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EFFECT OF MINT AND ROSEMARY EXTRACTS ON CHEMICAL, PHYSICAL, MICROBIOLOGICAL AND SENSORIAL PROPERTIES OF PREPARED BEEF BURGER

Abd El-gawwad, A.I.[1]; Faten Y. Ibrahim[1]; E. A. Aboulnaga[1]; Ban J. Majeed[1] and M.R.G. Youssif [2]

1Food. Industries Dept, Faculty of Agriculture Mansoura University

2Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

The objective of this work was to study the effects of mint and rosemary extracts on the microbiological , chemical properties sensory quality of prepared beef burger during storge. Two types of mint and rosemaryextracts at 3%(w/v) (water and ethanolic) were used in prepared beef burger samples.The products were evaluated chemically, physically and microbiol during storage at 5 ±1C°. The results indicated that rosemary extract showed moderate antioxidant activities being 52.38 mg/ml at 0.1% (1mg/ml) concentration, and high antioxidant activity of 81.52 mg/ml at 1.0% (10 mg/ml). While, mintextract had the lower antioxidant activitythanthose of others being 48.93 mg/ml and 66.72 mg/ml for 0.1% and 1.0% concentration, respectively. Also, both of mint and rosemary extracts markedly inhibited growth of most microorganisms tested.However, the effects differed with regard to the type of mint and rosemary extracts (water and ethanolic), concentration used and the type ofmicroorganisms. Storage burger at 5 ±1C° both cooking yield, water holding capacity (WHC), plasticity,while acid value, peroxide value, thiobarbituric acid (TBA) and total volatile nitrogen (TVN). At the same time, cooking loss was increased by increasing the storage period for all prepared beef burger samples but thisloss wasless in beef burger samples treated with mint and rosemary extracts. The results of organoleptic evaluation of beef burger samples showed that, there were no significant differences ($p>0.05$) between the control samples prepared without preservatives and with 100 ppm sodium nitrite) and all other treated beef burger samples.So,it could be concluded that addition of mint or rosemary extracts retarded microorganisms activity in beef burger samples with out significant effect on organoleptic properties.

Keywords: Mint, Rosemary, Beef burger, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Meat is considered as a biologically delicate product prone for rapid decomposition, microbiological activities, physiological and chemical changes Chatli and Joseph, (2014). Although several synthetic food additives have been widely used in the meat industry to extend food shelf life, inhibit lipid oxidation and delay or inhibit the growth of pathogenic microorganisms, the trend is to decrease their use because of the growing concern among consumers about such chemical additives. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years indicating that the application of natural food additives possessing both antioxidant and antimicrobial activities may be useful for maintaining meat

quality, extending shelf life and preventing economic loss Yin and Cheng, (2003) and Mielnik *et al.,*(2008).

An antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals which can perpetuate the reaction Fennema, (1996). Some vitamins (ascorbic acid, vitamin E) exhibit antioxidative activity. Many herbs and spices (rosemary, oregano, grapeseed, sage, thyme) contain antioxidant components Ahn *et al.,*(2007) and Rojas and Brewer, (2007).

The antioxidant potential of Rosemary (*Rosmarinus officinalis* L.) was investigated by various workers in turkey rolls Yu *et al.,(*2002) beef loins (Lawrence *et al.,* 2004). It contains carnosol, carnosic acid, rosmanol, isorosmanol, rosmariquinone, rosmaridiphenol and rosmary-diphenol. Rosemary extracts can chelate metal ions, Fe^{+2}, resulting in a reduced rate of formation of activated oxygen Formanek *et al., (*2003). Rosemary extract has also been used in the combination of various other antioxidants McBride *et al., (*2007) to have synergistic effect. Moreover, several studies reported that, some compounds such as phenolic diterpenoids present in rosemary extracts have antibacterial activity Cuvelier *et al., (*1996).

Rosemary extracts display a relatively poor inhibiting effect on Gram-negative bacteria but, at a level of 0.06-1%, they inhibit the growth of Gram-positive pathogens, such as: *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*. Moulds of the *Penicillium* and *Botrytis* genus are developing much slower in the environment containing a rosemary extract, while carnosol and carnosic acid (components of rosemary extracts) inhibit the vital activities of drug-resistant bacteria of the *Staphylococcus aureus* strain. Especially susceptible to the activity of rosemary extracts are also bacteria of the *Lactobacillus* and *Brocho thrix*genus Del Campo *et al.*(2000); Moyosoluwa *et al.*(2004) and Fernandez-Lopez *et al.*(2005).

Mint peppery (*Menthapiperita L*.) is one of the most widespread types of herbs. It is widely used in medicine, cookery, and household, as well as pharmaceutical and cosmetic industry. Mint is applied as a medication in the form of infusions and teas for treatment of gastroenteric diseases, as demulcent at palpitation, depression and insomnia, in sedatives, as anesthesia at burns and insect stings, as well as analgesic and antistress medicine. Antioxidant properties of mint allow to prevent cataract and other illnesses connected with ageing of an organism. Mint peppery basic component (2-3 %) is menthol defining its taste and anesthetizing properties. Other substances contained are ethers, phellandrene, pinene, jasmole, piperitone, menthofuran, *etc*. There are also tannic and resinous substances, carotin (0.01 %), ascorbic acid (0.01 %), routines (0.015 %), and other polyphenolic compounds Natalia *et al.,* (2011). Peppermint extracts are bacteriostatic against S*treptococcus pyrogens*, S*treptococcus aureus*, *Streptococcus pyrogens*, *Serratia marcescens*, *E.coli* and *Mycobacterium avium* MimicaDukic*et al.,*(2003).

The aim of this study is to evaluate the effects of mint and rosemary extracts as antioxidant and antimicrobial agents in beef burger during storageat 5 ±1C° for 6 days.

MATERIALS AND METHODS

MATERIALS:

Leaves of Mint (*Mentha longifolia* L.) and Rosemary (*Rosmarinus officinalis* L.) plants were obtained from Medicinal and Aromatic Plant Research Department, Agriculture Research Center, Giza, Egypt.

Frozen Meat Beef, All spices, Corn starch, Salt (Sodium chloride)Were collected from local market ,Cairo ,Egypt.

