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Effect of different plants' aromatic essential oils on frozen Awassi lamb meat's chemical and physical characteristics

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PAPER

Abstract

The effect of drenching Awassi lambs with three aromatic essential oils from sage (*Salvia officinalis L.*), clove (*Syzygium aromaticum L.*), and laurel (*Laurus nobilis L.*) was investigated on meat chemical and physical characteristics, and oxidative and deterioration measurements. Twenty-four Awassi lambs, five to six months old, were divided into four groups. A concentrated diet was provided to the lambs at a rate of 3% of the body weight. The treatments were as follows: T1 was served as the untreated control, while T2, T3, and T4 were drenched with oils of sage, clove, and laurel, respectively. Drenching was carried out using water-soluble capsules containing 500 mg oil/capsule/day. Treatments lasted 90 days. At the end of the treatment period, the animals were fasted overnight and slaughtered. The carcasses were cleaned and kept at 4°C for 24 h. The longissimus dorsi (LD) muscle was then separated and preserved in a plastic bag for three preservation periods: no freezing and 30 days and 60 days freezing at –18°C. Several physical, fat, and protein stability analyses of meat were done after the preservation periods. The results indicated no significant effect of drenching Awassi lambs with different aromatic essential oils on the meat's physical and chemical characteristics. However, these oils, especially clove oil, affected fat and protein stability with increasing preservation period by freezing.

Keywords: Awassi; essential oil; *Laurus nobilis L.*; meat; *Salvia officinalis L.*; *Syzygium aromaticum L.*

Introduction

Meat is classified as a perishable commodity due to its high moisture content and nutrient availability, making it suitable for microbial growth (Kumar *et al.*, 2015, 2017). Meat contamination might occur at different stages, starting from the field and ending with the preparation for consumption, including slaughter, transportation, handling, storage, processing, marketing, and consumer's handling (Niyonzima *et al.*, 2015). Treatments used to keep meat from contamination and spoilage vary with each stage, including heating, refrigeration, hydrostatic pressure, packaging, ionizing radiation, chemical preservatives, salts, and bioactive compounds. Selecting appropriate treatments to maintain the meat and meat products' hygiene depends on several factors (Chen *et al.*, 2012).

Fats' oxidation and destruction by free radicals in cell membranes are natural processes affecting membrane

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transport and functions. Cell membrane phospholipids are rapidly affected by the oxidation process closely related to the fatty acids' saturation level. Free radicals react with these fatty acids and produce hydroperoxides that decompose to volatile aromatic compounds such as alkanes and aldehydes. These toxic substances affect animal products' nutritional value (Aminzare *et al.*, 2019), safety for consumption, quality of meat, organoleptic characteristics, and storage period (Fernandes *et al.*, 2018). Controlling free radicals' biological damage has recently become a topic of interest to researchers. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used commercially to prevent or reduce lipid peroxidation's unwanted effects. However, the demand to find or use plant-based natural compounds has recently increased, with consumers trying to protect meat and meat products from oxidation, spoilage, and pathogens (Aminzare *et al.*, 2019; Veneziani *et al.*, 2017). Natural antioxidants play an essential role in this field, protecting food from spoilage and maintaining public health. Essential oils are natural sources rich in antioxidants that inhibit free radicals such as phenolic compounds. These oils preserve animal fatty tissue and protect animal products from off-flavor and reactive oxygen resulting from oxidation of polyunsaturated fatty acids (Nitiema *et al.*, 2012). Bioactive compounds such as essential oils improve food quality and protect consumers from the adverse effects of oxidative stress and microbial spoilage (Simitzis and Deligeorgis, 2011). Essential oils are complex mixtures of many ingredients and are mainly composed of terpenoids with low molecular weight aliphatic hydrocarbons such as aromatic aldehydes and phenols (Dorman and Deans, 2000). They have antimicrobial activities, as many studies have confirmed the antimicrobial effects of these oils when used with different foods, which extends the shelf life by reducing spoilage (Calo *et al.*, 2015). For example, adding 0.25% cassia oil to refrigerated fresh chicken sausages increases the shelf life by 5–6 days and lowers the microbial count (Sharma *et al.*, 2017). In another study, it was found that wrapping chicken meat burgers with edible film incorporated with 0.10% oregano and 0.15% thyme essential oils increased shelf life up to 30 days (Soni *et al.*, 2018).

