Improvement of Quality and Shelf-Life of Meat by Essential Oils of Laurel (*Laurus nobilis* L.) Leaves

Ebeed Saleh[1], Alaa Eldin Morshdy[2] and Atef M.K. Nassar[3], Hany Ramadan Salama[1]

[1]Food Hygiene and Control Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour, El-Beheira, Egypt.

[2]Food Hygiene and Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig City, Sharkia, Egypt.

[3]Plant Protection Department, Faculty of Agriculture, Damanhour University, Damanhour, El-Beheira, Egypt.

Abstract

Fresh meat is a highly perishable product due to its unique biochemical composition. Plant essential oils (EOs) serve as safe alternatives to synthetic antimicrobials (preservatives) that extend shelf-life of meat. Therefore, the aim of this study was to evaluate the effects of EOs of Laurel leaves (*Laurus nobilis* L.) on shelf-life, microbiological, chemical, and sensory characteristics of meat during chilling storage at 4 $\pm 1^0$ C. A total of 336 meat samples (divided into 4 trials) were collected from different butchers at Damanhur city, El-Beheira, Egypt. The effectiveness of Laurel's EO (LEO) at concentrations of 0.25, 0.5, and 1% as a natural preservative were studied compared to the non-treated samples. Chemical, microbial, and sensory evaluation were carried out after 0, 3, 6, 9, 12, 15, and 18 days of storage. Results showed significant preservative effects of 0.25, 0.5, and 1% of the LEO up to the 9th, 12th, and 15th day of storage, respectively. Moreover, significant enhancements of the microbiological, chemical and sensory parameters of treated meat samples were reported compared with the control group, which was spoiled at the 6th day of storage. Overall, the results of this study indicate that essential oils of leaves of Laurel could be applied on meat before storage to improve its qualities, safety, and storability.

Keywords: Meat, Shelf-life, *Laurus nobilis* L, Essential oils.

1. Introduction

Animal proteins such as meat, meat products, fish and fishery products are generally considered as high-risk commodities with regard to pathogen contents, natural toxins, and other possible contaminants, which affect seriously the food safety (Yousuf et al., 2008). Meat is subjected to enzymatic, chemical, and microbial changes that cause its deterioration. For example, microorganisms grow on meat causing organoleptic changes when they release

metabolites (Jackson et al., 2001). Spoilage of meat is due to microbial growth, oxidation and enzymatic autolysis. Moreover, the breakdown of fat, protein and carbohydrates in meat results in the development of off-odors, off-flavors and slime formation, which determine disagreeable meat for human consumption (Casaburi et al., 2015).

Therefore, preservation of meat is a must and achieved by many preservative methods, which significantly prolong the meat quality (Yousuf et al., 2008). Many complex processes are involved in food spoilage, and despite modern techniques of preservation, excessive amounts of foods are still wasted, mainly by the effect of microorganisms. Consumers nowadays favor food products with natural preservatives, natural antimicrobial agents and flavors due to their awareness about possible carcinogenic effects of synthetic preservatives and antibiotic resistance after long-term usage (Jayasena and Jo, 2013). Additionally, consumers preference is increasingly directed toward natural foods. Hence, the use of chemical additives is declining and the search for natural and safe alternative is growing (Viuda-Martos et al., 2008). Thus, the application of natural agents such as microbial metabolites, and plant or spice extracts has been rising significantly in the past few years (Cueva et al., 2011).

Essential oils (EOs) are well-known for its antimicrobial effects that could be applied to control food spoilage and food borne pathogenic bacteria (Burt, 2004). It has been demonstrated that several spices, herbs, and fruits containing essential oils efficiently inhibit microbial growth, although different results are observed depending on test conditions, microorganisms, and the source of the antimicrobial compound (Roller, 2003). The essential oils and various plant extracts have incited the food processors' interest as sources of natural products. They have been screened for its possible use as alternative cures in treating many infectious diseases and food preservatives, ensuring protection from the toxic effects of oxidants. In particular, the antimicrobial activities of plant essential oils and extracts have become the base for many applications, including raw and processed food preservatives, alternative, and natural medicines (Lis-Balchin and Deans, 1997).