Cultivation media:

Nutrient agar, broth media ,Potato dextrose medium, Violet Red Bile Agar medium, Bismuth sulphite agar medium and Barid-Parker agermedium. All cultivation used in this study were purchased from Alnasr Company for Chemical and Medical Preparation,Cairo,Egypt.

Microbial strains:

Ten pure cultures of *Bacillus subtilis* ATCC 14085, *Bacillus cereus* DSMZ 345, *Staphylococcus aureus* ATCC 6528, *Salmonella typhimurium* ATCC 14028, *Escherichia coli O157:H7* ATCC 51659, *Candida lipolytica* ATCC 10231, *Geotricum candidum* NRRL 552, *Aspergillus niger* ATCC 102, *Aspergillus flavus* ATCC 247 and *Fusarium moniliform* ATCC 206 were obtained from Microbiological Resource Center (Cairo-MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

All chemicals used in this study were of analytical grade were purchased from Alnasr Company for Chemical and Medical Preparation,Cairo , Egypt.

Methods:

Paper mint and rosemary leaves were air dried at 60 ˚C for 24 hours , grow, sieved to prepare dried powder.

Ethanolic extracts:

The air dried powders both of Mint and Rosemary leaveswere suspended in 80% ethanol (1:4 w/v) for 24 hr and filtered. The residue was re-extracted (about 3-4 times) with 80% ethanol until it was exhausted. Ethanol was evapuated using vacuum rotary evaporator at 45˚C (Fisher-Bioblock 4000, France). The ethanol free extract was then dried by using a lyophilizer, (Snijders, type 2040, Holland), and kept in the dark at 4˚C until used.

Water extracts:

The air dried ground both of Mint and Rosemary leaves were suspended in cold distilled water (1:4 w/v) at 5˚C for 4hr and homogenized for 1 min at the top speed of a waring blender. The mixture was filtered through cheesecloth and centrifuged for 15 min at 5000 rpm. The decanted supernatant was filtered through Whatman No. 1 filter paper and dried by lyophilizer and kept in dark at 4˚C until used.

Properation of beef burger:

Beef burger samples were prepared according to the method described by Ziprin *et al.,* (1981) with some modifications. Meat and fat tissues were cut into pieces of about egg-size and frozen at -18˚C for 24 hr. The frozen meat and fat were ground. Beef burger was prepared by blending (1.2%) of spices mixture in item with the following ingredients:

Ingredients%	
Lean meat	70.0
Fat tissues	12.0
Sodium chloride	2.3
Water (as ice)	10.0
Starch	2.0
Spices mixture	1.2
Garlic	0.5
Onion	2.0

The produced mixture was shaped to circular patties of 10 cm diameter, 0.5 cm thickness and about 50 g weight. Each piece was separated from the other using polyethylene layer before packaging in polyethylene bags and stored in home refrigerator under cooling at 5 ±1C˚ for 6 days until analysis. Samples in three replicates from each batch were subjected to chemical, physical and microbiological analysis initially and periodically after 3 and 6 days of cooling storagea.

The effect of addition of mint and rosemary extracts compared with negative control (without preservatives) and with positive control (with 100 ppm sodium nitrite as preservatives) samples on beef burger qualitywere studied.

Gross chemical analyses:

Moisture, crude protein, fat, ash contents were determined according to Total carbohydrates were calculated by differences Acid and peroxide values were estimated using the method described by A.O.A.C., (2000). Total volatile nitrogen and thiobarbituric acid were determined using the methods described by Harold *et al.,*(1987).

Physical propertion:

Cooking yield, cooking loss and shrinkage value of cooked beef burger were determined according to George and Berry, (2000). Water holding capacity and plasticity were measured according to the method described by Voloviskaya and Merkoolova, (1958).

Microbiological assay:

Total viable bacterial count, psychrophiliic bacterial count, coliforms, *Staphylococcus sp.* and *Salmonella sp.* were determined in beef burger samples.

Organoleptic evaluation of cooked beef burger:

Samples of beef burger were subjected to organoleptic evaluation by ten panelists according to Klein and Bardy , (1984).Scores Very good (10-9), Good (9-8), Fair (8-6), Poor (6-5).

Statistical analysis:

Data were analyzed by Analysis of Variance using General Liner Model (GLM) procedure according to the procedure reported by Snedecor and Cochran (1997). Means were separated using Duncan's test at a degree of significance (P≤ 0.05). Statistical analyses were made using the producer of the SAS software system program SAS, (1997).

RESULTS AND DISCUSSION

Chemical composition of Mint and Rosemaryleaves:

Moisture, crude protein, fat, ash and total carbohydrates were determined in mint and rosemary leaves and the results are reported in Table (1). From the obtained results, it could be noticed mint recorded the higher in content of ash 3.48% and total carbohydrates 88.39% while rosemary leaves. while rosemary leaves had higher content of moisture 5.23% crude protein 3.34% and fat6.17% than those of mint.

Also, the result presented in the same table show the composition of secondary metabolites phytochemicals namely, phenolic compounds, saponine, flavonoids and alkaloids which expected to have antibacterial activity were determined in mint and rosemary leaves. Data presented in Table (1) showed that mintleaves recorded the highest saponine content being 6.08 mg/g dry weight in comparison with rosemaryleaves which being 4.96 mg/g dry weight.

Table (1): Chemical composition of Mint and Rosemary powders:

Samples / Constituents	Mint	Rosemary
Moisture%	4.18 ± 0.03	5.23 ± 0.23
Crude protein%	1.75 ± 0.01	3.34 ± 0.24
Fat%	2.20 ± 0.02	6.17 ± 0.01
Ash%	3.48 ± 0.01	1.46 ± 0.04
Total carbohydrates%	88.39 ± 0.15	83.80 ± 0.26
Saponines mg/g	6.08 ± 0.32	4.96 ± 0.05
Flavonoids mg/g	17.90 ± 0.42	26.17 ± 0.38
Alkaloids mg/g	0.84 ± 0.19	0.73 ± 0.01
Phenolic acid mg/g	12.14 ± 0.44	55.53 ± 0.23

On the other hand, the highest value of flavonoids was recorded in rosemary followed by mint being (26.17 and 17.90 mg/g , respectively). Mint leaves had the highest alkaloids content followed by rosemary leaves(0.84 and 0.73 mg/g, respectively). Rosemary contained the highest phenolic acid content 55.53 mg/g dry weight followed by mint being 12.14 mg/g dry weight. the presence of those bioactive compendia in mint and rosemary powders stimulates of both herbs antibacterial agents. The action for phenolic and flavonoids compounds on microbes is due to their binding with cell wall and inactivate enzymes. While, the alkaloids were intereolate into the cell wall and bind with DNA Frankel *et al.,*(1996).

