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Study of sumac extract (Rhus coriaria L.), lactic acid and thyme oil as decontaminants for shelf life extension of refrigerated rabbit meat

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Abstract

In an attempt to improve the microbiological quality and extend the shelf-life of refrigerated rabbit meat, four groups of rabbit meat from New Zealand breed were prepared, the 1st group was untreated (control), while the other three groups were treated with lactic acid 0.5 %, thyme oil 0.5 % and water extract of sumac 8 % by dipping for 1, 1 and 10 min, respectively. Then the samples were refrigerated at 2 ± 1 °C to be periodically examined for their sensory and microbiological status. The control and treated samples showed excellent overall acceptability by panellists at zero-day of examination, off odours and tastes were noticed by the day 9, 12, 9 and 15 day of storage in control (C), lactic acid (LA), thyme oil (TO) and water extract of sumac (WES) treated groups, respectively. There was no significant differences in aerobic plate counts "APC" (at $p < 0.05$) between treated and untreated groups at zero and 3rd day of examination, while at 6th and 9th day of refrigerated storage, the values of APC were significantly lower in LA and WES treated groups than C and TO treated groups. The APC results confirm the concept that the slowest growth rate of the total bacterial population extends the shelf life of rabbit meat. Approximately, similar pattern to that of APC was observed in the results of coliforms (MPN). The MPN values of faecal coliforms and E. coli were relatively low, which could be attributed to the preparation of rabbit samples under strict hygienic measures. The obtained results in the present study suggest that WES can be used as a decontaminant for rabbit and poultry meat at each decontamination step instead of other chemical substances which could affect the acceptability of the final product and remain some hazardous residues.

Keywords: sumac, rabbit, quality, shelf life, thyme oil, lactic acid.

1. Introduction

Rabbit meat is a highly desirable food around the world with a high digestible quality which represents a respectable source of animal proteins of high biological value. It contains all essential amino acids required for human nutrition as well as a higher proportion of unsaturated fatty acids and less cholesterol than other classes of animal meat. It is traditionally considered to be one of the cheapest protein sources and can be used as a subsidiary animal food due to its short generation interval, high fecundity and rapid growth rate. However, Rabbit meat is nutritious not only for human, but also for a wide variety of microorganisms as well, due to its suitable pH value which lies within the growth range of most microbial species **(Gergis, 2004).**

Microbiological contamination is one of the main risk conditions that affect meat quality and consumers health **(Plym and Wierup, 2006)**, therefore, minimizing product contamination and delaying or stopping growth of spoilage microorganisms in the product are the main keys for improving fresh food shelf life **(Zambuchini et al., 2008).**

Production of a pathogen-free food product is not guaranteed under current production conditions **(Northcutt et al., 2003).** However, the incorporation of a decontamination step during slaughter, dressing and evisceration procedures can improve the microbial quality and safety of meat products **(Castillo et al., 2002).**

The technologies of carcass decontamination can be classified into chemical or physical methods. The application of chemical decontaminants in food processing is permitted in the United States, however, their use in commercial plants in European Union countries is prohibited. Lactic acid (LA) has become the most commonly used organic acid in commercial practice to improve the microbial quality, safety and shelf life of refrigerated carcasses **(Van Netten et al., 1994 and Coleman et al., 2003)**. Some of synthetic chemical antimicrobials are limited to be used in foods, because they may have some adverse effects on public health and reduce the acceptability of the final product, therefore, much attention in recent years has been directed toward plant extracts, which have been used for many periods to improve the sensory attributes and extend the shelf life of foods. The effects of herbal extracts against pathogenic bacteria in vitro are known, nevertheless few studies

have demonstrated the effect of these substances on pathogenic microorganisms associated with muscle foods such as **Cutter (2000) and Khalafalla et al. (2015).**

Numerous tanniniferous plants, including sumac (*Rhus coriaria* L.), have been known to contain natural compounds with antimicrobial actions and the milled spice is used as a seasoning and sprinkled over kebobs, grilled meats, soups, and some salads **(Wetherilt and Pala, 1994; Cowan, 1999; Digrak et al., 2001; and Nasar-Abbas and Halkman, 2004).** The bacteriostatic and bactericidal effects of water extract of sumac (WES) on foodborne pathogens, have been demonstrated in both broth and agar media **(Digrak et al., 2001 and Nasar-Abbas and Halkman, 2004)**, but we scarcely found studies in which WES is used as a meat surface decontaminant.

