Middle East Journal of Applied Sciences, 4(2): 142-150, 2014
ISSN: 2077-4613

Effect of Rosemary extract on microbiological, chemical and sensorial properties of chilled chicken meat

F.M. Elzamzamy

Department of Home Economics (Nutrition & Food Science), Faculty of Specific Education, Mansoura University, Egypt.

ABSTRACT

The effects of rosemary extract (RE) *Rosmarinus officinalis* addition on the microbiological, chemical and sensory quality of chicken meat stored at 7±2°C were assessed. Three levels of rosemary extract (0.00%, 0.01% and 0.02%w/w) were added to chicken meat. Raw and semi-cocked samples were subjected to microbiological, oxidative stability and sensorial evaluation after storage. The results showed that the 0.02% RE treated samples had significantly ($P<0.05$) lower microbial and antioxidative parameters. At the same time, a slight decrease in odor score was recorded in treated samples with insignificant differences in overall acceptability. Cooking delayed oxidation and caused marked reduction in microbial count. It can be concluded that combining of the addition of 0.02% rosemary extract and heat treatment was found to be shelf life extender of chicken meat with maintaining acceptable sensory attributes.

Key words: Rosemary extract, chicken meat, microbiological quality, lipid oxidation and sensorial.

Introduction

Poultry meat and poultry-based meat products are very vulnerable to spoilage (Spoilage can be defined as any change in a food product that makes it unacceptable to the consumer from a sensory point of view (Gram *et al.*, 2002) represent a risk to human health. Apart from color change, oxidation and physical damage, the other spoilage symptoms are attributable to the unacceptable levels of microorganism's undesired growth. Furthermore, microbial spoilage of meat produces off-odors and slime formation, which make the product undesirable for consumer (Hilario *et al*., 2004). The organoleptic changes may vary according to the association of microbial contaminating the meat and to the storage conditions. Types of spolilage and pathongenic bactria include mesophiles, psychrotrophs, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridiumperfringens*, *Yersinia enterocolitica* and *Bacillus cereus* (Waldroup, 1996; Russell, 1997; Mulder, 1999). The development of organoleptic spoilage is associated with microbial consumption of meat nutrients such as sugars and free amino acids and the emission of undesired volatile metabolites (Dainty *et al*., 1985). The inhibitory effect of water-soluble extracts of garlic bulbs, green garlic, green onions, hot peppers, ginger, Chinese parsley and basil on the growth of *Aspergi1lus niger* and *A. flavus* was observed by Yin & Cheng (1998). The combined neutral extract (*viz.* corni fructus, cinnamon and Chinese chive) showed antimicrobial effects on common foodborne microorganisms including bacteria, yeast and moulds (Hsieh *el al.*, 2001).

The main factors that determine food quality loss and shelf-life reduction are lipid oxidation and bacterial contamination Therefore, preventing and delaying these factors are highly pertinent to food processors. These factors in turn, contribute to the deterioration in sensory aspects of food products (Fernandez-Lopez *et al.*, 2004). A decrease in color and oxidative shelf-life depends upon increasing the degree of unsaturation of the fatty acids (Sampels *et al.*, 2004).

Rosemary (*Rosmarinus officinalis* L.) is plant belonging to the family Labiateae. This plant is widely used for various purposes. It is well known as a culinary spice and extremely utilized in the aromatherapy practice (Chan *et al.*, 2012) Rosemary is known to have antioxidant and antibacterial properties (Tavassoli & Emam Djomeh 2011). Also, it is used as general stimulant, for circulation improvement, hyperglycemia, skin care and treatment of rheumatic pains (Hamedo & Abdelmigid, 2009). In order to prevent or decrease microbial contamination and lipid oxidation in chicken meat, many additives are usually used. The recently trend is to decrease synthetic additives which have been vastly used because of the growing concern among consumers about their serious effects on human health. Consequently, find more natural additives, especially of plant source, has markedly increased in recent years. Therefore, the development of products with natural additives with antioxidants and antibacterial (Fernandez-Lopez *et al.*, 2004). Therefor, the purpose of this study was to investigate the effect of the addition of rosemary extract at different concentration on microbial, oxidative stability and sensory quality of chicken meat under refrigerated storage.

Corresponding Author: F.M. Elzamzamy, Department of Home Economics (Nutrition & Food Science), Faculty of Specific Education, Mansoura University, Egypt.
E-mail: Fatmamz95@yahoo.com

Materials and Methods

Rosemary plant was obtained from local market (Cairo, Egypt) and authenticated in agriculture research center (Giza, Egypt). Leaves were separated and dried at 30°C in a conventional oven in order to avoid activity losses caused by thermal treatment according to Ibanez *et al.,* (1999). Dried leaves were then blended in a blender and kept in refrigerator at 4°C until use (Tavassoli & Djomeh 2011).