Gross 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 (2). Chemical analysis cleared thatMoisture67.25%, crude fat 7.20%, ash 1.40%and carbohydrates 2.38% (WB). These results were within the permissible limits as recommended by Egyptian Organization for Standardization EOS (2005).

Table (2): Chemical composition of frozen minced beef meat:

Samples / Constituents %	Frozen Beef minced meat (Wet basis)	*EOS (2005)*
Moisture	67.25%	70% or less
Protein	21.77	Not less than 18%
Crude fat	7.20	20% or less
Ash	1.40	-
Carbohydrates	2.38	

***EOS: Egyptian Organization for Standardization.**

However, the crude Protein content 21.77% is higher than that EOS. These results are in agreement those of Ghoneim (2012), who showed that the frozen meat contain 21.60% crude protein.

Antioxdant activity of mint and rosemary extracts:

Results, presented in Table (3) show the antioxidant activity of mint and rosemary compared with than of BHT, ascorbic acid and α-tocopherol using the conjugated diene method. From the obtained data it could be observed that, rosemary extract showed moderate antioxidant activities being 52.38 mg/ml at 0.1% concentration, and high antioxidant activity of 81.52 mg/ml at 1.0% level.While, mint extract had lower antioxidant activity than other sources being 48.93 mg/ml and 66.72 mg/ml for 0.1% and 1.0% concentration, respectively.

These results are in agreement with those of Cai *et al.,* (2005), who mentioned that ,the antioxidant effect of herbs and spices is due primarily to phenolic -OH groups .Moreover, Dorman *et al.* (2003) stuted that, the antioxidant properties of rosemary (*Rosmarinus officinalis* L.) was not completely explained by the total phenolic content of the extracts, but appeared to be strongly dependent on rosmarinic acid. Phenolic acids are effective iron chelators Andjelkovic *et al.,* (2006).

Table (3): Antioxdant activity of mint, rosemary extract compared with BHT, ascorbic acid and α-tocopherol.

Concentrations (%)	Antioxdant Activity (%)				
	Mint extract	Rosemary extract	BHT	Ascorbic acid	α-tocopherol
0.1	48.93±0.13	52.38±0.12	65.04±0.04	64.91±0.02	65.17±0.02
0.2	55.79±0.05	63.25±0.04	81.09±0.02	80.19±0.03	80.95±0.06
0.4	58.98±0.08	71.86±0.07	88.05±0.08	85.11±0.07	86.83±0.03
0.8	61.76±0.07	77.19±0.11	93.47±0.06	89.86±0.04	91.69±0.04
1.0	66.72±0.02	81.52±0.15	95.58±0.04	92.10±0.05	94.64±0.01

Antimicrobial activity of water and ethanolic extracts of mint and rosemary:

Antimicrobial activity of mint and rosemary extracts against several bacterial species has been recognized and is considered as one of the most important properties linked directly to their possible biological applications. In vitro preliminary screening of the antimicrobial activities of mint and rosemary extracts with different types at different concentrations against five strains of

bacteria, two strains of yeasts and three strains of fungi were studied, the results are given in Tables (4 and 5).

Table (4): Antimicrobial activity of different concentrations of Mint and Rosemary water and ethanolic extracts.

Bacterial strains	*Diameter of Inhibition Zones (mm.)									MIC% (V/V)
	Conc.%	0.25	0.5	0.75	1.0	1.5	2.0	2.5	3.0	
Water extract of Mint										
B. subtilis ATCC 14085		0	0	0	0	12	15	16	17	2.5
B. cereus DSMZ 345		0	0	0	0	11	12	13	15	3.0
*E. coli*O157:H7 ATCC 51659		0	0	0	0	0	0	9	10	-
S. typhimurium ATCC 14028		0	0	0	0	0	0	0	9	-
St. aureus ATCC 6528		0	0	0	0	0	0	10	12	-
C. albicans ATCC 10231		0	0	0	0	0	11	13	14	3.0
G. candidum NRRL Y-552		0	0	0	0	0	12	13	13	-
Ethanolic extract of Mint										
B. subtilis ATCC 14085		0	0	16	24	28	32	32	33	1.5
B. cereus DSMZ 345		0	0	15	18	27	29	29	31	2.0
*E. coli*O157:H7 ATCC 51659		0	0	0	15	17	20	22	22	2.5
S. typhimurium ATCC 14028		0	0	0	0	12	16	16	16	2.0
St. aureus ATCC 6528		0	0	12	14	17	21	21	21	2.5
C. albicans ATCC 10231		0	0	14	15	17	20	22	22	2.5
G. candidum NRRL Y-552		0	14	16	18	20	21	21	21	2.0
Water extract of Rosemary										
B. subtilis ATCC 14085		0	10	12	15	18	21	22	25	1.5
B. cereus DSMZ 345		0	11	12	14	15	18	20	23	2.0
*E. coli*O157:H7 ATCC 51659		0	0	0	0	12	15	17	18	2.5
S. typhimurium ATCC 14028		0	0	0	10	13	15	18	20	2.5
St. aureus ATCC 6528		0	12	15	17	20	21	21	25	3.0
C. albicans ATCC 10231		0	10	14	17	20	22	23	27	3.0
G. candidum NRRL Y-552		0	0	10	13	16	18	21	22	2.0
Ethanolic extract of Rosemary										
B. subtilis ATCC 14085		12	12	16	21	27	27	27	31	1.5
B. cereus DSMZ 345		14	14	22	28	30	30	31	30	1.5
*E. coli*O157:H7 ATCC 51659		10	10	15	18	20	22	22	24	2.0
S. typhimurium ATCC 14028		0	0	14	17	23	25	25	25	2.0
St. aureus ATCC 6528		11	11	18	24	26	31	31	31	2.5
C. albicans ATCC 10231		14	14	18	25	30	30	30	32	2.5
G. candidum NRRL Y-552		10	10	17	22	26	29	29	30	2.0