Laurel *(Laurus Nobilis* L.*)* is an evergreen shrub or tree native of the southern Mediterranean area (Sellami et al., 2011). Its dried leaves are rich in the EOs and are used in the food industry as flavoring agent and used in traditional medicine (Ramos et al., 2012). Researchers reported that the potential application of laurel in food because of its rich content of secondary metabolites. Among them, Sellami et al. (2011) found that 1,8-cineole is the major component of laurel's EOs ranging between 31.4 and 56%. Other Compounds were present in appreciable amounts including linalool, trans-sabinene hydrate, methyl eugenol, α-terpinyl-acetate, eugenol, and sabinene. Also, some benzene compounds ranging between 1 and 12% were responsible for the spicy aroma of the leaves and are essential factors determining its sensory quality.

Considering the above-mentioned and through the available information, present study considered the first to investigate the effect of EOs of laurel leaves (LEOs) on meat quality and the extension of its shelf-life.

2. Materials and Methods

2.1. Extraction of essential oils

According to (Vilela et al., 2016) leaves of laurel were obtained, weighted, dried in an oven at approx. 40°C, grinded, and stored in the fridge until used. Then the material was submitted to hydro-distillation (1:10, w:v) using a Clevenger type apparatus for about 3 h. Plant materials were placed inside a volumetric flask (2L) with distilled water and heated to boiling degree on a mantle. The oil Clevenger was connected to a condenser. As the water boils, the formed vapor carries the volatile compounds retained in the sample, which condenses in the condenser and been collected in the Clevenger column. Then LEOs were collected and stored in 5-ml dark brown tubes at 4°C.

2.2. Preparation of meat samples

A total 84 meat fillets samples each weighing 90 ±10g were purchased from butchers in Damanhur City, El-Beheira government, Egypt. The samples were rapidly transferred in separate sterile and labeled plastic bags in an ice box to the postgraduate laboratory, Food Control Department, Faculty of Veterinary Medicine, Damanhur University, under complete aseptic conditions without undue delay. The slices were divided into 4 groups (control, 0.25, 0.5, and 1% of the EOs) and 21 samples in each group. Each treated sample was dipped for 15 min in the concentration solution, and then drained well for 5 min. Control groups were dipped in sterile distilled water. After dipping, meat slices were labeled, and each single sample was aerobically packed separately in polyethylene bags then stored at 4±1°C. Each group was subjected to sensory, chemical and bacteriological assessment at day zero (within 2 hours after treatment) then periodically every three days until decomposition (0, 3rd, 6th, 9th, 12th, 15th and18th). The scheme was replicated 4 times.

2.3. Sensory evaluation

About twenty volunteer panelists (adult, untrained) were asked to evaluate the sensory attributes of meat samples. The samples were blind–coded and panelists were informed about the experimental approach before their participation in the test. They were asked to give a score for each of color, odor, and consistency while the samples were raw. The samples without salt and spices were cooked then were served to the panelists to complete the evaluation of the sensory attributes. The panelists washed their mouths with warm water between samples. The taste test was carried out at 0, 3, 6, 9, 12, 15, and 18 day of the dipping in the EO concentrations.

Nine-point descriptive scale was used. A score of 7–9 indicated ‘‘very good’’, 4.0–6.9 ‘‘good’’, and 1.0–3.9 denoted as spoiled **(Amerina et al., 1965)**.

2.4. Chemical analysis

Meat quality parameters of pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and peroxide value (PV) were quantified. The pH was determined by using a pH meter (Digital, JENCO 609) according to the method recommended by (EOS 63/11, 2006). The TVB-N (mg/100g) was estimated by (EOS 63/10, 2006). TBA content was expressed as milligrams of malondialdehyde equivalents per kilogram of sample (EOS 63/9, 2006). The PV was expressed as milliequivalents of oxygen/kilogram of lipid (Sallam et al., 2004).

2.5. Microbial evaluation

Meat sample (10g) was aseptically transferred into homogenizer flask containing 90 ml sterile peptone water 0.1%. The content was homogenized at 4000 rpm for 2.5 min. To provide a dilution of 10^{-1} the original homogenate was allowed to stand for 5 min at room temperature then was mixed thoroughly by shaking, then ten folds serial dilution were done (ISO 6887-6, 2013).

Total viable count (TVC) was determined on plate count agar after incubation for 48 ± 2 h at 37 °C. Total *Psychrophilic* bacterial count (TP) was determined on pour plate technique and incubated at 4 °C for 5-7 days. TVC and TP were detected according to (Swanson et al., 1992). *Staphylococcal* count was enumerated on Baird Parker agar medium and incubated at 37 °C for 48 hrs according to ICMSF (1996). *Enterobacteriaceae* were enumerated on Violet Red Bile glucose agar medium and incubated at 37 °C for 24 hrs according to ISO 21528-2 (2004).

