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Anti-Oxidative Impact of Liquid Smoke and Thyme Essential Oil on the Quality Characteristics of Chicken and Turkey Meatballs Products during Frozen Storage

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to evaluate the effect of liquid smoke (LS) produced from beech sawdust and thyme essential oil (TEO) as natural antioxidants in chicken and turkey meatballs during frozen storage. The LS and TEO were added to chicken and turkey meatballs at levels of 1% and 0.1%, respectively, and storage at -18 ± 1°C for 6 months. pH value, water holding capacity (WHC), total volatile nitrogen (TVN), thiobarbituricacid (TBA) value, peroxide values (PV), total phenolic compounds (TPC) and antioxidant activity (DPPH %) were determined. The results demonstrated that the addition of LS or TEO had a positive effect on storage stability and a little change in the physical properties, quality attributes, and significantly ($p \le 0.05$) increased the values of total phenolic compounds and antioxidant activity of chicken and turkey meatballs during frozen storage compared to the control sample. The data revealed that the application of liquid smoke had decreased the value of TBA, TVN, and PV as well as increased shelf life and could be useful to achieve high stability of activity of chicken and turkey meatballs during storage and was better accepted compared to TEO and control.

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Keywords: Meatballs; liquid smoke; thyme essential oil; natural antioxidant; quality characteristics.

1. INTRODUCTION

Chicken meat consumption continues to rise, owing to its reduced cost, ease of preparation, and desired nutritional value, which includes a high content of easily absorbed protein, low fat content, and a comparatively high content of polyunsaturated fatty acids when compared to beef [1,2]. The poultry industry has evolved and adapted to satisfy customer needs for meat products; today, further processed meats have the largest market share, followed by chicken parts and whole carcasses, with 47.6%, 41.8%, and 13%, respectively [3,4]. Meatballs are one of the most popular meat products in the world, and there are many different sorts (including chicken meatballs, beef meatballs and fish meatballs). The chicken meatballs are the most popular and widely consumed. They are frequently served with noodles or vermicelli, veggies, and gravy [5].

Meat spoiling can be caused by a variety of internal and exogenous reasons, such as physical damage or chemical changes [6]. The main causes of meat quality deterioration and meat product shelf life limitations are lipid and protein oxidation. Oxidation processes are the principal cause of meat quality degradation and meat product shelf life reduction, resulting in negative changes in nutritional value, sensory, and physicochemical qualities of meat [7]. Physical and chemical variables such as color, pH, and water holding capacity, lipid oxidation, and microbial contamination affect the nutritional and sensory characteristics of foods, particularly meat and meat products [8]. The pH value is regarded important since it influences several properties of meat and meat products, including shelf life, color, water holding capacity, and texture [9]. The breakdown of glycogen may cause a reduction in pH in meat, whereas partial proteolysis may cause a rise in pH value [10]. Followed by an increase in total volatile nitrogen [11]. Meat degradation can be delayed by using synthetic or natural antioxidants. Synthetic antioxidants, on the other hand, haven't been used as much because they could be harmful to people and be toxic [12]. Consumers are becoming more interested in using natural products that are rich in bioactive compounds to keep meat products stable during freezing [13].

Smoke flavorings are produced on a large scale and used in a variety of foods, including meat, fish, and cheese [14,15]. Smoking as a food processing method contributes to guaranteeing food safety due to various advantages, such as being rich in bioactive compounds, low cost, and control of polycyclic aromatic hydrocarbon (PAH) content [16,17]. Liquid smoking has recently gained popularity in the food industry [15]. Food smoking contains antibacterial and antioxidant chemicals such as aldehyde, carboxylic acids, and phenols, which act as preservatives [18,19].

Essential oils are complex natural combinations of hydrocarbons (mostly terpenoids) and oxygenated molecules (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers) [20,21]. Thyme (*Thymus vulgaris*) essential oil (TEO) is a rich source of aromatic bioactive components such as thymol and carvacrol, both of which have ant oxidative and antibacterial properties [22,23]. The small concentration of TEO is not enough to significantly extend the meat products' shelf life [24]. The objective of this study was to evaluate the effect of adding liquid smoke or TEO as natural antioxidants on the stability of chicken and turkey meatballs during frozen storage, which includes quality characteristics.