*** Including disc diameter of (8 mm).**
MIC: Minimal Inhibitory concentrations

From the obtained results, it could be observed that, both of mint and rosemary extracts markedly inhibited growth of most bacteria tested; however, the effects differed with regard to the type of mint and rosemary extracts (water and ethanolic), concentration used and the type of bacteria. Ethanolic mint and rosemary extracts generally showed strong antibacterial activity for both Gram-positive and Gram-negative bacteria in the ratio ranged between (1.5 -2.5%).These results are in agreement with those obtained by

MimicaDukic *et al.,*(2003),who mentioned that, mint extracts are bacteriostatic against S*treptococcus pyrogens*, S*treptococcus aureus*, *Serratia marcescens*, *E.coli* and *Mycobacterium avium*. Also, Moreno *et al.* (2006) reported that, rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity could be attributed to carnosic acid and carnosol.

The minimum inhibitory concentrations (MICs) of mint and rosemary extracts (watery and ethanolic) at concentration of 0.25 – 3.0% are shown in Table (4), and the different MICs values ranged from 1.5 to 2.5%. *B. subtilis* ATCC 14085 showed MICs at 1.5% of mint ethanolic extract, where the value of MICs was at 2.0% for *B. cereus* DSMZ 345 and *S. typhimurium* ATCC 14028.

Table (5): Antifungal activity of different concentrations of Mint and Rosemary water and ethanolic extracts

Concentration % / Fungi strains		2.0	2.5	3.0	3.5	4.0	MIC % (V/V)
		Antifungal index(%)					
Water extract of Mint							
A.niger ATCC 102		0	0	9	10	12	-
A. flavus ATCC 247		0	0	11	12	14	4.0
F.moniliform ATCC 206		0	0	0	10	11	-
Ethanolic extract of Mint							
A.niger ATCC 102		9	11	12	15	17	3.5
A. flavus ATCC 247		11	14	15	17	18	3.0
F.moniliform ATCC 206		8	12	15	16	16	3.0
Water extract of Rosemary							
A.niger ATCC 102		12	20	24	28	30	4.0
A. flavus ATCC 247		18	25	30	38	40	3.5
F.moniliform ATCC 206		14	21	25	30	35	3.5
Ethanolic extract of Rosemary							
A.niger ATCC 102		18	27	31	35	35	3.5
A. flavus ATCC 247		26	33	38	45	45	3.5
F.moniliform ATCC 206		25	28	44	44	48	3.0

*** Including disc diameter of (8 mm).**
MIC: Minimal inhibitory concentrations

Concerining to the minimum inhibitory concentrations (MICs) of rosemary extracts (watery and ethanolic) at concentration of 0.25 – 3.0% which shown in Tables (4 and 5), and the different MICs values ranged from 1.5 to 2.5%. *B. subtilis*and *B. cereus* showed MICs at 1.5% of rosemary ethanolic extract, where the value of MICs was at 2.0% for *E. coli* and *S. typhimurium* while, it was 2.5% for *St. aureus*.

For the effect of mint and rosemary extracts on yeast. Data presented in Table(5), showed that, less activity of water mint extract was observed against *G. candidum* and *C. albicans*, where the diameter in inhibition zones were 13 and 14 mm, respectively. While, ethanolic mint extract was higher activity against the above mentioned strains where the diameter of inhibition zones were 21 and 22 mm, respectively. In addition, The minimum inhibitory

concentration (MICs) of mint extracts were examined at concentrations of 2.0 – 2.5% for *G. candidum NRRL Y-552* and *C. albicans ATCC 10231*, respectively.

At the same time, results in the same Table, showed that, less activity of rosemary watery extract was observed against *G. candidum* and *C. albicans*, where the diameter in inhibition zones were 22 and 27 mm, respectively. While, ethanolic rosemary extract was higher activity against the above mentioned strains where the diameter of inhibition zones were 30 and 32 mm, respectively.

The minimum inhibitory concentration (MICs) of rosemary extracts were examined at concentrations of 2.0 – 2.5% for *G. candidum* and *C. albicans*, respectively.

As seen in Table (5), it could be noticed that, the ethanolic mint and rosemary extracts at all evaluated concentrations inhibited the mycelia growth on three types of fungi. The antifungal index of mint and rosemary extracts showed differences in all concentrations compared to control.

Data presented in Table (5) showed the antifungal activity of the water mint extract against *A. niger*, *A. flavus also* and *F. moniliform*. It could be observed that, *A. flavus* showed the higher antifungal index (%) followed by *A. niger* and *F. moniliform* being 14, 12 and 11, respectively at concentration of 4.0%. The same trend was observed with the ethanolic mint extract. It could be observed that, *A. flavus* showed the higher antifungal index (%) followed by *A. niger* and *F. moniliform* being 18, 17 and 16, respectively at concentration of 4.0%. The results are in agreement with these of Hulin *et al.,* (1998).

For rosemary, data in the same table show that, the antifungal activity of the water rosemary extract against *A. niger*, *A. flavus* and *F. moniliform*. It could be observed that, *A. flavus* showed the higher antifungal index (%) followed by *F. moniliform*and *A. niger* being 40, 35 and 30, respectively at concentration of 4.0%. The same trend was observed with the ethanolic rosemary extract. It could be observed that, *F. moniliform*showed the higher antifungal index (%) followed by *A. flavus*and *A. niger* being 48, 45 and 35, respectively at concentration of 4.0%. These results are in agreement with those of Del Campo *et al.*, (2000) and Bozin *et al*., (2007).

Organolyptic evaluation of cooked beef burger samples:

Sensory evaluation is an important indicator of potential consumer preferences. In spite of its shortcomings, it will remain the most serious quality assessment technique for meat and meat products. The studied cooked beef burger samples were conducted to sensory evaluation for scores on appearance, color, juiciness, tenderness, flavor, taste and overall acceptability.