Feed processing with antioxidants such as essential oils is one of the easiest ways to deliver these antioxidants to membrane phospholipids, reducing the oxidation from free radicals and protecting animal products such as meat from fat oxidation (Fasolato *et al.*, 2015). Therefore, this method is considered the easiest and the most effective compared to the treatment of postmortem meat. There is a lack of sufficient research on using essential oils with Awassi sheep, the most common breed raised in Iraq. Fresh meat or meat refrigerated for 2–3 days is used for cooking. Finding a method to naturally increase the shelf-life without affecting the meat's quality and taste is important. Therefore, this research aimed to investigate the effects of three aromatic essential oils from sage (*Salvia officinalis L.*), clove (*Syzygium aromaticum L.*), and laurel (*Laurus nobilis L.*) on meat chemical and physical characteristics, and oxidative and deterioration measurements after different freezing periods.

Materials and Methods

Extraction of volatile oil

Dried seeds of clove (*Syzygium aromaticum L.*) and green leaves of sage (*Salvia officinalis L.*) and laurel (*Laurus nobilis L.*) were collected from local markets in Sulaymaniyah. The plants' identities were confirmed at the Department of Horticulture, Faculty of Agricultural Sciences, University of Sulaimani, where voucher specimens were deposited. The plants were dried in a freeze dryer and ground in a laboratory grinder. A hot oil extraction technique was used to extract oils. Then, water was added at a ratio of 5:1, and the mixture was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The volatile oil content was calculated as a relative percentage (v/w). Later, the essential oil was extracted from the milled sample by the hydro-distillation method using the Clevenger set in 1000 mL distilled water and refrigerated until use (Ranjitha and Vijiyalakshmi, 2014).

Animals and treatments

Twenty-four Awassi lambs, 5–6 months old, with an average weight of 28.4 kg, were divided according to weight into four treatment groups. The lambs were raised in individual cages and acclimatized for 2 weeks, followed by a treatment period lasting 90 days. During the experimental period, the lambs were fed a concentrated diet consisting of 35% wheat flour, 40% barley, 12% wheat bran, 10% soybean meal, 2.9% salt and limestone, and 0.1% premix. The energy content was 2791 cal/kg, and the protein content was 13.75/kg. Feed was provided to the lambs at 3% of their body weight. The lambs were subjected to weekly weight measurements, and the amount of feed was adjusted according to weight change.

The first group of lambs (T1) served as a control without drenching. Groups T2, T3, and T4 were drenched with sage oil, clove oil, and laurel oil, respectively. The drenching process was carried out using plastic syringes attached to a rubber tube. Each animal was given 0.5 mL of the extracted oil daily.

After the treatment period, the animals were fasted overnight and slaughtered. The carcasses were cleaned and

kept for 24 h at 4°C. Afterward, the longissimus dorsi (LD) muscle was separated, divided into several parts as appropriate, and each part was preserved in a plastic bag. Three preservation periods were used: zero freezing (P1), and 30-day freezing (P2) and 60-day freezing (P3) at –18°C.

Physical measurements

Several physical measurements were recorded on meat samples for each storage period, including pH, water holding capacity (WHC) (Dolatowski and Stasiak, 1998), thaw loss, and cooking loss (Purchas and Barton, 2012).

Chemical measurements

Several chemical measurements were also made on the stored meat samples at the end of each storage period, including meat chemical composition (moisture, protein, ether extract, and ash) (Horwitz and Latimer, 2005), thiobarbituric acid (TBA) (Gheisari *et al.*, 2010), total volatile nitrogen (TVN) (Pearson and Muslemuddin, 1971), and free fatty acids (FFA) (Pearson and Dustson, 1985).

Statistical analysis

SAS statistical analysis program was used to determine the effect of oil drenching and storage period on the studied measurements. Duncan's test was used to analyze the data to determine the effect of oil drenching and storage period on the studied measurements. Probability values of ≤0.05 were considered statistically significant.