2. MATERIALS AND METHODS

2.1 Materials

36 kg of fresh chicken breast and turkey meat were purchased from the local market in Assiut city, Assiut, Egypt during September 2020 and transported under refrigeration to the laboratory within 30 min. Meat of chicken and turkey were washed carefully then deboned**.** The chicken breast and turkey meat were minced separately using a meat mincer. Some samples were used directly for physiochemical analysis at zero time, while others were prepared for processing products [25].

2.1.2 Beech sawdust waste

Beech sawdust waste was used in liquid smoke preparation and was obtained from a local furniture manufacturer in Assiut, Egypt.

2.1.3 Essential oil

Thyme (*Thymus vulgaris*) was purchased from Natural oil extraction unit, National Research Centre, Cairo, Egypt. (2020).

2.1.4 Spices

The spice mixture was prepared using equal weights (black pepper, sweet paprika, chili paprika, and cumin). Also, salt, rusk flour, and onions were obtained from the local market during September 2020 in Assiut city, Egypt.

2.1.5 Chemicals and reagent

All chemicals and reagents used in the analytical methods (analytical grade) were produced by Sigma chemical co. (St. Louis, M., USA) and purchased from EL. gamhouria trading chemicals and drugs co.

2.2 Methods

2.2.1 Preparation of liquid smoke

The beech sawdust waste was moistened to a moisture content of about 20%. A small laboratory smoke generator was used to generate smoke, and the destructive distillation was condensed using a small condenser. The accompanying substance were removed from the obtained smoke condensates by settling about 7 days at 4˚C, followed by centrifugation at 2500 rpm for 10 min., filtration by Watman No.1 papers, and titration by carbonate solution to pH 4- 5.5. Finally, it was diluted 1:4 with distilled water (condensate: distilled water) to obtain liquid smoke [26]. Some modification in this method was carried out by lengthening the condenser tube until a large amount of compounds of large molecular weight is deposited on the wall of the condenser to reduce harmful compounds, also passing of filtered liquid smoke through active charcoal filtration to obtain liquid smoke completely free from harmful substances such as benzo[a]pyrene.

2.2.2 Preparation of chicken and turkey meatballs samples

The ingredients for the meatballs include (minced meat of chicken and turkey 71 gm, rusk flour 10 gm, salt 2.00 gm, onion 7gm, black pepper 0.40 gm, sweet paprika 0.25 gm, chilli paprika 0.25 gm, cumin 0.10 gm, and iced water 9.00 ml) [27].

2.2.3 Preparation of meatballs treated with LS or TEO oil

The samples were divided into three groups; the first group was treated with 1% LS or 0.1% TEO of their weight, for chicken meatballs; the second group was treated with either 1% LS or 0.1% TEO of their weight, for turkey meatballs; and the third group was kept as a control group. Each sample was packed in polyethylene bags and stored under the refrigerator stored at -18 ± 1°C for 6 months [28].

2.2.4 pH value

The pH of meatballs sample were measured by homogenizing 10 gm of the sample with 100 ml distilled water for 30 sec. The pH of the prepared sample was measured using a pH meter (OAKTON, pH/ mV/°C meter, USA) with a glass electrode at 20P o PC according to the method described by [29].

2.2.5 Determination of water holding capacity (WHC)

Water holding capacity (WHC) was measured according to the methods mentioned by [30,31].

2.2.6 Determination of total volatile nitrogen (TVN)

The total volatile nitrogen content of chicken and turkey meatballs samples was determined according to the method of [32]. The results were given in milligrams of TVN per 100g sample.

2.2.7 Determination of thiobarbituric acid (TBA)

The value of thiobarbituric acid (TBA mg MDA/kg) was determined by a distillation method, according to [33].