The scores analyses were presented as multidimensional model showing differences in quality attributes between samples. Sensory characters were evaluated by 10 panelists; the mean scores of each sensory character were subjected to a statistical analysis to detect the significant differences among means at level of 0.05 as shown in Table (6).

The results of appearance, juiciness, tenderness of cooked beef burger samples in this Table showed that, there were no significant differences ($p>0.05$) between the control samples (T1, prepared without preservatives

and T2, prepared with 100 ppm sodium nitrite as preservatives) and all other cooked prepared beef burger samples.

Table (6): Organolyptic evaluation of cooked beef burger samples:

Samples / Parameters	T1	T2	T3	T4	T5	T6
Appearance 10	9.50±0.42^{a}	9.50±0.67^{a}	9.10±0.66^{ab}	9.00±0.32^{ab}	9.40±0.70^{a}	9.40±0.52^{a}
Juiciness 10	9.60±0.84^{a}	9.60±0.70^{a}	9.50±0.42^{a}	9.30±0.48^{ab}	9.60±0.66^{a}	9.50±0.54^{a}
Tenderness 10	9.50±0.74^{a}	9.50±0.36^{a}	9.50±0.56^{a}	9.40±0.60^{a}	9.50±0.28^{a}	9.50±0.33^{a}
Color 10	9.50±0.52^{a}	9.50±0.63^{a}	9.50±0.35^{a}	9.50±0.52^{a}	9.50±0.67^{a}	9.50±0.42^{a}
Flavor 10	9.60±0.67^{a}	9.60±0.45^{a}	9.50±0.26^{a}	9.40±0.67^{a}	9.50±0.23^{a}	9.50±0.38^{a}
Taste 10	9.60±0.85^{a}	9.60±0.82^{a}	9.50±0.17^{a}	9.30±0.22^{ab}	9.60±0.45^{a}	9.50±0.52^{a}
Overall acceptability 10	9.60±0.20^{a}	9.60±0.42^{a}	9.50±0.32^{a}	9.40±0.60^{ab}	9.50±0.45^{a}	9.50±0.22^{a}

*** Means ± SD followed by different letters in the same raw are significantly by Duncan's multiple test (p≤0.05).**
T1: control beef burger sample without preservatives.
T2: control beef burger sample with 100 ppm sodium nitrite.
T3: with mint watery extract 3%.
T4: with mint ethanolic extract 3%.
T5: with rosemary watery extract3%.
T6: with rosemary ethanolic extract3%.

Color is one of the most important aspects of beef burger because color is one criterion a consumer used to select the burgers from the grocer's shelf. The color of burger samples primary provided by pigments. The results of color of beef burger samples in the same table showed that, there were no significant differences (p>0.05) between the control samples (T1 and T2) and the other samples. In addition, the obtained results indicated that, there were no significant differences (p>0.05) between control beef burger samples (T1 and T2) and samples T3, T4, T5 and T6 for flavor, taste and the overall acceptability So,finally if could be cachded that addition of mint or rosemary extract either water or ethanolic extract to burger samples had no significal effect on different organoleptic characterrstics of burger. These results are in agreement with those of Chen and Ockerman (1998) and George *et al.,* (2000).

Physical characteristics of beef burger samples:

Cooking yield and cooking loss of prepared beef burger as affected by storage under cooling at 5±1C˚ for 6 days are listed in Table (7). Control samples T1 (without preservatives) and T2 (with 100 ppm sodium nitrite); were showed the lowest initial cooking yield and achieved a level of 82.15 and 81.96%, respectively. While, cooked beef burger samples T4 and T6 showed higher initial cooking yield (83.38 and 84.25%, respectively). Also, all samples of cold beef burger were showed a remarkable decrease in cooking yield during storage at (5±1C˚ for 6 days).

The cooking loss was studied because of the relation between this factor and changes occurred in protins consequently meat tenderness, and the effect of cooling storage period at 5±1C˚ for 6 days on these parameters. Results of the cooking loss of beef burger show that, the cooking loss ofsamples were

increased as cold storage period progressed. Such increaseat zero time was 17.15 and 17.13% for control samples T1 and T2, respectively and reachedafter cold storage at 5±1C° for 6 days to 18.78 and 18.62% for control samples T1 and T2, respectively. Since, the beef burger sample T5 and T6 are showed the lowest initial cooking loss being 16.88 and15.70%, respectively.

All beef burger samples were showed a slight increase in cooking loss during during cold storage at 5±1C° for 6 days. These results are in agreement with those obtained by Gibriel *et al.,* (2007) they reported that, the cooking loss progressively increased as the period of storage increased.

Table (7): Physical characteristics of beef burger samples:

ColdStorge period (days)	Treatments*					
	T1	T2	T3	T4	T5	T6
% Cooking yield						
0	82.15±1.85	81.96±1.01	82.40±0.73	83.38±0.90	82.18±0.55	84.25±0.45
3	84.09±2.31	83.78±1.54	84.15±1.43	84.52±0.68	84.10±1.38	86.07±1.02
6	81.03±1.88	79.97±1.96	81.43±2.40	81.43±1.06	81.23±2.42	81.91±1.23
% Cooking loss						
0	17.15±1.85	17.13±1.01	17.04±0.73	17.00±0.90	16.88±0.55	15.70±0.45
3	16.47±2.31	16.38±1.54	16.33±1.43	15.52±0.68	14.34±1.38	13.54±1.02
6	18.78±1.88	18.62±1.96	18.57±2.40	18.10±1.06	17.85±2.42	16.28±1.23
% Shrinkage						
0	17.25±1.26	17.22±0.76	17.20±1.32	16.86±1.22	17.12±2.05	15.02±1.26
3	18.85±2.02	18.72±1.76	18.68±1.12	18.25±0.98	18.59±1.20	16.83±3.06
6	23.82±1.61	23.80±2.25	23.72±2.84	23.58±1.06	23.33±2.02	22.95±1.89
pH values						
0	5.85±0.14	5.90±0.15	5.82±0.23	5.46±0.03	5.76±0.05	5.42±0.04
3	5.67±0.02	5.86±0.22	5.60±0.09	5.39±0.08	5.45±0.16	5.28±0.11
6	6.30±0.07	6.38±0.19	6.28±0.40	6.10±0.06	6.25±0.06	6.15±0.20
Water holding capacity (WHC)						
0	7.45±0.22	7.43±0.20	7.49±0.14	7.35±0.10	7.44±0.15	7.15±0.14
3	7.92±0.12	8.02±0.29	7.88±0.26	7.69±0.33	7.75±0.02	7.63±0.02
6	8.60±0.61	8.79±0.37	8.39±0.12	8.26±0.06	8.46±0.24	8.02±0.23
Plasticity						
0	5.75±0.12	5.80±0.01	5.74±0.17	5.51±0.20	5.68±0.25	5.48±0.15
3	5.02±0.25	5.06±0.16	5.00±0.43	5.30±0.16	4.92±0.38	5.13±0.02
6	4.24±0.41	4.32±0.26	4.25±0.24	4.80±0.06	4.27±0.42	4.65±0.23