2.1. Preparation of Rosemary Extract (RE):

Dried blend rosemary leaves were subjected to Soxhlet extraction using pure methanol as the solvent. Fourteen grams of the plant material and 800 ml of methanol were used in the extraction. Methanol containing the extract was then filtered using Whatman filter paper no.1 and the solvent was vacuum-distilled at 40 °C in a rotary evaporator (Erkan *et al*., 2008). The remaining extract was finally dried in a vacuum oven at 30°C for two hours to ensure the removal of any residual solvent.

2.2. Chicken meat collection and preparation:

Ten kilograms of chicken breast meat slices were collected from Butcher shop (into polyethylene bags) in March 2013, transported to the lab within an hour and washed with tap water. All quantity was mixed with 2% salt, 2% black pepper and 10% onion was divided into three portions for three levels of rosemary extract (0.00% Control, 0.01% and 0.02% mixed with 5ml water). After adding extract, meat slices were hand flipped for 7 minutes to get equal distribution of extract. Half of each portion was heated at 90°C for 7 minutes and cooled to 30°C. Each of the chicken meat samples was packed into polyethylene bag. Both of raw and semi cooked samples were microbiologically and chemically evaluated at zero, 3, 5 and 7 days after storage in refrigerator at 4°C±1. Sensory evaluation was carried out for cooked samples at zero time.

2.3. Microbiological evaluation:

Chicken meat samples of 25g were blended with 225 ml of 0.1% peptone water for 1-2 minutes in a sterile blender jar. Further decimal serial dilutions prepared for testing. The plate count method was used for determination of the numbers of viable organisms. Appropriate media for the particular group of organisms was poured on one ml of each dilution to be tested as colony forming unit per gram (CFU/g). Plate count agar (PCA; Biolife) incubated at 30°C for 72 h for the enumeration of mesophilic aerobic bacteria and incubated at 5°C for 10 days for the enumeration of psychrotrophic aerobic bacteria according to A.P.H.A., (1992). Staphylococcus spp was determined by the spread plate method using Baird-Parker Agar (Oxoid) with egg yolk tellurite emulsion (Merck). The plates were incubated at 37°C for 48 h. as recommended by I.A.E.A (1990). Violet red bile glucose agar (VRBGA; Difico) for the total coliform was incubated at 30°C for 24 to 48 h, colonies were considered as round red to pink. Potato dextrose agar (PDA; Biolife) for yeasts and moulds was incubated at 25^{o}C for 5 days, count were enumerated according to the method of IDF (1990). Detection of *Salmonella* was done by using tetrathionate broth base as selective enrichment according to A.P.H.A. (1998). 10 ml of tetrathionate broth were mixed well with 1 ml from different sites of the sample and the mixture incubated at 35°C for 24 h. 1 ml of enriched samples was used for selective growth (pour plating method was used) from the same enriched samples on Salmonella-Shigella (SS) agar at 37 °C for 24 h (Andrews & Hammocks, 2003). Enumeration of typical colonies (pink on SS agar) was enumerated. Multiple standard biochemical procedures and gram staining were carried out. The dishes containing more than 30 and/or fewer than 300 colonies were counted. Results were reported as colony-forming units per gram of samples (CFU/g) as described by Feng *et al*. (1998).

2.4. Evaluation of oxidative stability:

Lipid oxidation was measured by determination of Thiobarbituric acid (TBA) calorimetrically by the Porkony and Dieffenbacher method as described by Kirk & Sawyer (1991). Results are expressed as mg malondialdehyde /kg (mg ma/kg) chicken meat muscle. Peroxide value (PV) was determined in the lipid extract according to the method described by AOAC (2000). Results are expressed as milli-equivalents peroxide per kg lipid (meq /kg lipid). Free Fatty Acid (FFA) content in the lipid extract was carried out according to Kirk & Sawyer (1991) method. Results are expressed as grams of % oleic acid.

2.5. Sensory evaluation:

Chicken meat samples were coded and served to 10 semi trained panelists. The samples were rated for the appearance, flavor, texture and overall acceptability. The evaluations were presented to ranging scale from 10 (extremely acceptable) to 1 (extremely unacceptable) according to Keeton (1983).

2.6. Statistical analysis:

The data were analyzed with IBM SPSS Statics version 20.0. The comparison between means of data was carried out using one way analysis of variance. Differences between means were separated using Duncan's Multiple Range Test. Standard deviation of all averages were calculated. Significance of variance value was determined at 0.05.

3. Results:

3.1. Microbiological evaluation:

Aerobic flora has been used as criteria to predict the mean life of products. Chicken meat samples treated with rosemary extract (0.01% and 0.02%) were analyzed in raw and cooked state under refrigerated storage at 4°C (Table 1). Data show that the mean concentration (log CFU/g) of Aerobic Plate Count (APC) detected from raw samples was significantly lower in samples treated with rosemary extract than control over the storage period. The counts were 4.783±0.002, 4.694±0.003 and 4.684±0.003 log CFU/g for control, 0.01% and 0.02%RE, respectively. On day 3 it ranged between 4.254±0.001 log CFU/g for 0.01%RE and 4.613±0.011 log CFU/g for control. The lowest count (6.447±0.001 log CFU/g) on day 7 was recorded for 0.02%RE, however the control had the highest count (6.555±0.002 log CFU/g). Cooking caused marked reduction in all samples. The lowest count was detected in 0.02%RE sample at all storage days; however the lowest was for control.

