}±0.009	55.97^{e}±0.007	58.90^{e}±0.012	64.43^{e}±0.015

R1= (Rosemary 100 ppm) R2= (Rosemary 200 ppm) B1= (Basil 100 ppm)
B2= (Basil 200 ppm) M1=(Mint 100ppm) M2=(Mint 200ppm)
Control + ppm*, means that Rosemary, Basil and Mint were added to the control according to its content of phenolic compounds as gallic acid.
Means of treatments having the same letter(s) within a column are not significantly different (P> 0.05).

Effect of adding different levels of Rosemary, basil and mint on microbiological properties of fresh beef sausage:

Data in Table (8) show the effect of adding different levels of rosemary, basil and mint on microbiological properties of fresh beef sausage.

Table (8): Effect of adding different levels of Rosemary, basil and mint on microbiological properties of fresh beef sausage:

Microbiological properties	Storage periods (days)	Treatments						
		control	R1 100ppm*	R2 200ppm*	B1 100ppm*	B2 200ppm*	M1 100ppm*	M2 200ppm*
		CFU/g						
TC×10^{4}	Zero	50	15	12	17	14	13	18
	1	54	25	19	31	27	25	30
	3	61	31	27	40	30	37	36
	5	72	34	31	45	39	41	45
Pr.b×10^{3}	Zero	31	25	14	21	16	18	24
	1	36	32	19	30	28	26	31
	3	54	44	32	37	33	29	33
	5	61	53	43	40	38	33	40
Ly.b×10^{3}	Zero	32	30	18	23	19	16	22
	1	36	35	21	29	24	21	25
	3	41	38	33	35	31	37	32
	5	59	46	39	43	40	40	46
M&Y×10^{3}	Zero	1	2	N.D	N.D	N.D	N.D	N.D
	1	3	2	1	3	1	3	2
	3	7	7	4	7	8	3	6
	5	14	10	6	11	10	5	8

R1= (Rosemary 100 ppm) R2= (Rosemary 200 ppm) TC=Total counts bacteria
B1= (Basil 100 ppm) B2= (Basil 200 ppm) Pr.b=proteolytic bacteria
M1=(Mint 100ppm) M2=(Mint 200ppm) Ly.b=lipolytic bacteria
CFU= Colony forming unit N.D= Not detected M&Y=mould and yeast
Control + ppm*, means that Rosemary, Basil and Mint were added to the control according to its content of phenolic compounds as gallic acid.

The obtained results indicated that control sausage samples had the highest total count, proteolytic and lipolytic bacteria compared with other treated samples. The total count bacteria were ranged from 12×10^4 to 72×10^4 CFU/g. These results were in agreement with EOS (2005b), which reported that the total count bacteria should not exceed 10^5 CFU/g. However, using herbs were decreased the total count bacteria and this was probably because these herbs contained some antimicrobial compounds. These results were in agreement with Liu *et al*. (2009), who found less microbial growth in sausage, due to the addition of rosemary or Chinese mahogany to the formula, thus led to less protein decomposition and lower TVN values. Also, results were in agreement with Georgantelis, *et al*. (2007), who reported that the fresh pork sausages with addition of rosemary extract at level of 260 mg/kg had significantly lower *Enterobacteriaceae, pseudomonace* and yeast /mould counts than the controls.

CONCLUSION

Depend on the obtained results in this study, it could be recommended the use of the following herbs (rosemary, basil and mint) as antioxidant, antimicrobial and flavoring agents to prolongate the shelf-life of meat products by using natural preservatives.

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تـأثير إضـافة مسـتويات مختلفـة مـن الروزمـارى ، الريحـان والنعنـاع علـى جـودة السجق البقري الطازج أثناء التخزين المبرد

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اجريت هذه الدراسة بغرض تقييم تأثير استخدام الروزمارى ، الريحان والنعناع بتركيزات ١٠٠, ٢٠٠ و٣٠٠ جزء في المليون (مركبات فينولية مقدرة كحمض جاليك) على جودة السجق البقري الطازج المخزن على ٤±١°م لمدة ٥ أيام. تم التعرف على المركبات الفينولية و تقديرها في الروزمارى ، الريحان والنعناع باستخدام جهاز الفصل الكروماتوجرافي عالي الكفاءة وكانت نسب المركبات الفينولية الكلية ١٠٣٠٤، ١٥٥٩ و١٩٣٨ جزء في المليون علي التوالي . وأكدت نتائج التقيم الحسي للخلطات انه لا توجد فروق معنوية بين العينة الضابطة والعينات المعاملة بالروزمارى ، الريحان والنعناع بنسب ١٠٠ و٢٠٠ جزء في المليون لذلك اجريت على هذه العينات الاختبارات الفزيقية ، الكيميائية والميكروبية مثل قوة مسك المنتج للماء ، درجة الأس الأيدروجيني ، النيتروجين المتطاير الكلي ، قيمة حمض الثيوباربيتيوريك بالاضافة إلى التقيم الميكروبى فى المعاملات المختلفة بعد صفر ، ١ ، ٣ و ٥ أيام من التخزين المبرد كمؤشر للفساد. النتائج المتحصل عليها اكدت أن اضافة الروزمارى ، الريحان والنعناع إلى خلطات السجق قد خفضت قيم الـ pH, TVN, TBA والعد الكلى للميكروبات بالمقارنة بالعينة الضابطة. بينما حدث زيادة في قوة مسك المنتج للماء. واعتماداً على النتائج المتحصل عليها من هذا البحث ننصح باستخدام الروزمارى ، الريحان والنعناع بنسب ١٠٠و٢٠٠ جزء في المليون فى صناعة السجق الطازج كعوامل مضادة للأكسدة ، مضادة للميكروبات ، مكسبه للطعم و الرائحة وإطالة فترة صلاحية المنتج.

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