*** Means of triplicate ± SD.**
T1-T2 see Table (6)

Reduction percentage in diameter (% shrinkage) of cooked beef burger samples compared to the raw sample is given in Table (7). Reduction in diameter was observed as a result of cooking of different beef burger samples. Control samples T1 and T2 showedat zero time the highest reduction in diameter (17.25

and 17.22%, respectively), and reached after cold storage at 5±1C° for 6 days to (23.82 and 23.80% for control samples T1 and T2, respectively). Whereas, the beef burger sample T4 and T6 are showed the lowest reduction at zero time being 16.86 and 15.02%, respectively and reached to 23.58 and 22.95%, respectively after cooled storage at 5±1C° for 6 days.

Reduction in diameter is an ultimate result of the losses in cooking yield and moisture loss. As expected, beef burger samples with low cooking yield and high moisture losses were showed the highest reduction in diameter after cold storage at 5±1C° for 6 days. These results are in agreement with the results obtained by Madkour *et al.,* (2000).

Changes in pH values of different beef burger samples during after cold storage at 5±1C° for 6 days are given in same table. The initial pH values slightly were decreased until 3 days for all samples, whereas after 6 days there was increased. The pH values of all samples ranged from 5.42 to 5.85 at zero time and from 5.10 to 6.38 after cold storage at 5±1C° for 6 days.

The slight decrease in pH values after the first 3days of cold storage of all samples might be attributed to the breakdown of glycogen with the information of lactic acid. Whereas, the slight increase in pH values after 6 days during cooling storage of all samples, might be attribute due to the partial protein hydrolysis with the formation of free alkaline group. These results are in accordance with those of Madkour *et al.,* (2000) and Gibriel *et al.,* (2007).

The water holding capacity (WHC) in the beef burger samples determined as area of released water in cm^2/0.3g sample and plasticity of beef burger samples were followed during cooling storage at (5±1C° for 6 days) and the results are given in the above mentioned Table. It could be noticed that, the WHC of all samples progressively decreased with the increase of outer zones, resulted from secretion of water from samples, throughout the storage period. Control samples of beef burger (T1 and T2) had the highest WHC values after cold storage at 5±1C° for 6 days. However, beef burger samples T4 and T6 are showed the lowest WHC at zero time being (7.35 and 7.15, respectively) and reached to (8.26 and 8.02, respectively) after cooling storage at 5±1C° for 6 days.

On the other hand, plasticity (cm^2/0.3g sample) of all beef burger samples under investigation tended to a progressively decrease during cooling storage at (5±1C° for 6 days). This might be explained on the basis of denaturation or aggregation of protein during cooling storage. The decrease of plasticity was clearly pronounced in the control sample T1 (prepared without preservatives) followed by control samples T2 (prepared with 100 ppm sodium nitrite as preservatives) at the end of cooling period. These results agree with those of Madkour *et al.,* (2000); Georgantelis *et al.,* (2007) and Gibriel *et al.,* (2007).

Chemical characteristics of prepared beef burger samples:

Acid value (% as oleic acid) is considered as one of the important chemical constants for quality assurance of food lipids and as a good indicator for the hydrolysis extent takes place in these lipids, during processing and cooled period. In addition, acid value determines the free fatty acids content which partially resulted from hydrolysis of food lipid (which

enhances by moisture content of food stuffs) as well as from further oxidation of the secondary oxidation products (aldehydes and ketones) formed during cold storage Kun, (1988).

As shown in Table (8), there was a progressive elevation in the acid values of the studied prepared beef burger samples during storage. The percentage of oleic acid for control sample T1 (without preservatives) was increased from 0.92 at zero time to 5.10% during cold storage at (5±1 ˚Cfor 6 days).. Control sample T1 (without preservatives) and control sample T2 (within 100 ppm sodium nitrite) were showed the higher acid values during cold storage at (5±1C˚ for 6 days)., and achieved a level of 5.10 and 5.21%, respectively. While, the prepared beef burger samples T4 and T6 were showed lowest acid values during cold storage at (5±1C˚ for 6 days). being (4.78 and 3.08%, respectively). These results are in agreement with those obtained by McBride *et al., (*2007) and Sokovic *et al.,* (2009).

Table (8): Chemical characteristics of prepared beef burger samples

Samples / Cool Period days	T1	T2	T3	T4	T5	T6
Acid values as (% Oleic acid)						
0	0.92±0.04*	0.90±0.01*	0.91±0.70*	0.97±0.09*	0.95±0.15*	0.95±0.04*
3	2.56±0.32*	2.66±0.20*	2.60±0.42*	2.29±0.22*	2.71±0.06*	1.83±0.18*
6	5.10±0.02*	5.21±0.15*	5.18±0.38*	4.78±0.06*	5.12±0..42*	3.08±0.23*
Peroxide values (m.eq/kg)						
0	2.21±0.08*	2.23±0.01*	2.24±0.70*	2.11±0.15*	2.21±0.55*	2.07±0.25*
3	3.64±0.18*	3.71±0..54*	3.75±0.35*	3.25±0.28*	3.14±0.38*	2.89±0.02*
6	6.18±0.60*	6.25±0.07*	6.30±0.02*	5.44±0.06*	6.15±0.41*	4.35±0.23*
Thiobarbituric acid (TBA) values as (mg malonaldehyde/kg)						
0	0.97±0.20*	0.96±0.28*	0.94±0.04*	0.97±0.09*	0.95±0.25*	0.91±0.42*
3	2.71±0.11*	2.73±0.19*	2.73±0.42*	2.55±0.61*	1.86±0.38*	1.74±0.02*
6	4.18±0.22*	4.23±0.06*	3.81±0.13*	3.49±0.06*	2.89±0.42*	2.58±0.23*
Total volatile nitrogen (TVN) content						
0	16.65±0.42*	16.73±0.14*	16.25±0.43*	16.37±0.33*	16.51±0.50*	16.18±0.48*
3	21.64±0.23*	21.81±0.22*	21.56±0.21*	19.56±0.68*	20.43±0.38*	18.27±0.12*
6	24.87±0.51*	24.98±0.31*	24.47±0.40*	20.82±0.26*	23.85±0.42*	19.62±0.23*