Table 1: Counts of total aerobic mesophilic bacteria of raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C

Storage days	Control		0.01% RE		0.02 %RE	
	R	C	R	C	R	C
Initial	4.783^{f} ±0.002	4.204^{c} ±0.003	4.694^{e} ±0.003	3.905^{b} ±.002	4.684^{d} ±0.003	3.891^{a} ±0.002
3	4.613^{f} ±0.011	4.379^{c} ±0.002	4.254^{b} ±0.001	3.913^{a} ±0.011	4.408^{e} ±0.001	3.903^{a} ±0.002
5	4.880^{f} ±0.002	4.698^{c} ±0.001	4.792^{d} ±0.001	4.429^{b} ±0.001	4.806^{e} ±0.005	4.302^{a} ±0.002
7	6.555^{e} ±0.002	4.608^{c} ±0.01	6.698^{f} ±0.001	4.520^{b} ±0.002	6.447^{d} ±0.001	4.303^{a} ±0.002
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Data show no significant differences in psychrotrofs counts between raw samples at initial (Table 2). However 0.02%RE sample recorded the lowest count on day 3 (4.940±0.003 log CFU/g), followed by 0.01%RE sample (5.095±0.082 log CFU/g). The same trend was observed on day 7. The lowest psychrotrofs counts in cooked samples was found for 0.02%RE sample at initial (2.043±0.140 log CFU/g), on day 3 (4.177±0.001) and 5 (4.476±0.002), however control sample had the highest counts over the storage period. Results indicated a gradual increase for all samples during storage. Salmonella was positive in all raw samples; however it couldn't be detected in any of cooked samples (Table 4).

Table 2: Counts of total aerobic psychrophilic bacteria of raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	4.263^{c} ±0.002	3.3140^{b} ±0.002	4.256^{c} ±0.003	2.044^{a} ±0.116	4.202^{c} ±0.002	2.043^{a} ±0.140
3	5.295^{f} ±0.010	4.602^{c} ±0.002	5.095^{e} ±0.082	4.313^{b} ±0.002	4.940^{d} ±0.003	4.177^{a} ±0.001
5	5.3087^{e} ±0.056	4.877^{d} ±0.002	4.3427^{a} ±0.002	4.495^{b} ±0.011	4.6117^{c} ±0.001	4.476^{b} ±0.002
7	6.953^{f} ±0.107	5.476^{c} ±0.001	6.792^{e} ±0.002	5.321^{b} ±0.005	6.599^{d} ±0.007	4.845^{a} ±0.003
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Table 3: Counts of total coliform bacteria of raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	3.214^{e} ±0.002	2.602^{c} ±0.002	3.002^{d} ±0.003	1.230^{b} ±0.002	3.393^{f} ±.001	0.906^{a} ±0.007
3	4.162^{e} ±0.002	3.832^{c} ±0.001	4.083^{d} ±0.002	1.040^{b} ±0.001	4.081^{d} ±0.001	0.823^{a} ±0.01
5	4.232^{f} ±.0.002	3.123^{c} ±0.004	4.221^{e} ±0.002	1.006^{b} ±0.005	4.201^{d} ±0.001	0.632^{a} ±0.004
7	3.422^{e} ±0.003	2.452^{c} ±0.004	3.611^{f} ±0.001	0.823^{b} ±0.004	3.408^{d} ±0.007	0.421^{a} ±0.002
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Table 4: Detiction salmonella in raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	+	-	+	-	+	-
3	+	-	+	-	+	-
5	-	-	-	-	-	-
7	-	-	-	-	-	-

R: Raw; C: cooked.

Coliform count (log CFU/g) in raw samples varied from 3.00±0.003 in 0.01%RE to 3.214±0.002 log CFU/g in control at initial (Table 3). A decrease in coliform count was parallel to the increase in rosemary extract level as counts ranged from 4.081±0.001, 4.202±0.001 and 3.408±0.007 log CFU/g on days 3, 5 and 7, respectively in 0.02%RE to 4.162±0.002, 4.232±.0.002 and 3.422±0.003 log CFU/g on days 3, 5 and 7, respectively in control. Cooking temperatures led a marked reduction in coliform counts and all other bacteria. In cooked samples data show marked reduction in coliform count parallel to the increase in extract level.

Staphylococcus spp counts in raw samples varied from 3.00±0.017 in 0.01%RE to 3.204±0.002 log CFU/g in control at initial as shown in Table 5. The lowest count detected on day 3 was in 0.02%RE sample (3.38±0.002 log CFU/g), followed by control (4.477±0.002 log CFU/g). Counts raged between 5.14±0.003 and 5.505±0.002 log CFU/g on day 5 and between 5.448±0.001 and 6.362±0.003 log CFU/g on day 7 in 0.02%RE and control respectively. Data indicate rosemary extract addition was significantly effective in cooked samples. Counts in all cooked samples are gradually increased during storage.