2.6. Statistical analysis

Data was statistically analyzed using the Statistical Analysis System (SAS, Cary, USA, version 9.3) software (SAS, 2016). The chemical, microbiological, organoleptic parameters were presented as mean ± SD. Significant means were compared using Tukey’s Studentized Range (HSD) *post-hoc* Test ($P \leq 0.05$).

3. Results and discussion

3.1. Sensory evaluation

The results of organoleptic examination of meat samples stored at 4 °C revealed that the control sample was completely spoiled (rejected) at the 6th day of storage (Fig 1). The addition of LEOs at 0.25, 0.5, and 1% significantly improved the appearance (Fig 1A), smell (Fig 1B), taste (Fig 1C), texture (Fig 1D), and overall acceptability (Fig 1E) sensory properties till the 9th, 12th,

and 15th day of storage, respectively. There were significant differences between the control and treated groups of meat samples. These results were in agreement with (Silveira et al., 2014) who reported that the addition of LEOs had a significant impact in the improvement of the sensory properties of fresh Tuscan sausage. Similarly, Alparslan et al. (2014) reported that texture, odor, color, and overall acceptability of gelatin film containing 1% of LEOs enhanced the acceptability of Rainbow Trout fillets up to 22 days. Also, Ozogul et al. (2013) reported that LEOs extended the shelf-life of vacuum-packed and refrigerated European eel (*Anguilla anguilla*) fillets from 16 days after the treatment.

3.2. Chemical analysis

The initial pH values of control and treated samples LEOs (0.25, 0.5, and 1%) were 5.69, 5.65, 5.63, and 5.59, respectively. Along the storage, pH values were increased with different degrees within untreated and treated meat samples due to endogenous enzymes effects and bacterial metabolites such as hydrogen sulfides, organic sulfides, and other volatile organic compounds like amines (Gill, 1986). The addition of LEOs resulted in significantly decreased pH compared to the control (Fig. 2A). meat is not suitable for consumption when the pH is over 6.4 or below 5.6 (Skrökki, 1993). The pH value of the control was 6.83 at day 6 of storage while, in the treated samples LEOs (0.25, 0.5, and 1%) it was increased close to safety margin 6.39, 6.38, and 6.35 at days 9, 12, and 15 of storage, respectively. These results were similar to that of Silveira et al. (2014) who reported that the pH values of control were significantly higher than in those treated with laurel EOs. Also, Alparslan et al. (2014) reported that during storage, pH was lower in Rainbow Trout (*Oncorhynchus mykiss*) fillets that were wrapped in gelatin film containing LEO and there was a statistically significant difference among the groups ($p<0.05$).

Total volatile nitrogen (TVBN mg/100g) is used as a quality indicator of meat and meat products. It is associated with decarboxylation of amino acid activity of microorganisms (Jay, 1992). The acceptable limit of TVBN is 20 mg according to the EOS (2013). Results reported in Fig 2B showed that the initial TVBN values of treated samples with 0, 0.25, 0.5, and 1% of LEOs were 6.49, 6.43, 6.40, and 6.11, respectively. Moreover, after storage the TVBN of all groups of samples progressively increased with different rates depending on the nature of treatments. The control group showed the highest incremental rate compared to other treated groups where the TVBN value of the control reached 23.78 mg after 6 days in storage (Fig 2 B). Moreover, TVBN values in the 0.25, 0.5, and 1% treated samples were 18.94, 19.38, and 19.35 at 9, 12, and 15 days of storage, respectively. The addition of LEOs resulted in significant decrease in the accumulation of basic volatile nitrogen compared to the control. Ozogul et al. (2013) found significant differences ($P < 0.05$) in TVBN levels after 4 days of storage and

control group deteriorated more rapidly than did European eel (*Anguilla anguilla*) fillets treated with LEO.

TBA (mg malonaldehyde equ/Kg) values is used as an indicator of lipid oxidation in meat during storage and when TBA values reach 0.9 the rancid flavor is initially detected in meat (EOS, 2013). The initial TBA values of control and LEOs (0.25, 0.5, and 1%) were 0.28, 0.27, 0.24, and 0.20, respectively. There was a significant difference between the control and treated samples after zero day of storage. TBA value of the control was 1.06 at day 6 of storage (Fig. 2C). While TBA values in the treated samples were 0.83, 0.80, and 0.85 at days 9, 12, and 15 of storage for LEOs (0.25, 0.5, and 1%), respectively. These results regarding the antioxidant effect of laurel EOs. Also, Alparslan et al. (2014) found that a significant increase in thiobarbituric acid content in control samples compared to the Rainbow Trout samples wrapped in gelatin film only or gelatin film containing different amounts of LEO ($p<0.05$) during storage.