*** Means of triplicate ± SD.**
T1-T2 see Table (6)

Therefore, the peroxide value of prepared beef burger samples as affected by cold storage at 5±1C˚ for 6 days was determined, and the obtained results are presented in Table (6). From the obtained results, it could be easily noted that, peroxide value of all beef burger samples was increased as a result to effect of cold storage. Treatments T4 and T6 were exhibited the lowest values of peroxide value. The best antioxidative effect was obtained by the ethanoil rosemary extract (T6), which had peroxide value (4.35 m.eq./kg), were

lower peroxide value at the end of storage period, than those obtained by control samples T1 and T2 (6.18 and 6.25 m.eq./kg, respectively). These results agree with the results of Georgantelis *et al.,* (2007).

TBA values (expressed as mg malonaldehyde/kg) of beef burger samples were measured during cooling storage and the results are given in Table (7). The obtained data revealed the effect of plant extract treatment and cold storage on the TBA value. The results indicate that, all prepared beef burger samples had a closed TBA values tended to increase during storage period. Control samples T1 and T2 recorded the higher TBA values after cold storage at 5±1C° for 6 days being (4.18 and 4.23 mg malonaldehyde/kg, respectively), whereas the samples T5 and T6 recorded the lowest TBA values after cold storage being 2.89 and 2.58 mg malonaldehyde/kg, respectively. These results are in agreement with those of Lin and Chao (2001) and Gibriel *et al.,* (2007).

It is well know that, total volatile nitrogen (TVN) content could be widely used as an indicator for protein decomposition caused by microorganisms as well as protein breakdown caused by tissue proteolytic enzymes during storage (Gibriel *et al.,* 2007). Total volatile nitrogen content of beef burger samples was determined at several times during cold experiment (6 days) and the results are presented in Table (7). The obtained results show that, all beef burger samples had closed TVN content at zero time (16.18 to 16.73 mg TVN/100g sample). Furthermore, the obtained data indicate that, TVN content was increased during storage of different samples. Results also revealed that, the control beef burger samples (T1 and T2) had a higher TVN content (16.65 and 16.73 mg/100g, at zero time of cold storage respectively), and continuousy increased to 24.87 and 24.98 mg/100g, respectively after 6 days). While the corresponding value for the beef burger samples T4 and T6 had a lowest TVN content during storge pariod.

The increase in TVN during cooling storage of prepared beef burger samples might be attributed to the break-down of nitrogenous substances by microbial activity. These results are in agreement with those of Madkour *et al.,* (2000) and Gibriel *et al.,* (2007).

Microbiological examination of prepared beef burger samples:

Total viable bacterial count presented in prepared beef burger samples during cooling storage for 6 days was and the resultant data are shown in Table (7). The recorded results revealed that, the initial total viable bacterial count at zero time of cooling storage was ranged from (4×10^4 to 7.6×10^4cfu/g), from the same table, it could be noted that, all treatments (at zero time) exhibited closely or similar total viable bacterial count. This may be related to the good sanitary conditions followed during beef burger preparation.

By prolonging cooling storage period increased, it is obvious that, TVBC of control samples (T1) which prepared without preservatives was recorded the highest TVBC being 7.6×10^4cfu/g at zero time to 3.3×10^4cfu/g at the end of storage period. On the other hand, the obtained data revealed that, other treatments showed decrease progressively during cold storage. Also, it could be observed that, addition of 100 ppm sodium nitrite to beef burger sample (T2) caused a decrease in TVBC after storage for 6 days at 5±1C° compared with

the control sample which prepared without adding any preservatives (T1). These results agree with those obtained by Govaris *et al.,* (2010).

Psychrophilic bacterial are primarily responsible for spoilage of meat and meat products. All prepared beef burger samples were subjected to Psychrophilic bacterial count test. The obtained results are recorded in the same table. The recorded results revealed that, the initial Psychrophilic bacterial count at zero time was ranged from (1.9×10^3 to 2.6×10^3cfu/g), from the same given results, it could be noted that all treatments (at zero time) exhibited closely Psychrophilic bacterial count.

During cold storage period, it is obvious that, Psychrophilic bacterial count of control samples (T1) which prepared without preservatives was still recorded the highest PBC during all storage period. On the other hand, the obtained data revealed that, other treatments showed decrease progressively overtime during cooling storage.

Table (9): Microbiological analysis of beef burger samples

treatment / Cold period days	T1	T2	T3	T4	T5	T6
Total viable bacterial count (cfu/g)						
0	7.6×10^4	4.0×10^4	6.4×10^4	5.6×10^4	6.3×10^4	5.4×10^4
3	5.8×10^4	2.6×10^1	4.2×10^3	3.2×10^2	7.8×10^2	6.1×10^1
6	3.3×10^4	$< 10^1$	1.8×10^2	4.0×10^1	2.3×10^1	$< 10^1$
Psychrophilic bacterial count (cfu/g)						
0	2.5×10^3	1.9×10^3	2.6×10^3	2.5×10^3	2.6×10^3	2.4×10^3
3	2.3×10^3	1.2×10^1	5.2×10^1	3.2×10^1	2.8×10^1	1.5×10^1
6	2.2×10^3	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
Total Coliforms count (cfu/g)						
0	6.2×10^1	6.0×10^1	6.4×10^1	6.1×10^1	6.3×10^1	6.0×10^1
3	4.5×10^1	$< 10^1$	4.2×10^1	2.3×10^1	3.7×10^1	$< 10^1$
6	3.8×10^1	ND	6.2×10^0	$< 10^1$	1.3×10^0	ND
Viable count of *Staphylococcus aureus* (cfu/g)						
0	7.3×10^0	2.5×10^0	6.3×10^0	5.6×10^0	6.0×10^0	4.1×10^0
3	6.5×10^0	ND	2.4×10^0	ND	1.2×10^0	ND
6	4.3×10^0	ND	ND	ND	ND	ND