Table 5: Count of staphelecoccus bacteria ssp of raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	3.204^{d} ±0.002	3.123^{c} ±0.002	3.001^{b} ±0.017	1.602^{a} ±0.002	3.294^{f} ±0.011	1.598^{a} ±0.006
3	4.477^{d} ±0.002	3.981^{c} ±0.001	4.478^{d} ±0.578	3.476^{b} ±0.001	3.380^{b} ±0.002	2.697^{a} ±0.001
5	5.505^{f} ±0.002	4.476^{c} ±0.002	5.493^{e} ±0.001	3.490^{b} ±0.003	5.146^{d} ±0.003	3.296^{a} ±0.011
7	6.362^{f} ±0.003	4.520^{c} ±0.003	6.146^{e} ±0.002	4.042^{b} ±0.002	5.448^{d} ±0.001	3.698^{a} ±0.005
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Table 6: Counts of yeast&mold of raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	2.902^{f} ±0.002	2.780^{e} ±0.002	2.602^{d} ±0.002	2.320^{c} ±0.003	2.299^{b} ±0.006	2.096^{a} ±0.025
3	4.681^{d} ±0.002	4.505^{f} ±0.002	4.747^{e} ±0.002	3.792^{b} ±0.018	4.176^{c} ±0.002	2.230^{a} ±0.003
5	4.845^{d} ±0.002	4.292^{c} ±0.018	4.845^{d} ±0.002	3.959^{b} ±0.003	5.146^{e} ±0.002	3.302^{a} ±0.002
7	6.006^{f} ±0.005	5.296^{c} ±0.013	5.902^{e} ±0.002	4.295^{a} ±0.012	5.777^{d} ±0.001	4.602^{b} ±0.002
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Table 7: Thiobarbituric acid values for raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	0.244^{e} ±.001	0.242^{c} ±0.002	0.270^{b} ±0.003	0.138^{b} ±0.002	0.134^{a} ±0.001	0.102^{a} ±0.002
3	0.490^{f} ±.002	0.276^{e} ±0.002	0.2030^{d} ±0.002	0.177^{c} ±0.001	0.144^{b} ±0.002	0.081^{a} ±0.003
5	0.520^{d} ±.010	0.268^{c} ±0.017	0.223^{b} ±0.007	0.208^{b} ±0.001	0.152^{a} ±0.005	0.141^{a} ±0.003
7	0.553^{d} ±0.015	0.335^{c} ±0.002	0.256^{b} ±0.008	0.255^{b} ±0.006	0.163^{a} ±0.005	0.154^{a} ±0.005
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Significant differences were found between samples for yeast and mold counts (Table 6). In raw samples counts ranged from 2.299±0.006 and 2.902±0.002 log CFU/g in 0.02%RE and control, respectively at initial and increased gradually during storage. On day 7 counts were 5.777±0.001, 5.902±0.002 and 6.006±0.005 log CFU/g for 0.02%RE, 0.01%RE and control respectively. On the other hand the lower count in cooked samples was found in 0.02%RE sample over the storage period. Meanwhile the control had the highest counts.

3.2. Evaluation of lipid oxidation:

TBA value is widely used as an indicator of the degree of lipid oxidation (Can, 2011). In the present study, TBA values (TBA, mg malonaldehyde/kg) of chicken meat increased during storage at 4ºC (Table 7). The lowest TBA values were recorded for 0.02%RE sample during storage period (0.144±0.002, 0.152±0.005 and 0.163±0.005) however the control had the highest values (0.490 ±.002, 0.520±.010 and 0.553±0.015 at initial and on days 3, 5 and 7, respectively. Cooking decreased TBA values. Peroxide values (PV) increased during storage in both of raw and cooked samples (Table 8). At initial, values of raw samples were ranged between 2.05±.041 for 0.02%RE and 2.387±.002 meq/kg for control. However on day 7 the values ranged between 2.300±.050 for 0.02%RE and 4.844±.05 for control. Cooked samples take the same trend. Free fatty acids (FFA) values (% oleic acid) for raw samples are significantly different over the storage period (Table 9). FFA values at initial were 1.795±.041, 2.702±.010 and 2.810±.020 % oleic acid for 0.02%RE, 0.01%RE and control, respectively in raw samples. These values increased throughout storage. Cooked 0.02%RE sample had the lowest FFA value, followed by cooked 0.01%RE sample on day 3, 5 and 7. Data show that heat treatment with rosemary extract at level of 0.02% recorded the lowest FFA values over the storage period.