Among essential oils, thyme oil (TO) has increasingly gained the interest of researchers and food processors as a potential natural antimicrobial and antioxidant agent, as it contains high concentrations of phenolic compounds including carvacrol, thymol, p-cymene and g-terpinene **(Marino et al., 1999).**

Therefore, the present study was designed to assess the effect of some natural herbs including water extract of sumac (WES) and thyme oil (TO), as well as an organic acid (lactic acid, LA), as meat decontaminants, on the microbial quality and shelf-life of refrigerated rabbit meat.

2. Material and methods

2.1. Collection and preparation of rabbit meat samples

A total of 24 New Zealand rabbits were purchased from a small rabbit farm in Beni-Suef Governorate, they were of average body weight 2000 g. Before slaughtering, the rabbits were fasted for 6 h and supplied with fresh cool water, and then slaughtered by the Islamic method of slaughter, after that, dressing and evisceration were carried out at a rabbit market. Then cutting of each carcass into 4 quarters was done using market facilities and with the assistance of market workers. Then the samples were wrapped in sterile polyethylene bags and directly transferred to the laboratory in sterile icebox with a minimum of delay for further preparation and examination.

2.2. Preparation of meat decontaminants and samples grouping

Rabbit quarters were divided into 4 groups to be treated using the following solutions:

1. Control group (sterile DW): distilled water was autoclaved at 120 °C for 15 min then left to cool and refrigerated at 4 °C until use.
2. Lactic Acid treated group (Lactic acid 0.5 %): lactic acid 0.5 % (v/v) solution was prepared using pure lactic acid liquid L (-) (2013/1, ADWIC, Egypt) in distilled water of 4 °C, to be used at the same day of preparation.
3. Thyme treated group (thyme oil 0.5 %): 0.5 % concentration of thyme oil in DW was prepared. The mixture was cooled to 4 °C and used at the same day.
4. Sumac treated group (water extract of sumac 8 % "Rhus coriaria L."): the ripened (reddish-brown), chopped native sumac fruit was bought from a local retailer in Beni-Suef City, and added to sterile DW at the ratio of 8:100 (wt/vol) in a sterile bag and left at 45°C for 12 h. After that, the bag was squeezed by hand to crush its contents. The milled contents were then filtered through a piece of gauze into sterile Erlenmeyer flask. The contents of the flask were heated to 90 °C, cooled to 4 °C, and were used at the same day.

After preparation of rabbit samples and decontaminant treatments, dipping of each group of quarters was done on refrigerator shelves at 4 °C for 10 min in case of C and WES groups, while for 1 min in case of TO and LA groups.

2.3. Packaging and storage

After application of treatment solutions for the four groups; each group was aerobically packed as triplicates, as each three quarters were separately packed in a clean foam dishes and wrapped with cellophane casing. Then, the packages were labeled and stored at 2 ± 1 °C inside the refrigerator. The treated groups were examined at zero day (after one hour of dipping) then periodical examination every three days at 3rd, 6th, 9th, 12th, 15th and 18th day of storage was done until the time of spoilage.

2.4. Examination of treated rabbit samples

2.4.1. Sensory evaluation

Sensory evaluation of treated rabbit samples was performed according to the technique recommended by AMSA

(1995) with some modifications. Briefly; each rabbit quarter was cut and placed in an electric oven at 160 ºC for 25 minutes and served warm to the panelists. A total of five panelists (staff members from the laboratory) were used to evaluate the sensory attributes of rabbit samples. The rabbit samples were blind-coded by special codes and the panelists were not informed about the experimental approach. The sensory evaluation was carried out in artificial light, and the temperature of the packed product was similar to ambient temperature. Rabbit sample from each treatment was evaluated independently of the other on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely for appearance, odor, flavor, and texture. Average score (overall acceptability) below 6 indicates unacceptability.