Evaluation of spoilage rate peroxide value (milli Equ O_2/Kg lipid) indicates the overall picture of rancidity of fat present in meat. Lipid peroxidation is one of the prime mechanisms of quality deterioration in stored meat and meat products (Pearson et al., 1983). The initial PV values of control and LEOs (0.25, 0.5, and 1%) were 0.36, 0.34, 0.30, and 0.26 respectively (Fig. 2D). There was a significant difference between the control and treated samples after zero day of storage. PV value of the control was 1.60 at day 6 of storage while, in 0.25, 0.5, and 1% treated samples were 1.40, 1.39, and 1.52 (milli Equ O_2/Kg lipid) at days 9, 12, and 15 days of storage, respectively. These results were in agreement with Alparslan et al. (2014) who reported that the PV increase of the gelatin film containing different amounts of LEO in rainbow trout fillets was slower ($P<0.05$) than the control.

3.3. Microbial evaluation

The initial count of TVC of the control and treated samples (0.25, 0.5, and 1%) were 5.79, 5.76, 5.71, and 5.65 log CFU/g, respectively (Fig. 3A). The microbial populations were significantly lower ($P<0.05$) in the treated samples than the control except for day zero. Significant differences were observed between the treated meat samples with 0.25, 0.5, and 1% LEO after 6 days of storage. The high concentration of LEO (1%) was more effective in decreasing the total viable count compared to 0.25 and 0.5% levels. TVC increased with storage time for all groups. The TVC exceeded the value of 7 log CFU/g that is considered as the upper microbiological limit for good quality meat, as determined by ICMSF (1986). TVC was 7.17 log cfu/g for the control group at day 6, 6.87 log cfu/g for 0.25% of LEO at day 9, 6.88 log cfu/g for LEO 0.5% at day 12, and 6.81 log cfu/g for 1% LEO at day 15 of storage (Fig 3A). These values exceeded the acceptable limit after 12, 15, and 18 days of storage for 0.25, 0.5, and 1% LEOs, respectively. These results were similar to that of Vilela et al. (2016). They reported that LEOs

reduced the TVC in fresh Maronesa beef burgers by almost 1 log CFU/g at 2 °C. Also, Silveira et al. (2014) found that the Tuscan sausage had an initial contamination with TVC close to 4 log CFU/g after 2, 8, 10, and 12 days of storage and this was significantly lower in sausages containing laurel EOs than in the control sample.

With respect to the *Psychrophilic*, the initial count for control sample was 5.31 log CFU/g while the treated samples with 0.25, 0.5, and 1% of LEOs were 5.28, 5.24, and 5.18 log CFU/g, respectively (Fig. 3B). The changes in TP were approximately similar to those of TVC. There were significant differences ($P<0.05$) between the control and treated groups after zero day. The TP counts were 6.43 log CFU/g for the control group at day 6, 6.32 log CFU/g for 0.25 % of LEO at day 9, 6.29 log CFU/g for 0.5% of LEO at day 12, and 6.16 log CFU/g for 1% of LEO after 15 days of storage. These results agreed with Vilela et al. (2016) who stated that laurel EOs had positive effects in reducing counts of *Psychrophilic* after 48 h of storage in fresh Maronesa beef burgers. Similarly, Silveira et al. (2014) reported that the initial count of *Psychrophilic* was 4 log CFU/g for all samples and the treated sausages with laural EOs were significantly lower than in the control except for days 0 and 4. Also, Alparslan et al. (2014) reported that samples of Rainbow Trout (*Oncorhynchus mykiss*) fillets that were wrapped in gelatin film containing 1% LEO had the lowest *Psychrophilic* count when compared to the control during storage.