Table 8: Peroxide acid values for raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01%RE		0.02%RE	
	R	C	R	C	R	C
Initial	2.387^{e} ±.002	2.839^{f} ±.05	2.312^{d} ±.05	2.122^{c} ±.05	2.05^{b} ±.041	1.351^{a} ±.05
3	4.542^{f} ±.04	4.313^{e} ±.061	3.137^{d} ±.037	2.211^{b} ±.04	2.230^{c} ±.05	1.369^{a} ±.05
5	4.697^{e} ±.02	5.404^{f} ±.04	2.694^{d} ±.06	2.520^{c} ±.02	2.340^{b} ±.04	1.949^{a} ±.05
7	4.844^{e} ±.05	5.849^{f} ±.03	2.733^{c} ±.03	2.545^{b} ±.03	2.35^{a} ±.02	2.300^{d} ±.050
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Table 9: Free fatty acids concentrations for raw and semi-cooked chicken meat samples treated with rosemary extract during refrigerated storage at 7°C.

Storage days	Control		0.01% RE		0.02% RE	
	R	C	R	C	R	C
Initial	2.810^{d} ±0.020	2.666^{c} ±0.015	2.702^{c} ±0.010	1.977^{b} ±0.015	1.795^{a} ±0.041	1.760^{a} ±0.020
3	3.512^{e} ±0.030	3.572^{f} ±0.020	2.342^{d} ±0.030	2.183^{c} ±0.030	1.951^{b} ±0.010	1.642^{a} ±0.010
5	3.625^{c} ±0.015	3.916^{d} ±0.020	2.410^{b} ±0.020	1.876^{a} ±0.586	1.934^{a} ±.0150	1.681^{a} ±0.001
7	4.058^{c} ±0.025	4.560^{d} ±0.010	2.520^{b} ±0.010	2.513^{b} ±0.035	1.956^{a} ±0.035	1.948^{a} ±0.030
LSD	0.000	0.000	0.000	0.000	0.000	0.000

RE: Rosemary extract; R: Raw; C: cooked; Superscripts (a, b, c, d, e, f) in the same row are significantly different ($p<0.05$); N= three replicates.

Table 10: Sensory evaluation of semi-cooked chicken meat samples treated with rosemary extract after cooking.

Samples	Taste	Odor	Color	Appea	OA
Control	9.590[a] ±0.284	9.750[b] ±0.232	9.770[a] ±0.266	9.650[a] ±0.212	9.655[a] ±0.136
0.01% RE	9.650[a] ±0.464	9.600[b] ±0.76[4]	9.690[a] ±0.246	9.630[a] ±0.182	9.742[a] ±0.124
0.02% RE	9.600[a] ±0.365	8.970[a] ±0.661	9.590[a] ±0.237	9.655[a] ±0.214	9.402[a] ±0.256

RE: Rosemary extract; Superscripts (a, b, c, d, e, f) in the same column are significantly different ($p<0.05$); N= three replicates.

3.3. Sensory evaluation:

Sensory evaluation was carried out for chicken meat samples treated with rosemary extract after cooking (Table 10). No significant differences were found in taste, color, appearance and overall acceptability (OA) scores for 0.02%RE, 0.01%RE and control samples. Odor scores were 8.970±0.661, 9.600±0.764 and 9.750±0.232 for 0.02%RE, 0.01%RE and control, respectively.

4. Discussion:

It is recommended that the flesh total aerobic bacteria count should not exceed 10^6/g wet weight ICMSF (1998). This recommendation was met by our results of cooked samples. Our findings are lower than those reported by Al-jasser (2012); however they are parallel to Yavas & Bilgin, (2010) who recorded that APC (log CFU/g) in chicken nuggets reached at 6.18 in control samples. Dughaym & Altabari (2010) found that the mean total bacterial count in the nuggets was 2.7×10^4 and $3.3\cdot10^7$ CFU/g in burger. Presence of these counts in cooked samples can be explained by Can, (2011) who suggest that the temperature was not totally inactivate the micro-organisms; it only thermally injured them and thus micro-organisms were capable of recovering throughout storage. A gradual increase in the total aerobic counts throughout the storage period was observed (Simpson *et al*. 1994). A recent guideline to assess microbial safety of ready-to-eat foods placed on the market was published by the Health Protection Agency from the United Kingdom (Health Protection Agency, 2009). The document suggest levels of aerobic plate counts $<10^5$ CFU/g in deli meats to be considered as satisfactory (<5.0 $\log_{10}$ CFU/g); 10^5 to $<10^7$ CFU/g as borderline (5.0 to <7.0 log CFU/g); and $\geq10^7$ as unsatisfactory levels (≥7.0 log CFU/g). On the other hand the aerobic plate counts, feacal coliforms, *S. aureus*, should not exceed 5.7, 2 and 2 log $CFU.g^{-1}$ respectively in raw ground meats according to Moroccan regulatory standards for microbiological safety criteria for foods, (Moroccan Department order, 2004). 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. Tavassoli & Djomeh (2011) recorded that rosemary leaves extract can be used as a potential antimicrobial of natural origin in foods. Can (2011) and Rosnes (1999) reported that the psychrotrophs counts were lower than the mesophile counts which in line with our results. Coliforms are supposedly the most used index of food quality even with some disadvantages (Tompkin, 1983; Reinbold, 1983; Matches & Abeyta, 1983). Our results are in line with Yuksek *et al*., (2009) who observed that cooking temperatures led a 4 and 5 log reduction in coliform counts and most of other bacteria. Throughout the storage period, the counts for coliforms were well below the levels 3 log cfu/g in chicken meat nuggets treated with Preservative Mixture (containing calcium lactate, sodium diacetate and sodium chloride) stored at 4±2°C (Yavas & Bilgin, 2010). *Salmonella* and *E. coli* in chicken meat were detected at the first day according to Javanmard *et al*., (2006). Saudi standard (SASO No. 1556) states that the total counts in chicken meat must not exceed 10^6 CFU/g, *S. aureus* and *E. coli* counts should be less than 10^2 CFU/g and *Salmonella* should be negative. Colmenero, 1996; Modi *et al*., 2006 indicated that *Salmonella* increased parallel to increasing storage time and it was detected in shop samples but negative in supermarket samples. *E. coli, S. aureus, Salmonella*, and yeast and moulds were negative in all nugget samples during storage period. Similar results were noticed in cooked meat products under storage at lower temperatures.