Results

There was no significant effect of oil treatments on the LD muscle pH in all preservation periods. In contrast, a significant increase in pH values ($P \leq 0.05$) occurred for most treatments with increasing freezing periods (Table 1). Also, different oils did not affect the LD muscle WHC according to the control for all preservation periods, while a significant decrease in WHC was observed for most treatments with increasing freezing periods.

Values are presented as mean ± SEM. Different lowercase letters indicate significant differences between means within columns, while different uppercase letters indicate significant differences between means within rows ($P \leq 0.05$). WHC, water holding capacity.

No significant effects on thaw loss were observed for different oils and preservation periods, while a significant linear increase ($P \leq 0.05$) in thaw loss was observed for all oils with increasing preservation periods of up to 60 days (Table 1). No significant cooking loss was observed in the treated groups for all preservation periods, while a significant decrease was diagnosed with increasing preservation periods.

Table 2 illustrates the effect of different oils and freeze-preservation periods on TBA, FFA, and TVN values of the LD muscle. The results indicated a decreasing effect of oil treatments on TBA, FFA, and TVN values in all treatment groups. Group T3 drenched with clove oil recorded the lowest values than the rest of the oil drenching treatments and the control for all preservation periods. Data also recorded a significant linear increase in TBA, FFA, and TVN values for all treatments, increasing the preservation period to 60 days.

Values are presented as mean ± SEM. Different lowercase letters indicate significant differences between means within columns, while different uppercase letters indicate significant differences between means within rows ($P \leq 0.05$). TBA, thiobarbituric acid; TVN, total volatile nitrogen; FFA, free fatty acids.

Figure 1 shows the effect of different oil treatments and freeze-preservation periods on the LD muscles' chemical composition. There was no significant effect of oil treatments and preservation periods on the muscles' chemical components, despite decreasing moisture content and increased protein and fat content for all treatments with increasing preservation period.

Discussion

Maintaining meat's ultimate pH is very important because of its relationship to meat quality, color, and shelf life. Parvar *et al.* (2018) stated that feed additives, such as essential oils, had no significant effect on meat's final pH 24 h postmortem. Smeti *et al.* (2018) noted no significant effect of rosemary essential oils on lamb meat's final pH. Moreover, de Oliveira Monteschio *et al.* (2017) reported no significant effect of feeding clove and rosemary essential oils on final meat pH. These results agree with the results achieved in this research (Table 1). The results also agreed with Rivaroli *et al.* (2020) and Ornaghi *et al.* (2020), who indicated that the average pH of LD is between 5.5 and 5.8. This value is influenced by chilling and the stress to which the animal is subjected before slaughter. The research data indicated that the meat pH of the animals was within the normal limits, indicating the animals were calm when slaughtered and the carcasses were cooled well. Microorganisms and meat enzymes cause proteolysis, producing organic sulfides, ammonia, and amines, which raise the pH (Muela

Table 1. Effect of different oils and freeze-preservation periods on longissimus dorsi muscles' pH, WHC, thaw lass, and cooking loss.

Parameter	pH			WHC (%)			Thaw loss (%)			Cooking loss (%)	
Periods Groups	No freezing (P1)	30-day freezing (P2)	60-day freezing (P3)	No freezing (P1)	30-day freezing (P2)	60-day freezing (P3)	30-day freezing (P2)	60-day freezing (P3)	No freezing (P1)	30-day freezing (P2)	60-day freezing (P3)
Control (T1)	aB 5.72 ± 0.04	aAB 5.80 ± 0.05	aA 5.91 ± 0.02	aA 34.52 ± 0.74	aAB 34.32 ± 0.35	aB 33.85 ± 0.96	aB 3.89 ± 0.04	aA 4.49 ± 0.08	aA 34.75 ± 0.24	aA 34.16 ± 0.30	aB 33.41 ± 0.08
Sage oil (T2)	aB 5.58 ± 0.06	aAB 5.77 ± 0.03	aA 5.95 ± 0.02	aA 34.33 ± 0.96	aAB 34.10 ± 0.96	aB 33.56 ± 0.74	aB 3.75 ± 0.04	aA 4.10 ± 0.03	a A 34.53 ± 0.14	aAB 33.95 ± 0.15	aB 33.10 ± 0.08
Clove oil (T3)	aB 5.40 ± 0.06	aA 5.71 ± 0.12	aA 5.78 ± 0.05	aA 34.63 ± 1.00	aA 34.53 ± 0.96	aA 33.75 ± 0.96	aB 3.48 ± 0.03	aA 4.28 ± 0.07	aA 34.49 ± 0.18	aA 34.10 ± 0.08	aB 33.38 ± 0.08
Laurel oil (T4)	aA 5.63 ± 0.04	aA 5.81 ± 0.31	aA 5.79 ± 0.35	aA 34.55 ± 0.74	aB 34.22 ± 0.74	aC 33.85 ± 0.96	aB 3.62 ± 0.10	aA 4.37 ± 0.01	aA 34.69 ± 0.07	aAB 34.22 ± 0.10	aB 33.65 ± 0.05