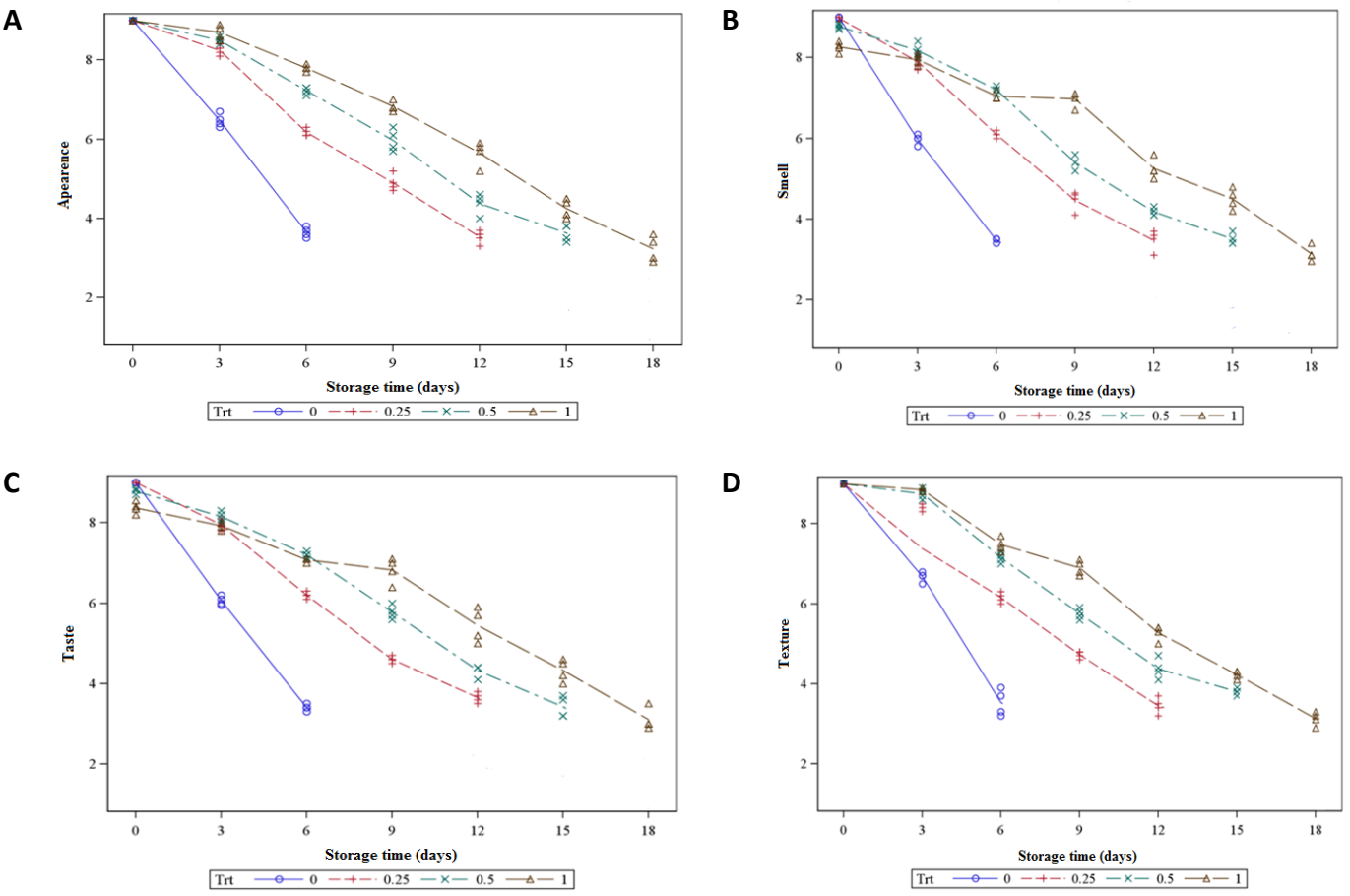
Enterobacteriaceae, regarded as a hygienic indicator, is one of the meat-causing spoilage microorganisms. The initial count for control sample was 3.84 log CFU/g while in the treated samples (0.25, 0.5, and 1% LEOs) were 3.81, 3.77, and 3.71 log CFU/g, respectively (Fig. 3C). The effect of the addition of LEOs (0.25, 0.5, and 1%) was significantly suppressing the growth of *Enterobacteriaceae*. Counts of *Enterobacteriaceae* were 4.71, 4.51, 4.55, and 4.48 log CFU/g for the control, 0.25, 0.5, and 1% of LEOs after 6, 9, 12, and 15 days in storage. These results agreed with Vilela et al. (2016) who reported that laurel EO maintained *Enterobacteriaceae* counts below control samples counts in fresh Maronesa beef burgers.