2.2.8 Determination of peroxide value (PV)

The peroxide concentrations were measured in meq O_2/Kg of sample according to method described by [34]. The PV was calculated using the following formula:

$$\textbf{Peroxide value meq O2/ Kg muscle} = (\mathbf{V} - \mathbf{B}) \times \mathbf{Nf/W}) \ * \mathbf{1000}$$

where V is the volume of sodium thiosulfate consumed, B is the volume of normal sodium thiosulfate consumed during a blank titration, W is the weight of the sample (g) and Nf is the normality of sodium thiosulfate multiplied by a factor.

2.2.9 Determination of total phenolic compounds in meatballs

The Folin–Ciocalteu method [35] was used to determine the total phenol content. Gallic acid was used as a standard to produce the calibration curve. The total phenol content was measured in mg of Gallic acid equivalents (GAE) per 100 g of a fresh weight sample.

2.2.10 Determination of antioxidant activity in meatballs

Determination of DPPH radical scavenging activitymethod was used according to [36] with certain adjustments. In a 99 % ethanol solution, a 60-µmol/L DPPH solution was prepared. Extract from chicken and turkey meatballs (100µL) was reacted with 3.9 ml of the DPPH solution. The solution was incubated for 60 min in a dark environment. The absorbance (A) at 515 nm was measured using a spectrophotometer against a blank of 95% ethanol. Antioxidant activity was calculated as follows:

DPPH radical-scavenging activity (%) **= (A control-A sample)/ A control) *100**

Where, A is the absorbance at 515 nm. All DPPH tests were carried out in triplicate.

2.2.11 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using a three way completely randomised factorial design. Basic statistics and ANOVA were performed to test the significance within replications and between types meat, treatments and storage periods [37]. (L.S.D) tests were used to determine the differences among means at the level of 0.05%.

3. RESULTS AND DISCUSSION

3.1 Effect of LS or TEO on pH Value of Chicken and Turkey Meatballs

The pH values of chicken and turkey meatballs for control and treated with 1% LS or 0.1% TEO at zero time and during frozen storage up to 180 days are shown in Table 1. From these results, it could be observed that the sample treated with 1% liquid smoke or 0.1% TEO had a lower pH value than the control. With a significant difference (P<0.05) between treatments. The maximum TEO concentration was more successful in decreasing pH values than lower concentrations compared to untreated samples [38]. The pH of the meat treated with the higher concentration of liquid smoke will be lower. This is caused by the presence of phenol and organic acid in liquid smoke, which cause the pH to lower [39].

The pH values of all chicken and turkey meatball samples gradually increased as the frozen storage period was extended, with a significant difference (P<0.05) observed between the samples and frozen storage periods. The highest increased was observed in the control samples and the lowest in liquid smoke then TEO. The pH value rises during freezing storage, which could be due to microorganisms and endogenous enzymes breaking down meat proteins, releasing ammonia, organic sulfides, and amines, all of which raise pH values [40]. During different intervals of analysis, samples treated with thyme essential oil had lower pH values than control samples. This might be owing to the activation action of thyme oil as an antibacterial agent producing protein breakdown and the development of alkyl groups [41].

3.2 Effect of LS or TEO on Water Holding Capacity of Chicken and Turkey Meatballs

The ability of meat to bind and retain moisture (Water-holding capacity, WHC) is the most important quality of meat products, particularly frozen meat, because it influences consumer acceptance and final product weight [42].

Data given in Table 2 displayed the effect of addition 1% LS, and 0.1% TEO for chicken and turkey meatballs compared to control sample on (WHC) during frozen storage. The obtained results showed that the chicken and turkey meatballs with the addition of 1% LS or 0.1% TEO, the WHC increased.

During frozen storage, (WHC) slow decreased. With a significant effect (p<0.05) between treatments and frozen storage periods. The effects of freezing storage on the physical properties of turkey breast meat, such as WHC and cooking loss percentages, significantly decreased while the pH was significantly increased those obtained by [43]. When the pH of a muscle drops from 7 to 5.5, there is always some loss of WHC and the water is expelled into the intracellular space those suggest by [44].

3.3 Effect of LS or TEO on Total Volatile Nitrogen of Chicken and Turkey Meatballs

The total volatile nitrogen (TVN) is a positive predictor of meat and meat products quality during storage [45].