T1-T6 see Table (6).
ND= Not Deleated

Data presented in Table (8) shows the presence of coliforms in all prepared beef burger samples under investigation in the accepted limit at zero time of cold storage. The recorded results revealed that, the initial coliforms count at zero time was ranged from (6.0×10^1 to 6.4×10^1cfu/g), it could be noted that, all treatments (at zero time) exhibited closely coliforms count. During cold storage period, it is obvious that, total coliforms count of control samples (T1) which prepared without preservatives was still recorded

the highest coliforms count during all storage period. On the other hand, the obtained data revealed that, all treatments showed decrease progressively overtime during cooling storage. These results agree with the results obtained by Gibriel *et al.,* (2007) and Govaris *et al.,* (2010).

Concerining to *Staphylococcus aureus*, the data presented in the same Table shows the presence of *Staphylococcus aureus* in all prepared beef burger samples under investigation in the accepted limit at zero time of cold storage. The receded results revealed that, the initial *Staphylococcus aureus* count at zero time of storage was ranged from (2.5×10^0 to 7.3×10^0cfu/g), from the same given results, it could be noted that, all treatments (at zero time) exhibited closely *Staphylococcus aureus* count. While, during cooling storage period, it is obvious that, total *Staphylococcus aureus* count of control samples (T1) which prepared without preservatives was still recorded the highest *Staphylococcus aureus* count during all storage period. On the other hand, the obtained data revealed that, all treatments showed decrease progressively overtime during cooling storage.For the detection on Salmonella, the obtained result relative that, all beef burger samples were subjected to Salmonella detection test. Also, the same test was carried out for the same studied samples during cooling storage experiment up to 6 days, and the results were negative for Salmonella detection test. These results agree with Govaris et al., (2010).

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تأثير أستخدام مستخلصات نبات النعناع والروزماري(اكليل الجبل) على الخصائص الكيميائية، الفيزيائية، الميكروبيولوجية والحسية لبرجر اللحم المجهزة .

عبد الحميد ابراهيم عبد الجواد[١]، فاتن يوسف ابراهيم[١]، الحسيني احمد ابو النجا[١] , بان جلال مجيد[١]و محمد رشادجودة يوسف[٢]

١. قسم الصناعات الغذائية – كلية الزراعة – جامعة المنصورة – مصر

٢. **معهد بحوث تكنولوجيا الاغذية – مركز البحوث الزراعة – الجيزة – مصر**

الهدف من هذا البحث هو دراسة تأثير إضافة مستخلصات النعناع و الروزماري علي خصائص الجودة الميكروبيولوجية، الكيميائية والحسية لبرجر اللحم المخزن علي 5م. تم استخدام نوعين من مستخلصات نبات النعناع والروزماري بنسبة ٣%(مستخلص مائي و اخر كحولي) في إعداد عينات برجر اللحم. تم تقدير التركيب الكيميائي، الخصائص الطبيعية بالإضافة إلي الخصائص الميكروبية. وقد أظهرت النتائج المتحصل عليها أن مستخلص الروزماري أظهرنشاط متوسط كمضاد للأكسدة ٥٢.٣٨ ملليجرام / مل عند تركيز ١.٠٪ (١ملليجرام / مل)، وارتفاع النشاط المضاد للأكسدة إلي ٨١.٥٢ ملليجرام / مل عند تركيز ١.٠٪ (١٠ ملليجرام / مل). بينما سجلت النتائج أن نشاط مستخلص النعناع كمضاد للأكسدة أقل من نشاط باقي المصادر الأخرى حيث وصل إلي ٤٨.٩٣ ملليجرام / مل و ٦٦.٧٢ ملليجرام / مل لكلا من تركيز١.٠٪ و ١.٠٪ على التوالي وأكدت النتائج ايضا أن كلا من مستخلص النعناع والروزماري أظهر تأثير مثبط ملحوظ تجاة معظم سلالات الميكروبات المختبرة. وقد اختلف التأثير أعتمادا علي نوع المستحلص المستخدم (مائي أو كحولي)، التركيز المستخدم ونوع الميكروب. كذلك اوضحت النتائج المتحصل عليها حدوث تدهور في ناتج الطهي - القدرة على الاحتفاظ بالماء (WHC) – اللدانة – رقم الحامض – رقم البيروكسيد – حامض الثيوباربيتيورك (TBA) و النيتروجين الكلي المتطاير (TVN) وهذا الانخفاض راجع الى عملية التخزين المبرد للمنتج على درجة ٥ °1 ±م وليس الى اضافة مستخلص النعناع أو الروزماري الى عينات البرجر. وفي الوقت نفسه، لوحظ زيادة قيم فقد الطبخ بزيادة فترة التخزين لجميع عينات برجر اللحم المجهزه ولكن هذه التأثيرات كانت محدودة في العينات التي تم إعدادها باستخدام مستخلصات النعناع والروزماري. كما أظهرت نتائج التقييم الحسي لعينات البرجر المجهزة باستخدام مستخلصات النعناع والروزماري عدم وجود فروق معنوية ($p < 0.05$) بينها وبين عينات الكنترول (T1 التي تم تجهيزها بدون استخدام مواد حافظة وT2 التي تم تجهيزها بإضافة ١٠٠ جزء في المليون نتريت الصوديوم).وهكذا يمكن ان نخلص الى ان اضافة مستخلص النعناع أو الروزماري يمكن ان يقلل من النشاط الميكروبي بعينات البرجر البقري دون أن يؤثر معنوياً على الصفات الحسية للمنتج.

الكلمات الدالة: النعناع، الروزماري، برجر اللحم، النشاط المضاد للميكروبات والنشاط المضاد للأكسدة.