With regard to the antimicrobial actions of the LEOs on *Staphylococcus*, the initial count for control sample was 2.69 log CFU/g while in the treated samples (0.25, 0.5, and 1% LEOs) were 2.66, 2.62, and 2.56 log CFU/g, respectively (Fig. 3D). The microbial populations were significantly lower ($P<0.05$) in the treated samples compared to the control. *Staphylococcus* counts were 3.42, 2.85, 2.81, and 2.72 log CFU/g for the control, 0.25, 0.5, and 1% groups of LEOs after 6, 9, 12, and 15 days of storage. These results highlight the significant protective effect of LEO against meat content of *Staphylococcus*. The antibacterial activity of Laurels essential oils against *Staphylococcus aurus* among others by disc diffusion assay was studied by Derwich et al. (2009) who found that *Staphylococcus aureus* was the most sensitive strain tested

to *Laurus nobilis* EOs with the strongest inhibition zone. Also, Ghadiri et al. (2014) found that *Laurus nobilis* extract had antibacterial activity against *staphylococcus aureus* by disc diffusion assay. According to Keskin et al. (2010) found that the antimicrobial activity of LEO was active against *Staphylococcus aureus* with inhibition zones 18 mm.

Conclusions

In conclusion, essential oils of natural products such as *Laurus nobilis* would be used in the preparation of foods and labelled as "Generally Recognized as Safe" (GRAS). The results of current study demonstrated that the application of LEOs on meat at the concentration of 0.25, 0.5 and 0.1 % provide additional protection of the product against microbial growth and increase its shelf life. In addition to, the treated meat samples with LEOs was considered acceptable by consumers at the three tested concentrations. The overall results indicate that *Laurus nobilis* essential oils can potentially be applied in meat to improve its safety and shelf life.



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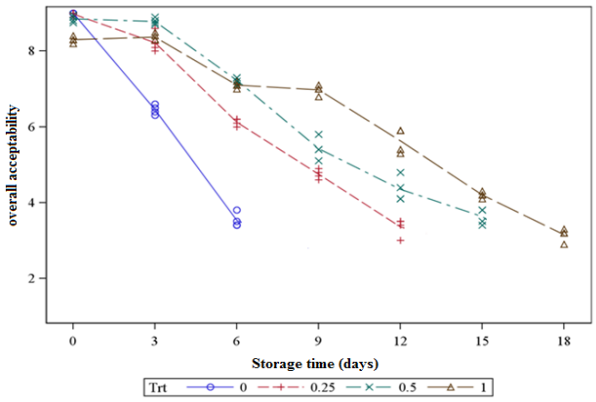
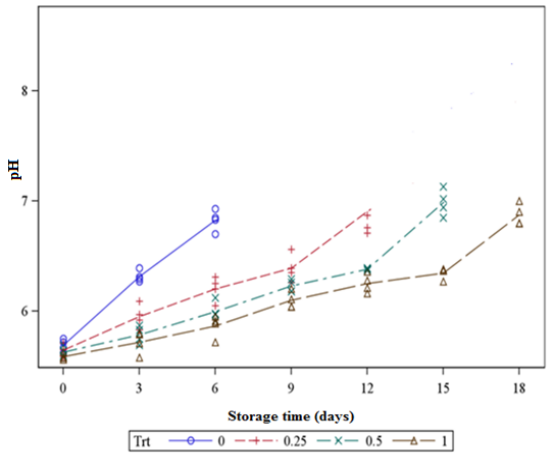


Fig. (1): Sensory evaluation of meat fillets stored at 4 °C. Control (0) and with the addition of *Laurus nobilis* EO 0.25, 0.5, and 1%. (A) Appearance, (B) Smell, (C) Taste, (D) Texture, and (E) Overall acceptability.

A



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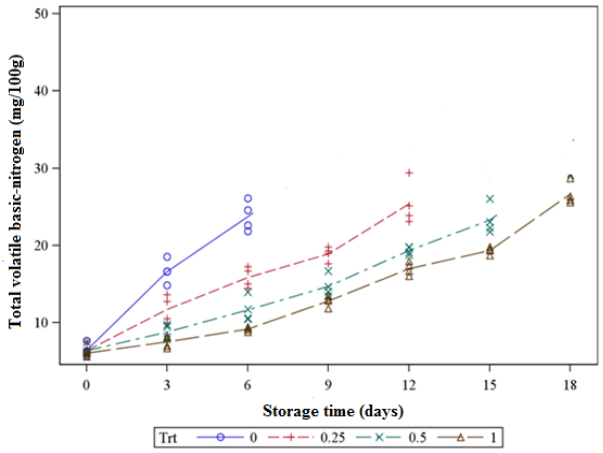