Table 1. Changes in pH values of chicken and turkey meatballs treated with LS or TEO during frozen storage up to 180 days

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	5.87	5.92	5.97	6.09	6.14	6.21	6.23	**6.06**
	LS	5.63	5.74	5.75	5.77	5.77	5.85	5.83	**5.76**
	TEO	5.73	5.86	5.85	5.83	5.84	5.92	5.96	**5.85**
Mean		**5.75**	**5.84**	**5.86**	**5.90**	**5.92**	**6.00**	**5.90**	**5.88**
Turkey	**C**	5.72	5.83	5.81	5.91	5.98	6.13	6.10	**5.92**
	LS	5.54	5.59	5.59	5.61	5.63	5.67	5.72	**5.62**
	TEO	5.67	5.70	5.73	5.78	5.81	5.90	5.90	**5.78**
Mean		**5.65**	**5.87**	**5.71**	**5.77**	**5.81**	**5.90**	**5.95**	**5.80**
Main effects	**C**	5.80	5.67	5.89	6.00	6.06	6.17	6.17	**5.96**
	LS	5.58	5.78	5.67	5.69	5.70	5.76	5.77	**5.71**
	TEO	5.70	5.77	5.79	5.80	5.82	5.91	5.93	**5.82**
Mean		**5.69**	**5.92**	**5.78**	**5.83**	**5.86**	**5.95**	**5.95**	
F-test A(Var.)=**		**L.S.D0.05**	**B= 0.016**	**AB=0.022**	**C =0.024**	**AC=n.s**	**BC=0.041**	**ABC=0.059**	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

F-test	**A= Types meat**	**
L.S.D0.05	**B=** Treatments	**0.016**
	C= Storage periods	**0.024**
	AB= interaction between types meat x treatments	**0.022**
	AC= interaction between types meat x storage periods	**n.s**
	BC= interaction between treatments x storage periods	**0.041**
	ABC= interaction between types meat x treatments x storage periods	**0.059**

Table 2. Changes in water holding capacity (WHC %) of chicken and turkey meatballs treated with LS or TEO during frozen storage

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	83.87	79.16	78.11	75.44	74.38	72.48	68.36	**75.97**
	LS	84.62	84.00	84.34	82.13	81.06	80.96	79.28	**82.34**
	TEO	81.64	81.30	81.30	80.74	79.67	80.05	79.00	**80.53**
Mean		**83.37**	**81.49**	**81.25**	**79.44**	**78.37**	**77.83**	**78.56**	**80.04**
Turkey	**C**	79.55	75.32	76.64	74.95	73.63	72.81	69.81	**74.67**
	LS	82.96	81.89	82.19	81.13	80.09	81.42	77.90	**81.08**
	TEO	84.25	83.53	83.08	80.28	80.59	81.46	78.36	**81.65**
Mean		**82.25**	**77.24**	**80.64**	**78.79**	**78.10**	**78.56**	**75.90**	**78.78**
Main effects	**C**	81.71	82.95	77.38	75.20	74.01	72.65	69.08	**76.14**
	LS	83.79	82.42	83.26	81.63	80.58	81.19	78.59	**81.64**
	TEO	82.94	80.87	82.19	80.51	80.13	80.75	78.68	**80.87**
Mean		**82.81**	**79.16**	**80.94**	**79.11**	**78.24**	**78.20**	**75.45**	
F-test A(Var.)=**		**L.S.D0.05**	**B= 0.37**	**AB= 0.53**	**C (P)=0.57**	**AC=0.80**	**BC=0.98**	**ABC=1.39**	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

The data presented in Table 3 showed that the TVN for chicken and turkey meatballs was affected by using LS and 0.1% TEO oil compared with the control during frozen storage periods.