2.4.2. Bacteriological examination

2.4.2.1. Preparation of the samples

Preparation of rabbit meat sample for bacteriological examination was performed according to the muscle maceration technique recommended by ICMSF (1978).

2.4.2.2. Bacteriological techniques

For determination of aerobic plate count at 35 ºC (APC), the pouring plate technique recommended by (AOAC, 2000) was applied, also for Most Probable Number (MPN) of coliforms, f. coliforms and E. coli; the three tubes method recommended by (AOAC, 2000) were carried out.

2.5. Statistical Analysis

Data were subjected to analysis of variances (one way-ANOVA) according to Knapp and Miller (1992) using (SPSS Statistics 17.0) software program. Data was expressed as mean values (n=3) with standard error. Differences among the mean values of the various treatments were determined by the least significant difference (LSD) test, and the significance was defined at $P < 0.05$.

3. Results

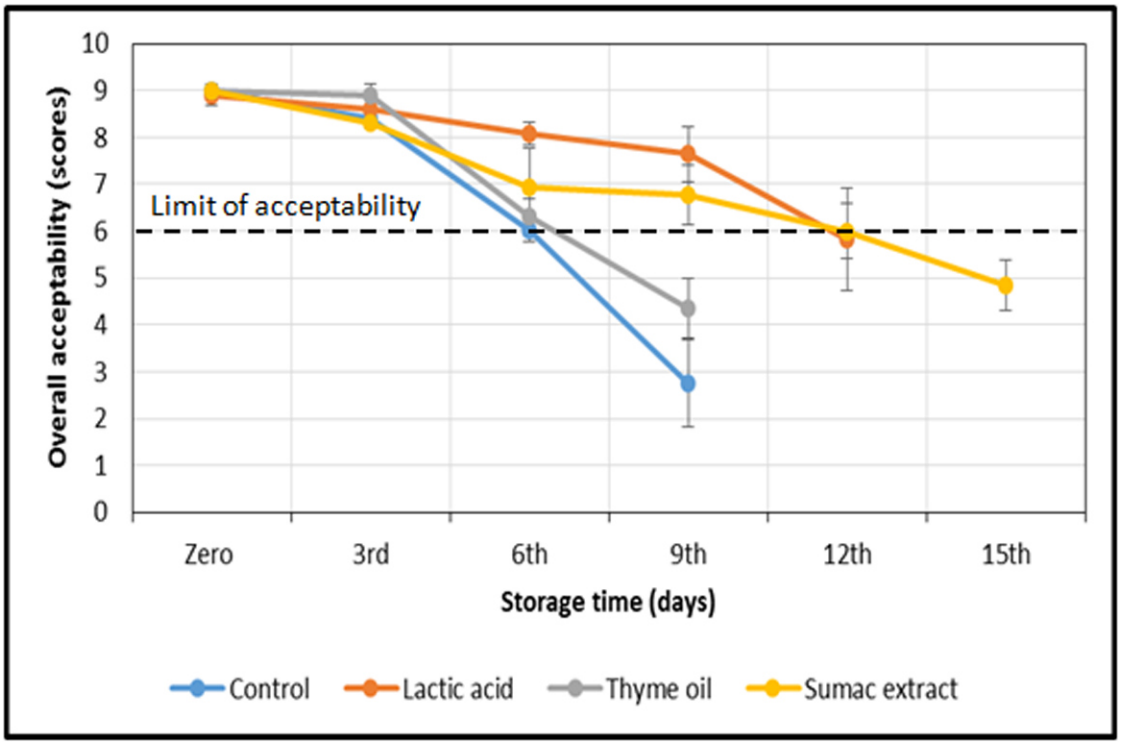

Figure (1): The changes in the overall acceptability of control and rabbit meat treated samples during refrigerated storage at 2 ± 1 °C. The error bars represent standard errors of triplicates.