Table 2. Effect of different oils and freezing periods on longissimus dorsi muscles' TBA, FFA, and TVN.

Parameter	TBA mg malonaldehyde/kg			FFA (%)			TVN mg N/100 g		
Periods Groups	No freezing (P1)	30-day freezing (P2)	60-day freezing (P3)	No freezing (P1)	30-day freezing (P2)	60-day freezing (P3)	No freezing (P1)	30-day freezing (P2)	60-day freezing (P3)
Control (T1)	aC 0.96 ± 0.01	aB 1.35 ± 0.01	aA 1.58 ± 0.02	aC 0.90 ± 0.31	aB 1.19 ± 0.71	aA 1.42 ± 0.28	aC 8.92 ± 0.05	aB 10.87 ± 0.18	aA 12.15 ± 0.39
Sage oil (T2)	bC 0.66 ± 0.01	bB 1.06 ± 0.08	bA 1.34 ± 0.03	bcC 0.66 ± 0.25	cB 0.84 ± 0.36	cA 0.98 ± 0.21	abC 7.89 ± 0.04	bB 9.45 ± 0.16	bA 10.94 ± 0.58
Clove oil (T3)	dB 0.47 ± 0.02	dA 0.77 ± 0.02	dA 0.76 ± 0.08	cC 0.51 ± 0.17	dB 0.68 ± 0.85	dA 0.82 ± 0.34	cB 5.24 ± 0.59	dA 6.75 ± 0.19	dA 7.22 ± 0.43
Laurel oil (T4)	cC 0.56 ± 0.01	cB 0.92 ± 0.01	cA 1.08 ± 0.01	abC 0.82 ± 0.20	bB 0.96 ± 0.43	bA 1.21 ± 0.22	bC 7.04 ± 0.19	cB 8.17 ± 0.23	cA 9.32 ± 0.30

et al., 2010). This research agreed with the results that indicated increasing pH values corresponding to an increased storage period. Increase in pH value with increasing storage period of oil treatments was not at the same level in the control group. The increase in control treatment pH value may have resulted from proteolysis, reduced by the effect of oils and their active substances. The antimicrobial activity of essential oils is due to the presence of hydroxyl groups. This antimicrobial activity is mediated by several mechanisms, including influence on the cytoplasmic membrane and active transport (Sharma *et al.*, 2020).

WHC is one of the most important measurements that determine meat quality and other characteristics. It is affected by many postmortem factors, including the extent of pH decline, proteolysis, and others (Huff-Lonergan and Lonergan, 2005). The results obtained in this research (Table 1) agree with Rivaroli *et al.* (2020), who indicated no significant effect of different essential oils on meat WHC. Ripoll *et al.* (2012) confirmed that WHC is greatly affected by meat pH. The average pH values of the treated lambs' meat may explain the absence of differences in WHC values between these treatments. The results also agree with Muela *et al.* (2015), who observed a decrease in WHC with an increased freezing period. The degradation of meat protein and its decreased ability to hold water may explain this outcome. There is a positive relationship between WHC and pH, as lactic acid production leads to a decrease in pH. As the pH reaches the isoelectric point of meat proteins (especially myosin), the excess charges that bind the protein with water are minimal, resulting in decreased WHC. With an increase in the pH and its rise above the isoelectric point of proteins, the negative or positive charges that are not associated with the protein increase, enabling the proteins to bind more water molecules, resulting in increased WHC. In the case of freezing (as in this research), although the pH increased as a result of proteolysis, a decrease in WHC occurred.