Odunfa (1988) reported that *Staphylococcus aureus* levels of 10^8ml are considered potential hazardous to consumers as its presence is an indication of food handler's contamination. Al-Dughaym & Altabari (2010) stated that the *Staphylococcus aureus* count was less than 10^2 CFU/g. Our results are in agreement with those reported by Arain *et al*., (2010) who recorded that the overall yeasts and molds count in goat meat ranged between 4.0×10^3 to 3.0×10^3 cfug-1 (mean $1.5 \times 10^3 \pm 1.5 \times 10^2$ cfug-1). Oxidative deterioration in lipid food manifest itself in the form of primary and secondary oxidation products. The peroxide value (PV) is a quantitation for the primary oxidation products while TBA is a measure of the secondary oxidation products.

Meat products are mostly vulnerable to lipid oxidation due to their considerable fat content which leads to quality deterioration in flavor, taste, texture, color and nutritional aspects of the product (Olafsdottir *et al*., 1997; Yilmaz, 1998). Results in current study are in accordance with those of Hamilton & Kirstein (2008) who recorded that the PV in all samples were much lower than the recommended acceptable level of nutritionists and

consumers which had PV of between 5 and 20 meq O_2/kg of fat as established maximum levels. And PV values were significantly increased ($P<0.05$) during storage period (21 days). Free fatty acids values represent an indicator about fat stability during storage due to the enzymatic products or microbial degradation of lipids (Das *et al*., 2008). Many studies stated significant increase in FFA during refrigerated storage (Sahoo & Anjaneyuldu 1997, Yavas & Bilgin, 2010, Al-jasser 2012). Rosemary can prevent lipid oxidation, chelate metals and scavenge superoxide radicals (Peschel *et al*., 2007). Nakatani (2003) reported that phenolic diterpenes from rosemary are essentially antioxidative. The antioxidant activity of carnosic acid has antioxidative capacity several times of BHT and BHA but less than TBHQ (Richheimer *et al*., 1996). Based on TBA values and sensory attributes, Pizzocaro *et al*. (1994) explained that ground fresh rosemary leaves (0.3%) and rosemary plus sage (0.3% + 0.3%) could preserve the oxidative quality of beef hamburger in frozen storage. Sensory results revealed that the insignificant differences between OA scores reversed a slight effect of odor score for 0.02%RE on its overall sensory. This may be due to the effect of other spices (onion and black pepper) which limited rosemary strong flavor.

5. Conclusion:

The current study concluded that the addition of 0.02% rosemary extract improved the oxidative stability, microbiological quality of chicken meat and maintained high sensory scores. Results indicated that 0.02% RE samples had significantly ($P<0.05$) lower organisms counts of total aerobic mesophilic bacteria, psychrophilic bacteria, coliforms, staphylococci, Salmonella spp, yeast and mold. Values of lipid oxidation parameters indicted a positive effect of rosemary extract especially in cooked samples. Based on shelf life and sensory evaluation the results obtained in this work show that it is possible to produce safer and acceptable chicken meat using natural extracts. However, additional research is needed to produce clear information about the effects of various storage temperatures and packaging materials and procedures.

References

AL-Dughaym, A.M., G.F. Altabari, 2010. Safety and quality of some chicken meat products in AL-Ahsa markets –Saudi Arabia. Saudia J Biol Sci., 17: 37-42.

Andrews, W.H., T.S. Hammack, 2003. Bacteriological Analytical Manual online. 8th edition. United States Department of Health and Human services.

Arain, M.A., I.R. Rajput, M. Khaskheli1, S. Faraz, K. Devrajani, S.A. Fazlani, 2010. Evaluation of Microbial Quality of Goat Meat at Local Market of Tando Jam. Pakistan Journal of Nutrition 9: 287-29.

AOAC, 2000. Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists, Washington, Arlington, USA.