Table (1): Changes in aerobic plate count at 35 °C (mean ± SE) of control and treated rabbit meat samples during refrigerated storage at 2±1 °C (CFU/g meat) (n=3) .

Storage days	Zero day	3rd day	6th day	9th day	12th day	15th day
Control (C)	6.3×10^{4a} $\pm2.8\times10^{4}$	6.4×10^{4a} $\pm2.7\times10^{4}$	4×10^{5a} $\pm4.7\times10^{4}$	1.15×10^{7a} $\pm3.36\times10^{6}$	-	-
Lactic acid (LA) 0.5%	2.66×10^{4a} $\pm2.37\times10^{4}$	3.7×10^{4a} $\pm3\times10^{4}$	3.9×10^{4b} $\pm3\times10^{4}$	9×10^{4b} $\pm6.4\times10^{4}$	2.9×10^{5a} $\pm1.1\times10^{5}$	-
Thyme oil (TO) 0.5%	5.17×10^{3a} $\pm2.6\times10^{3}$	5.97×10^{3a} $\pm2.6\times10^{3}$	1.7×10^{5a} $\pm1.1\times10^{4}$	3.95×10^{6a} $\pm1.97\times10^{5}$	-	-
Water extract of (WES) 8 %	2.9×10^{4a} $\pm2.88\times10^{4}$	3.3×10^{4a} ± 2.7×10^{4}	5.4×10^{4b} $\pm2\times10^{4}$	8.46×10^{4b} $\pm5.3\times10^{3}$	2.36×10^{5a} $\pm8\times10^{4}$	9.76×10^{5} $\pm3\times10^{5}$

(-): Indicates that it was not examined due to spoilage. Different small letters superscripts (a, b, c & d) within the same column indicates significant difference of means at p <0.05.

Table (2): Changes in coliforms (MPN) (mean ± SE) of control and treated rabbit meat samples during refrigerated storage at 2±1 °C (m.os./g meat) (n=3).

Storage days	Zero day	3rd day	6th day	9th day	12th day	15th day
Control (C)	1.8×10^{2a} $\pm7.1\times10$	6.3×10^{a} $\pm4.3\times10$	4×10^{2a} $\pm1.5\times10^{2}$	1×10^{3a} $\pm6.5\times10^{2}$	-	-
Lactic acid (LA) 0.5%	2.27×10^{b} $\pm1.2\times10$	1.2×10^{a} $\pm1.2\times10$	2.2×10^{b} $\pm1.3\times10$	3.3×10^{a} $\pm1.8\times10$	4.4×10^{a} $\pm2.6\times10$	-
Thyme oil (TO) 0.5%	1.9×10^{b} ±10.4	4×10^{a} $\pm2.5\times10$	2×10^{2a} $\pm2.7\times10$	2.7×10^{2a} $\pm6.2\times10$	-	-
Water extract of (WES) 8 %	1.1×10^{b} ±4.7	1.3×10^{a} $\pm1.2\times10$	5×10^{b} $\pm2\times10$	1.6×10^{2a} $\pm1.5\times10^{2}$	2.5×10^{3b} $\pm1.7\times10^{2}$	4×10^{3} $\pm2.6\times10^{3}$

(-): indicates that it was not examined due to spoilage. Different small letters superscripts (a, b, c & d) within the same column indicates significant difference of means at p <0.05.

Table (3): Changes in faecal coliforms (MPN) (mean ± SE) of control and treated rabbit meat samples during refrigerated storage at 2±1 °C (m.os./g meat) (n=3) .