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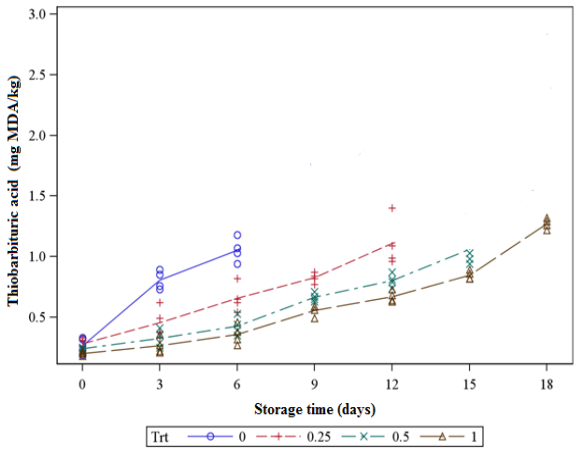

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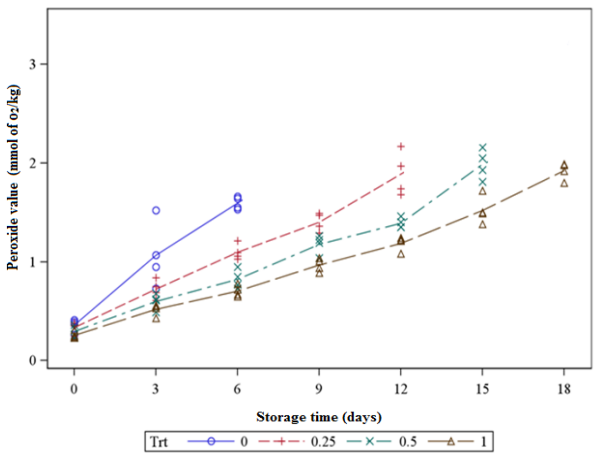

Fig. (2): Changes in chemical parameters of (A) pH, (B) Total volatile basic nitrogen (TVBN), (C) thiobarbituric acid (TBA), and (D) peroxide value (PV) contents of meat fillets stored at 4 °C after 0, 3, 6, 9, 12, 15, and 18 days of dipping in 0, 0.25, 0.5, and 1% of EOs of *Laurus nobilis*.