A.P.H.A., 1992. Compendium of methods for the microbiological. examination of foods, 3rd edn. Amer. spp (Stph.s), Public. Health Assoc. Washington.

Al-jasser, M.S., 2012. Effect of cooling and freezing temperatures on microbial and chemical properties of chicken meat during storage. *Journal of Food*, Agriculture & Environment, 10: 113-116.

Burt, S., 2004. *Essential oils:* their antibacterial properties and potential applications in foods a review. *Int. J. Food Microbiol*., 94: 223-253.

Campo, D.J., M.J. Amiot, C. Nguyen, 2000. Antimicrobial effect of rosemary extracts. *Journal of Food Protection*, 10: 1359-1368.

Can, Ö.P., 2011. Evaluation of the Microbiological, Chemical and Sensory Quality of Carp Processed by the Sous Vide Method. World Academy of Science, Engineering and Technology, 80.

Chan, E.W.C., L.Q. Kong, K.Y. W.Y. Yee, Chua, T.Y. Loo, 2012. Rosemary and Sage Outperformed Six other Culinary Herbs in Antioxidant and Antibacterial Properties, In. J. Biotechnology for Wellness Industries, 1: 142-151.

Colmenero, F.J., 1996. Technologies for developing low fat meat products. Trends Food Sci. Technol., 7: 41-48.

Dainty, R.H., R.A. Edwards, C.M. Hibbard, 1985. Time course of volatile compounds formation during refrigerated storage of naturally contaminated beef in air. J. Appl. Bacteriol., 59: 303-309.

Das, A.K., A.S.R. Anjaneyulu, Y.P. Gadekar, R.P. Singh, H. Pragati, 2008. Effect of full-fat soy paste and textured soy granules on quality and shelf-life of goat meat nuggets in frozen storage. Meat Sci., 80: 607-614.

Erkan, N., G. Ayranci, E. Ayranci, 2008. Antioxidant of rosemary (Rosmarinus officinalis L.) extract, black seed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *J. Food Chem.*, 110: 76-82.

Feng, P., S.D. Weagant, M.A. Grant, 1998. Enumeration of E. coli and the Coliform B acteria in Bacetreriological Analytical Manual. *Food and Drug Adminstration*, 8th Edn., Revision A. (monograph on the internet). Retrieved from: http://www.cfsan.fda. gov/-ebam/bam-4.html. (Accessed date: April 13, 2009).

Fernandez-Lopez, J., N. Zhi, L. Aleson-Carbonell, J.A. Perez-Alvarez, V. Kuri, 2004. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science*, 69: 371-380.

Gram, L., L. Ravn, M. Rasch, J.B. Bruhn, A.B. Christensen, M. Givskov, 2002. Food spoilage-interactions between food spoilage bacteria. *Int. J. Food Microbiol*., 78: 79-97.

Hamedo, H.A., H.M. Abdelmigid, 2009. Use of antimicrobial and genotoxicity potentiality for evaluation of essential oils as food preservatives. Open Biotechnology Journal, 3(50-56): 1874-0707.

Hamilton, C.R., D. Kirstein, 2008. Does rancidity, as measured by peroxide value, affect animal performance? [Internet document] URL http://www.darlingii.com/pdf. Accessed 01/02/2009

Hsieh, P.C., J.L. Mau, S.H. Huang, 2001. Antimicrobial effect of various combinations of plant extracts. Food Microbiology, 18: 35-43.

Hilario, E., T.R. Buckley, J.M. Young, 2004. Improved resolution of the phylogenetic relationships among Pseudomonas by the combined analysis of atpD, carA, recA and 16S rDNA. *Antonie Leeuwenhoek*, 86: 51-64.

I.A.E.A., 1990. Microbiological specification and testing methods for irradiated foods. Technical Report series No. 104, I.A.E.A, m Vienna.

ICMSF, 1998. Microorganisms in Foods, Microbial Specifications of Commodities. International Commission on Microbiological Specifications for Foods (ICMSF) Blackie Academic & Professional, London, 3.

IDF, 1990. Enumeration of yeast and molds in milk and milk products. Yeast and molds at 25^{o}C. In: International Dairy Federation, Brussels (Belgium).

Javanmard, M., N. Rokni, S. Bokaie, G. Shahhosseini, 2006. Effects of gamma irradiation and frozen storage on microbial, chemical and sensory quality of chicken meat in Iran. Food Control, 17: 469-473.

Javanmard, M., N. Rokni, S. Bokaie, G. Shahhosseini, 2006. Effects of gamma irradiation and frozen storage on microbial, chemical and sensory quality of chicken meat in Iran. *Food Control*, 17: 469-473.

Keeton, J.T., 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J. Food Science, 48: 879-881.

Kirk, R., R. Sawyer, 1991. *Pearsons composition and Analysis of Foods*, 9 ed. Longman scientific and technical, 642-643.

Matches, J.R., C. Abeyta, 1983. Indicator organisms in fish and shellfish. Food technology, 37: 114.