Storage days	Zero day	3rd day	6th day	9th day	12th day	15th day
Control (C)	3.1×10^{a} $\pm3.1\times10$	$<3^{a}\pm2.4$	$9.3^{a}\pm9.3$	5×10^{a} $\pm5\times10$	-	-
Lactic acid (LA) 0.5%	$<3^{a}\pm0.0$	$<3^{a}\pm0.0$	$<3^{a}\pm1.2$	$<3^{a}\pm2.46$	$3.66^{a}\pm3.66$	-
Thyme oil (TO) 0.5%	$<3^{a}\pm0.0$	$<3^{a}\pm2.47$	$6.7^{a}\pm6.7$	$9^{a}\pm9$	-	-
Water extract of (WES) 8 %	$<3^{a}\pm0.0$	$<3^{a}\pm1.2$	$<3^{a}\pm2.47$	$3.67^{a}\pm3.67$	$5^{a}\pm5$	9 ± 9

(-): indicates that it was not examined due to spoilage. Different small letters superscripts (a, b, c & d) within the same column indicates significant difference of means at p <0.05.

Table (4): Changes in E. coli (MPN) (mean ± SE) of control and treated rabbit meat samples during refrigerated storage at 2±1 °C (m.os./g meat) (n=3) .

Storage days	Zero day	3rd day	6th day	9th day	12th day	15th day
Control (C)	$<3^{a}$ ± 1.2	$<3^{a}$ ± 1.2	$<3.07^{a}$ ±3.07	6.67^{a} ±6.67	-	-
Lactic acid (LA) 0.5%	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	-
Thyme oil (TO) 0.5%	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	3.07^{a} ±3.07	-	-
Water extract of (WES) 8 %	$<3^{a}$ ± 0.0	$<3^{a}$ ± 0.0	$<3^{a}$ ± 1.2	$<3^{a}$ ± 2.47	6.13^{b} ± 1.27	8.6± 0.6

(-): indicates that it was not examined due to spoilage. Different small letters superscripts (a, b, c & d) within the same column indicates significant difference of means at $p < 0.05$.

4. Discussion

4.1. Sensory characteristics

Sensory evaluation is a simple, fast and the most popular method of assessing the freshness of poultry and rabbit meat as it provides immediate information about food quality (Reineccius, 1990). The illustrated data in Figure (1) outlines the changes in the sensory attributes of C and treated rabbit meat groups during refrigerated storage at 2 ±1 ºC. The average score of sensory attributes (overall acceptability) given by the panelists at zero-day of storage was 9 for each of C, WES and TO -treated samples, while it was 8.91 for LA treated samples, which could be attributed to the sour odor and flavor observed by the panelists in LA group. A sharp decline in the overall acceptability was observed in both C and TO-treated samples by the 6th day of storage. Moreover, the scores of sensory attributes (appearance, odor, texture and flavor) given by the panelists reduced with storage time in all groups, thus the overall acceptability scores declined to be below the limit of acceptability score (6), they were 2.76, 5.82, 4.36 & 4.84 for C, LA, TO and WES groups at 9th, 12th, 9th and 15th day of refrigeration storage at 2 ± 1 °C, respectively.

In conclusion, according the sensory evaluation results, WES and LA extended the shelf-life of rabbit meat for about 3 and 6 days in comparing with untreated group (C) during refrigeration storage at 2 ± 1 °C, respectively, while TO treated group showed similar shelf-life like C samples. Similar shelf-life extension of similar food item (poultry) during cold storage (3 ± 1 °C) by WES and LA was previously recorded by Gulmez et al. (2006).

4.2. Bacteriological status

4.2.1. Aerobic plate count at 35 °C

About one third and more of the food production around the world is lost annually as a result of microbial spoilage. Microbial enzymes are responsible for deterioration of most fresh and of lightly preserved foods. For this reason, the total number of microorganisms, named aerobic plate counts (APC), have been used in mandatory food standards in most European Countries, Japan and USA (Lund et al. 2000).