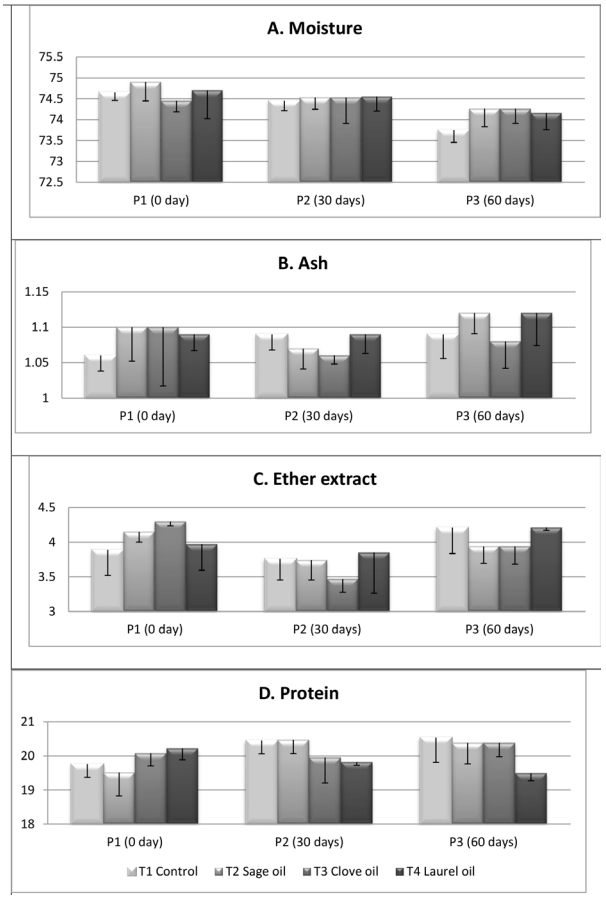

Figure 1. Effect of different oil treatments on the percentages of A. moisture, B. Ash, C. Ether extract, and D. Protein content of the treated lambs' longissimus dorsi muscles after different preservation periods.

This decrease may be due to the myofibril proteins shrinkage resulting from the presence of ice crystals that cause damage to muscle cells and denatured proteins, which increases in size with increasing storage period (Ripoll *et al.*, 2012).

This study indicated no significant effect of treatment with essential oils in cooking loss, which agrees with previous studies (de Oliveira Monteschio *et al.*, 2017; Smeti *et al.*, 2018). The results confirmed thawing losses with increasing preservation period and indicated a rise in total thaw loss with increased freezing time. These results coincide with previous studies (Muela *et al.*, 2015; Ornaghi *et al.*, 2020). Freezing increases thaw loss due to muscle membranes' mechanical damage by ice crystals, which leads to increased loss. Membrane damage may lead to increased protein denaturation and decreased WHC. Also, prolonged freezing causes increased water loss in meat by ice crystals damaging the cell membranes (Lu *et al.*, 2019).

Fat and protein oxidations affect meat quality and are related to meat deterioration. TBA level is an indicator of the extent of lipid oxidation through malondialdehyde concentration and color intensity measurement. A TBA content of 0.6–2.0 mg malondialdehyde/kg is considered to be within normal limits (Falowo *et al.*, 2014). The results revealed that the different essential oils decreased TBA levels. These results agreed with Parvar *et al.* (2018) and Ranucci *et al.* (2019). The oxidative indicator's decrease is because the essential oils are absorbed into the circulatory system ingestion, then distributed to muscles and the rest of the tissues (Velasco and Williams, 2011). Phenolic compounds are considered antioxidants due to their ability to inhibit free radicals by incorporating them into the aromatic ring (Maqsood *et al.*, 2014). Nieto *et al.* (2010) stated that feed additives allow antioxidants such as essential oils to get into tissues and cellular membranes, protecting them from oxidative stress by reactive oxygen. This process has proven to be more effective than treating meat with antioxidants postmortem (Kumar *et al.*, 2018).