At the end of frozen storage period, the control sample was recorded 12.54 and 11.98 mg / 100g. While, for samples containing 1% liquid smoke or TEO 0.1% the TVN was 9.71, 10.07 and 9.62, 10.08 mg/100g, respectively; for chicken and turkey meatballs. The TVN in meat products may be increased as the days of storage increased where protein break down (ammonia) may occur due to microbial growth and its proteolytic enzymes [11]. According to Table 3 Egyptian Organization for Standardization [46], for poultry meat, all the examined samples were within the accepted level as TVN were lower than 20 mg%. Freezing process led to the formation of ice crystals that have prevented the emergence of these bacteria. This proportion rises with the length of freezing storage, halting microbe development and reducing their numbers while creating adverse circumstances for the rest of them to growth [47].

From the obtained results, it was also observed that samples treated with LS or TEO caused a decrease in total volatile nitrogen compared to control samples during frozen storage periods with significantly ($P<0.05$) between types meat x treatments x storage periods. This could be due to the ability of phenol compounds to inhibit microbial activity. Phenols from liquid smoke are antimicrobial agents able to inhibit microbial activities, which is very effective in preserving meat [48]. During storage, the greatest rate of TVN values was recorded in control samples, and thyme oil was more effective in lowering TVN values [41].

3.4 Effect of LS or TEO on Thiobarbituric Acid (TBA) of Chicken and Turkey Meatballs

The change in TBA values for chicken and turkey meatballs samples were evaluated for 180 days during frozen storage, and the data are summarized in Table 4. At zero time of frozen storage, there were no significant differences between chicken and turkey meatballs samples treated with liquid smoke or TEO compared with control samples.

Moreover, throughout frozen storage periods, TBA values of the control sample increased significantly over time, reaching their highest values (0.87 and 0.91 mg malonaldhyde/kg sample) for chicken and turkey meatballs, at the end of frozen storage period. Although, during frozen storage the other treatments displayed a slight increase in TBA values. These increases were significantly smaller than that in the control group. The highest significant difference ($P<0.05$) was observed in the interaction between treatments and frozen storage periods. This might explain these observations due to the frozen storage and high antioxidant effect of liquid smoke and TEO, which is connected to the nature of their phenolic content. Freezing of foods does not stop the change in physicochemical, but it does slow down the activities that cause food to deteriorate [49,50]. TBA values of minced beef in the control sample increased rapidly with storage time, although TBA values of thyme treatment samples on day 0 were substantially lower than those of the control sample [51]. The phenols, which contain fewer carboxylic acids, are known to be strong antioxidants, so that liquid smoked products are more resistant to rancidity. Anti-oxidizing properties of the smoke are attributed more to the componentsdispersed than to the dispersing phase. Among the phenol group, the strongestantioxidants are 3-methylpyrocatechol and pyrogallol,then, in decreasing rate: Hydroquinone and its homologous, guaiac resins and their homologous mono-hydroxylphenols. Antioxidizing properties display alsoformic acid, benzoate acid, salicylic acid, vanilla. Theanti-oxidizing properties of smoke retards autooxidationof fats those suggest by [52]. Liquid smoke had a remarkable result in reduction of oxidation by affecting 2-thiobarbituric acid (TBARs) [53].

3.5 Effect of LS or TEO on Peroxide Values of Chicken and Turkey Meatballs

The changes of peroxide value for chicken and turkey meatballs mixed with LS 1% or TEO 0.1 % and control sample during frozen storage up to 180 days are shown in Table 5. At the end of frozen storage periods, peroxide values reached (14.15, 9.47, 10.10, and 13.27, 9.88, and 10.23 m.equiv./Kg) for chicken and turkey meatballs control, LS, and TEO, respectively. The increase in peroxide value (PV) in chicken during frozen storage is probably due to the faster rate of formation of peroxides during months 1, 2, and 3 of storage than degradation of peroxides into secondary oxidation products these agreement with [54].

Moreover, the lowest PV during the frozen storage period with significant differences ($P<0.05$) were noticed in samples treated with liquid smoke, followed by samples treated with thyme essential oil during frozen storage periods. Such findings may be attributed to the high antioxidant effect. The slow rate of oxidation during storage might be related to the smoke antioxidants' efficacy [55]. Smoking process can impact on lipid oxidation as the activity of phenolic compounds [56]. Liquid smoke possesses antioxidant and antimicrobial capabilities due to the presence of phenol, carbonyl, organic acids, and a low pH value. Liquid smoke contains bioactive chemicals that can inhibit microbiological development [57]. Adding thyme extracts was the most successful in reducing the PV during storage those found by [58].