The estimated data in Table (1) showed that the mean values of APC at 35 °C of C, LA, TO and WES – treated rabbit meat groups were 6.3×10^{4}, 2.66×10^{4}, 5.17×10^{3} and 2.9×10^{4} CFU/g meat at zero-day of refrigeration storage, respectively, without significant difference (at $p < 0.05$) in between. It was noticed that these values gradually increased with storage time, without significant difference at 3rd day of storage (at $p < 0.05$), while at 6th and 9th days of cold storage, the APC of C and TO- treated groups was significantly higher than LA and WES-treated groups (at $p < 0.05$). Similar findings were reported by Gulmez et al. (2006). The present results confirm the previously reported concept that the slower growth rate of the total bacterial population the longer shelf life of rabbit meat. Besides, it was demonstrated that determination of the numbers and types of microorganisms of animal carcasses is important, not only from the standpoint of public health and for judging the effectiveness of sanitary measures during processing, but also for evaluating quality including shelf-life (Buttler et al., 1979).

Slight increase in APC of WES-treated group during refrigeration could be attributed to the antimicrobial activity of sumac (Rhus coriaria L.), which previously reported by Chung et al., (1998); Cowan et al. (1999) and Zalacain et al. (2003). Furthermore, Nasar-Abbas and Halkman (2004) mentioned that the bactericidal and

bacteriostatic effects of WES on foodborne bacteria, have been demonstrated on liquid and solid culturing media. Similar pattern of APC changes during storage to that of WES-treated groups was observed in LA-treated rabbit meat samples, which could be attributed to the obtained acidic pH by the addition of lactic acid that hinder the growth and multiplication of most species of microorganisms, this substantiate the hypothesis reported by Jay (1986) who demonstrated that the antimicrobial action of lactic acid is attributed to the undissociated lactic acid molecule and the reduction of pH below the level at which the growth of many bacteria is inhibited.

Although APC of any food item is not an assured index of its safety for consumption, nonetheless it is of a good significance in evaluating the hygienic measures under which it has been produced, handled and stored (Levine, 1961). In this respect, Özogul et al. (2004) stated that when APC reaches 10^6 CFU/g or ml in a food product, it is assumed to be at, or near, spoilage. In our present study, the APC at 35 °C of only control (untreated) samples that exceeded such limit at the 9th day of refrigerated storage (when off odours and taste), however, all of the treated groups did not exceed such limit even after spoilage. On the other hand, Zambuchini et al. (2008) reported that standards, guidelines and specifications often use much lower bacterial population or total plate count as indices of acceptability.

4.2.2. Coliforms (MPN)

Coliform group of bacteria in rabbit meat has been considered important in microbiological analysis on account of their significance as indicator organisms for pin pointing the unhygienic conditions during handling and processing. Therefore, presence of coliforms in rabbit meat may be responsible for their inferior quality resulting in economic losses besides their presence in a great number may raise the public health risks.

The obtained results in Table (2) clarified that coliforms (MPN) mean values in C, LA, TO and WES –treated rabbit meat groups were 1.8×10^2, 2.27×10, 1.9×10 and 1.1×10 m.os./ g, respectively at zero- day of refrigeration storage, and the value in C group was significantly higher than treated rabbit meat groups at $p < 0.05$. At 3rd day of refrigeration storage, it was observed that the MPN of coliforms were 6.3×10, 1.2×10, 4×10 and 1.3×10 m.os./ g, respectively, without significant difference ($p < 0.05$) between control and treated samples. Then the values of coliforms MPN was gradually increased with storage time, with significantly higher values were observed in C and TO-treated samples than other treated samples at 6th day of storage at $p< 0.05$, while there was no significant difference between all groups at 9th day of cold storage, however at 12th day of storage, LA treated group had significantly higher coliforms MPN than WES-treated samples.

In general, LA and WES-treated groups had the lowest coliforms MPN during refrigerated storage, which could be attributed to their antimicrobial activity that was previously reported by several studies include Cowan et al. (1999); Jay (1986); Nykänen et al. (1998); Sorrells et al. (1989) and Zalacain et al. (2003). In this respect, it was recorded that WES is rich in tannins, and the antimicrobial property of tannins is well documented (Chung et al., 1998). Moreover, Nasar-Abbas and Halkman (2004) have reported that not only the organic acids but also other agents in sumac are effective antimicrobial substances.