Modi, V.K., N.M. Sachindera, A.D. Sathisha, N.S. Mahendrakar and D.N. Rao, 2006. Changes in quality of chicken curry during frozen storage. *J. Muscle Foods*, 17: 141-154.

Moreno, S., T. Scheyer, C.S. Romano, A.A. Vojnov, 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research, 40: 223-231.

Moroccan Department order No. 624-04, 2004. Relative of animal and animal origin foods. Official Bull., 5214: 727-745.

Mulder, R.W., 1999. European directives to regulate food safety. World Poultry Sci. J., 15: 50-51.

Nakatani, N., 2003. Biologically functional constituents of spices and herbs. J. Japanese Soc. Nutr. Food Sci., 56(6): 389-395.

Nychas, G.J.E., 1995. *Natural antimicrobials from plants. In:New methods of food preservation*. Gould G.W. (ed.). London, Blackie Academic and Professional, 58-89.

Odunfa, S.A., 1988. Microbiological quality of Yoghurt and milk drink samples in Ibadan, a Nigerian City *J. Agric.*, 2: 43-46.

Olafsdottir, G., E. Martinsdottir, J. Oehlenschlager, P. Dalgaard, B. Jensen, I. Undeland, I.M. Mackie, G. Henehan, J. Nielsen, H. Nilsen, 1997. Methods to evaluate fish freshness in research and industry. Trends in Food Sci. Technol., 8: 258-265.

Pizzocaro, F., E. Senesi, G. Babbini, 1994. Protective effect of fresh rosemary and sage on frozen beef hamburgers. Industrie Alimentari, 33: 289-294.

Peschel, W., W. Dieckmann, M. Sonnenschein, A. Plescher, 2007. High antioxidant potential of pressing residues from evening primrose in comparison to other oilseed cakes and plant antioxidants. Indust. *Crops Prod*. 25: 44.

Reinbold, G.W., 1983. Indicator organisms in dairy products. *Food technology*, 37: 111.

Richheimer, S.L., A.M.W. Bernart, G.A. King, M.C. Kent, D.T. Bailey, 1996. Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. J. Am. Oil Chem. Soc., 73: 507-514.

Rosnes, J.T., H. Kleiberg, H. Bergslein, J. Vidvei, 1999. Microbiological safety of two sous vide fish based meals. In: Third European Symposium on Sous-vide Proceedings, Leuven, Belgium, 195-204.

Russell, S.M., 1997. A rapid method for predicting the potential shelf life of fresh broiler chicken carcasses. J. Food Protec., 60: 148-152.

Sampels, S., J. Pickova, E. Wiklund, 2004. Fatty acids, antioxidant and oxidation stability of processed reindeer meat. *Meat Science*, 67: 523-532.

Sahoo, J. and A.S.R. Anjaneyuldu, 1997. Effect of natural antioxidants and vacuum packaging on the quality of buffalo meat nuggets during refrigerated storage. Meat Sci., 47: 223-230.

SASO, 1998. Saudi Arabia Standards for Microbial Levels of Foodstuffs-Part One, No.1556.

Shelef, L.A., 1983. Antimicrobial effects of spices. *J. Food Safety*, 6: 29-44.

Simpson, M.V., J.P. Smith, B.K. Simpson, H. Ramaswamy, K.L. Dodds, 1994. Storage studies on a sous vide spaghetti and meat sauce product, Food Microbiol., 11: 5-14.

Tavassoli, S., Z.A. Djomeh, 2011. Total Phenols, Antioxidant Potential and Antimicrobial Activity of Methanol Extract of Rosemary (*Rosmarinus officinalis* L.), *Global Veterinaria*, 7: 337-341.

Tompkin, R.B., 1983. Indicator organisms in meat and poultry products. Food technology, 37: 111.

Waldroup, A.L., 1996. Contamination of raw poultry with pathogens. World Poult. Sci. J., 52: 7-25.

Yavas, E., B. Bilgin, 2010. Effect of Calcium Lactate, Sodium Diacetate and Sodium Chloride Mixture on the Microbiological, Chemical and Sensory Properties of Chicken Nuggets Stored in Refrigeration and under Modified Atmospheres International Journal of Poultry Science, 9: 66-71.

Yilmaz, I., 1998. Farkli ambalajlama yontemi ve depolama sicakliginin Tekirdag koftesinin bazi mikrobiyolojik, fiziksel ve kimyasal ozellikleri uzerine etkilerinin belirlenmesi. Doktora Tezi. T. Uni. Zir. Fak. Tekirdag, pp: 204.

Yuksek N., S.S. Evrensel, S. Temelli, S. Anar, M.K. Cem, A. Sen, 2009. Microbiological Evaluation on the Ready-To-Eat Red Meat and Chicken Donair Kebabs from a Local Catering Company in Bursa *J. Biol. Environ*. SCI., 3: 7-10.

Yin M.C., W.S. Cheng, 1998. Inhibition of Aspergillus niger and Aspergillus flavus by some herbs and spices. *J. Food Protect*., 61: 123-125.