3.6 Effect of LS or TEO on Phenolic Compounds of Chicken and Turkey Meatballs

Phenolic chemicals are antioxidants that have a wide range of biological effects, including antibacterial, antiviral, ant carcinogenic, vasodilatory, and anti-inflammatory properties [59,60]. The results in Table 6 showed the amounts of total phenolic compounds (TPC) in the control and treated samples of chicken and turkey meatballs at zero time and during frozen storage. The levels of TPC after the addition of 1% LS and 0.1% TEO caused a significant increase in TPC content in chicken and turkey meatballs at zero time, and it showed that the control contained 82.30 and 78.80 mg/100 g TPC, while samples treated with LS or TEO had higher levels of TPC, reaching 312.3, 291.2, and 319.5, 275.1 mg/100g for chicken and turkey meatballs, respectively. This may be due to the

Table 3. Changes in total volatile nitrogen (per100 g sample) of chicken and turkey meatballs treated with LS or TEO during frozen storage

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	8.48	8.95	9.53	10.40	10.89	11.83	12.54	**10.38**
	LS	6.68	6.91	7.15	7.46	7.84	8.87	9.71	**7.80**
	TEO	7.08	7.20	7.51	7.92	8.55	9.43	10.07	**8.25**
Mean		**7.41**	**7.69**	**8.07**	**8.60**	**9.10**	**10.05**	**9.89**	**8.68**
Turkey	**C**	7.78	7.98	8.34	8.75	9.80	11.14	11.98	**9.39**
	LS	5.49	5.74	6.14	6.91	7.61	8.86	9.62	**7.19**
	TEO	5.69	5.93	6.69	7.82	8.21	9.66	10.08	**7.72**
Mean		**6.32**	**8.46**	**7.06**	**7.83**	**8.54**	**9.89**	**10.56**	**8.38**
Main effects	**C**	8.13	6.32	8.94	9.58	10.35	11.48	12.26	**9.58**
	LS	6.08	6.57	6.64	7.18	7.73	8.87	9.67	**7.53**
	TEO	6.38	7.12	7.10	7.87	8.38	9.54	10.08	**8.07**
Mean		**6.87**	**8.95**	**7.56**	**8.21**	**8.82**	**9.96**	**10.67**	
F-test A(Var.)=**		**L.S.D0.05**	**B =0.13**	**AB=0.19**	**C =0.20**	**AC=0.29**	**BC=n.s**	**ABC=0.50**	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

Table 4. Changes in TBA values (mg malonaldhyde per kg. sample) of chicken and turkey meatballs treated with LS and TEO during storage up to 180days

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	0.56	0.60	0.68	0.75	0.78	0.85	0.87	**0.73**
	LS	0.49	0.50	0.55	0.60	0.64	0.66	0.69	**0.59**
	TEO	0.49	0.53	0.56	0.58	0.65	0.6	0.72	**0.60**
Mean		**0.52**	**0.55**	**0.60**	**0.65**	**0.69**	**0.73**	**0.76**	**0.64**
Turkey	**C**	0.66	0.69	0.74	0.78	0.82	0.87	0.91	**0.78**
	LS	0.63	0.61	0.64	0.64	0.66	0.67	0.69	**0.65**
	TEO	0.61	0.63	0.65	0.68	0.69	0.71	0.73	**0.67**
Mean		**0.64**	**0.64**	**0.68**	**0.70**	**0.73**	**0.76**	**0.77**	**0.70**
Main effects	**C**	0.61	0.56	0.71	0.77	0.80	0.86	0.89	**0.74**
	LS	0.56	0.58	0.59	0.62	0.65	0.67	0.69	**0.62**
	TEO	0.55	0.59	0.61	0.63	0.67	0.69	0.73	**0.64**
Mean		**0.57**	**0.60**	**0.64**	**0.67**	**0.71**	**0.74**	**0.77**	
F-test A(Var.)=**		**L.S.D0.05**	**B =0.010**	**AB=n.s**	**C =0.016**	**AC=0.022**	**BC=0.027**	**ABC=n.s**	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