4.2.3. Faecal coliforms (MPN)

The presented results in Table (3) showed the MPN of faecal coliforms (mean ± SE) in untreated (control) and treated rabbit meat samples. Form such data, it is obvious that the faecal coliforms (MPN) in treated rabbit meat samples (LA, TO & WES) was negative (<3 ± 0.0 m.os./g meat) at zero-day of refrigeration storage, while in control samples was 3.1×10 ± 3.1×10. At 3rd day of storage, the recorded MPN values of faecal coliforms were <3 ± 2.4, <3 ± 0.0, <3 ± 2.47 and <3 ± 1.2 m.os./g meat in C, LA, TO and WES, respectively. These values were not dramatically changed at 6th day of storage, but they still approximately constant in case of LA and WES-treated rabbit meat groups, however they slightly increased to be 9.3 ± 9.3 and 6.7 ± 6.7 m.os./g meat in C and TO-treated rabbit meat groups, the same scenario was detected at 9th day of refrigeration storage at 2 ± 1 °C. Mostly, there was no significant difference (at $p< 0.05$) observed between untreated and treated samples at each occasion of examination. The most obvious figure in the results of faecal coliforms shown in Table (3) is that the level of faecal coliforms in the examined groups were very low, which could be attributed to their preparation of samples under strict hygienic measures., as the presence of faecal coliforms in food samples is indication of faecal contamination (Ali and Ibrahim, 2004; Mousa, 1986 and Youssef et al., 1985).

4.2.4. E. coli (MPN)

As noticed from the data outlined in Table (4) that the mean values of E. coli (MPN) were < 3 m.os/g meat until the 6th day of refrigeration, this value still constant in case of LA-treated group until the day of off odor and taste (time of spoilage by sensory evaluation results). Similar findings were reported by (Sorrells et al., 1989) who mentioned that the addition of organic acids to foods, either by fermentation or by deliberate addition, is an

important mechanism for controlling foodborne pathogens in a variety of foods. While in case of control and TO-treated groups, they increased to be 6.67 and 3.07 m.os./g meat, respectively at the day of spoilage. Concerning WES-treated group, it was observed that the MPN of E. coli mean value exceeded < 3 m.os/g meat at 12th and 15th day of cold storage to be 6.13 and 8.6 m.os/g meat, respectively. Similar figure to that of faecal coliforms was also recorded in E.coli (MPN) values, that they were greatly reduced or almost absent in most occasions of examination in all treated and untreated samples, and this result refers to the strict hygienic measures adopted during slaughtering, dressing, evisceration, preparation and storage of samples. The obtained results held the view reported by Rodríguez-Calleja et al. (2004) that it is important to consider carcass handling during slaughtering and hygienic measures during processing, which have direct influence on the potential pathogen load in food products and thus affecting human health. This risk can be avoided if adequate hygiene practices are adopted in animals` slaughterhouses, and supported by maintaining the cold storage temperature during transport, distribution and marketing.

5. Conclusion

From the obtained results in the present study it could be concluded that WES and LA extended the shelf-life of rabbit meat for about 3 and 6 days in comparing with C group during refrigeration storage at 2 ± 1 °C, respectively, while TO treated group showed similar shelf-life as C group, according to the sensory evaluation results. The APC of C and TO- treated rabbit meat groups were significantly higher than LA and WES-treated groups (at $p < 0.05$) at most occasions of examination. The APC results come in accordance with the results of sensory evaluation. Approximately, similar pattern to that of APC was observed in coliforms (MPN) results. The demonstrated MPN values of faecal coliforms and E. coli were low, which could be attributed to the preparation of rabbit samples under strict hygienic measures. WES and LA showed efficient antibacterial properties, and the sensory acceptability by panelists was the highest in WES group, besides, the adverse effects of chemical substances (LA) as a food additives such as their residues, so we recommend WES to be used as a decontaminant for rabbit and poultry meat at each decontamination step and even in kitchens because it is a natural, safe, and easily prepared product.

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