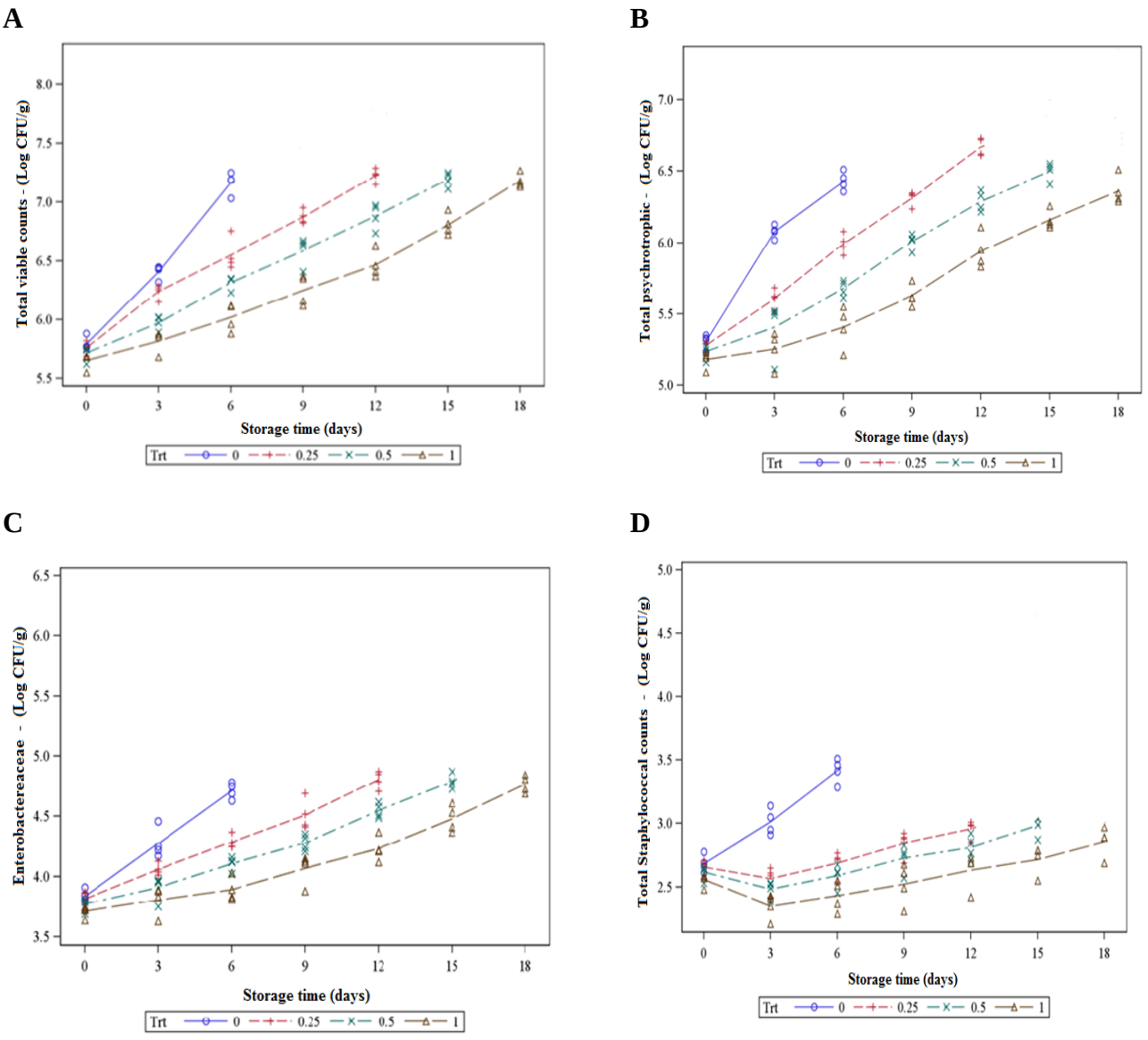

Fig. (3): Microbiolgical quality of (A) total viable (TVC), (B) total *Psychrophilic*, (C) *Enterobacteriaceae*, and (D) *Staphylococcal* counts of meat fillets after 0, 3, 6, 9, 12, 15, and 18 days of dipping in 0, 0.25, 0.5, and 1% of EOs of *Laurus nobilis* leaves at 4 °C.

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تحسين جودة وفترة حفظ اللحوم باستخدام الزيوت الطيارة لاوراق نبات اللوري

أ.د. عبيد صالح[1] ، أ.د. علاء الدين مرشدي[2]، أ.د.م. عاطف نصار[3]، د. هاني رمضان سلامه[1]

[1]قسم سلامة الغذاء ، كلية الطب البيطرى ، جامعة دمنهور ، دمنهور ، البحيرة ، مصر.[2]قسم سلامة الغذاء ، كلية الطب البيطرى ، جامعة الزقازيق ، الزقازيق ، الشرقية ، مصر. [3]قسم وقاية النبات كلية الزراعة ، جامعة دمنهور ، دمنهور ، البحيرة ، مصر.

تعتبر اللحوم من المنتجات سريعة التلف مما يدعو الي ضرورة اضافة المواد الحافظة اليها للحفاظ علي خواصها وجودتها. وتعمل الزيوت النباتية الطيارة (EOs) كبديل آمن لحفظ اللحوم عن المواد الكيميائية أو الاصطناعية حيث تثبط أكسدة الدهون وبالتالي تزيد من صلاحيتها. لذلك كان الهدف من هذه الدراسة هو تقييم تأثير الزيوت الطيارة لاوراق نبات اللوري على صلاحية وجودة اللحوم بعد حفظها (4±1°م) لفترات زمنية 0 ، 3 ، 6 ، 9 ، 12 ، 15 ،و 18 يوم من المعاملة. تم جمع 363 عينة من اللحوم من جزارين مختلفين من مدينة دمنهور ، محافظة البحيرة ، مصر وتم تقسيمها إلى 4 تجارب. تم دراسة تأثير زيت اللوري بتركيزات 0.0 ، 0.25 ، 0.5 ،و 1 ٪ كمادة حافظة طبيعية. وتم أجراء تحليل المكونات الكيميائية والعد الميكروبي والتقييم الحسي بعد الفترات التخزينية. أوضحت النتائج أن التركيزات المطبقة من زيت اللوري (0.25 ، 0.5 ،و 1٪) حفظت اللحوم صالحة للاستهلاك حتى اليوم الخامس عشر من المعاملة بالمقارنة بالمجموعة الضابطة (التي فسدت عند اليوم السادس من التخزين). أيضا أوضحت النتائج تحسن معنوى في الخواص الميكروبية ، الكيماوية ،و الحسية للعينات المعاملة الي 15 يوم من المعاملة. تشير نتائج هذه الدراسة الي أن زيت اللوري يمكن استخدامه كمادة حفظ طبيعية وبالاضافة الي قدرته علي تحسين الخواص المختلفة للحوم وزيادة فترة صلاحيتها مما قد يؤدى الي الاقبال المتزايد من المستهلكين عليها.