The even distribution of antioxidants within the tissues and cells ensures the effectiveness of these compounds against fat and protein oxidation. The results in Table 2 also agreed with that reported by Politeo *et al.* (2006), who ranked 12 essential oils in the descending order according to their capacity as antioxidants, including the oils used in this research. They showed that the antioxidant efficacy of clove oil was the highest. Other studies recorded an increase in TBA relative to the increased storage time (Ranucci *et al.*, 2019; Rivaroli *et al.*, 2020). O'sullivan *et al.* (2004) stated that increased storage time leads to increased lipid oxidation, breakdown of peroxides, and increased secondary compounds resulting from oxidation, represented by malondialdehyde.

FFA is formed from fat and oil hydrolysis and is considered a measure of degradation by microorganisms and lipolytic enzymes (Rahman *et al.*, 2015). The FFA results in Table 2 agreed with Ozogul *et al.* (2017), who observed a significant decrease in FFA for the different nano oil treatments and all preservation periods, compared with the control treatment. The results also indicated that a decrease in FFA is due to additives' ability to inhibit lipolytic bacteria growth (Maqsood *et al.*, 2015; Rahman *et al.*, 2020). Several hypotheses explain the essential oils and phenolic compounds' antimicrobial activity, but they are not yet proven (Kalogianni *et al.*, 2020). Olatunde and Benjakul (2018) reported that essential oils could destroy bacteria by interacting with bacterial cell wall proteins. This interaction increases membranes' permeability and leaking of cytoplasmic structures and potassium ions, causing cell death (Bajpai *et al.*, 2008). At the same time, other researchers reported that the decrease in pH and increase of phenols increased essential oils' hydrophobicity, favoring their attachment to pathogen's lipid cell membranes and increasing antimicrobial activity (Gutierrez *et al.*, 2009). Due to their hydrophobic properties, phenolic compounds are bound with microbial membrane lipids causing their disruption (Devi *et al.*, 2010; Trombetta *et al.*, 2005). This disruption leads to intracellular compounds efflux, protein functional dysregulation, and cell death (Devi *et al.*, 2010).

TVN is a product of meat and nonprotein nitrogenous substance degradation and a measure of meat deterioration. Degradation of nitrogenous substances is caused by microbial and endogenous proteolytic enzyme activity. This study's results agreed with Saleh *et al*. (2021), who reported decreased TVN values by feed additives, and Ozogul *et al.* (2017), who indicated a decrease in TVN following different nano oil treatments and storage times. The maximum permissible meat TVN content is 150 mg/kg. Research reports the antimicrobial effectiveness of phenolic compounds, which possibly destabilizes the bacterial cell membrane (Pisoschi *et al.*, 2018), leading to permanent damage of the cell membrane and intracellular organelles and bacterial internal enzyme inhibition causing cell death. Besides, phenolic compounds' antioxidant efficacy may increase proteins' stability and reduce TVN by reducing radicals (Moroney *et al.*, 2013). Our results confirmed increases in TVN values with increasing storage period. Custódio *et al.* (2018) indicated that TVN increases due to protein degradation by the internal meat enzymes and microorganisms' action.

This study's results revealed no significant effect of essential oils as feed additives on LD chemical composition. Other studies also reported similar results (Ranucci *et al.*, 2019; Rivaroli *et al.*, 2020). A decrease in moisture content with increasing storage time might occur due to protein denaturation, lack of WHC, and decomposition by microorganisms. This decrease in moisture leads to increased dry matter (protein ratios, ether extract, and ash) content (Al-Rubeii *et al.*, 2009; Sharma *et al.*, 2015).

Conclusion

The results of this research indicated no significant effect of drenching Awassi lambs with different aromatic essential oils of sage, clove, and laurel at a concentration of 500 mg/head/day on the physical and chemical characteristics of meat. However, these oils' effect was positive on fat oxidation and protein stability, increasing the preservation period, especially when using clove oil.

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