Table 5. Changes in peroxide values (m. equiv. per Kg. of sample) of chicken and turkey meatballs treated with LS or TEO during frozen storage up to 180 days

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	7.71	8.42	9.22	10.47	11.22	13.69	14.15	**10.69**
	LS	6.42	6.45	6.96	7.29	8.13	8.83	9.47	**7.65**
	TEO	5.59	6.03	6.78	7.37	8.94	9.78	10.10	**7.80**
Mean		**6.57**	**6.97**	**7.66**	**8.38**	**9.440**	**10.76**	**11.24**	**8.71**
Turkey	**C**	6.83	7.59	8.50	9.30	12.19	12.15	13.27	**9.97**
	LS	6.33	6.50	7.15	7.46	8.32	9.42	9.88	**7.87**
	TEO	5.98	6.29	6.98	7.56	8.49	9.13	10.23	**7.81**
Mean		**6.38**	**6.79**	**7.54**	**8.11**	**9.67**	**10.23**	**11.12**	**8.55**
Main effects	**C**	7.27	8.00	8.86	9.88	11.70	12.92	13.71	**10.33**
	LS	6.37	6.47	7.05	7.37	8.23	9.12	9.67	**7.75**
	TEO	5.69	6.45	6.83	7.41	8.83	9.62	10.13	7.85
Mean		**6.44**	**6.97**	**7.58**	**8.22**	**9.58**	**10.55**	**11.17**	
F-test A(Var.)=n.s		**L.S.D0.05**	**B =0.35**	**AB=***	**C =0.54**	**AC=n.s**	**BC=*** **	**ABC=n.s**	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

high level of phenolic compounds in LS or TEO added to meatballs. Adding 0.1% from by-products such as pomegranate peel was able to increase the shelf life of chicken meat products. By-products are good sources of phenolic compounds that have very potent antioxidant and antimicrobial activity [61]. The application of liquid smoke that is rich in phenolic derivative compounds will make a significant difference to the content of products derived from phenolic compounds. Compared to that, which is not applied to liquid smoke [62]. The ratio of the optimal concentration of liquid smoke during the process may be determined by the greatest total phenol value and the lowest pH value [63].

From the same Table, it could be observed that TPC was decreased in all samples during frozen storage, with significant differences (P<0.5) between treatments and storage periods. The reduction in TPC may be attributed to the decomposition of TPC during the storage periods this is consistent with [64]. Storage period affects the total phenolic content and antioxidant activities. The reason for decrease in antioxidant activity during storage may be attributed to possible oxidation of antioxidant components under favorable conditions during storage such as temperature and duration of storage [65]. The phenol compounds are volatile, the amount of phenol in meat decreases with time as a result of evaporation those reported by [66].

Table 6. Changes in phenolic compounds (mg/100 g) of chicken and turkey meatballs treated with LS or TEO during frozen storage up to 180days

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	82.30	70.8	59.4	50.9	55.8	49.9	40.8	**58.6**
	LS	312.3	190.8	188.5	205.9	221.2	168.1	160.1	**206.7**
	TEO	291.2	187.2	177.9	158.3	221.8	171.0	159.0	**195.2**
Mean		**228.6**	**149.6**	**141.9**	**138.4**	**166.3**	**129.7**	**119.2**	**153.4**
Turkey	**C**	78.80	67.8	60.8	58.3	58.6	44.6	38.8	**58.2**
	LS	319.5	178.4	210.9	213.6	214.5	156.7	140.8	**204.9**
	TEO	275.1	176.5	201.4	210.7	203.4	147.3	136.6	**193.0**
Mean		**224.5**	**140.9**	**157.7**	**160.9**	**158.8**	**116.2**	**105.4**	**152.0**
Main effects	**C**	80.55	69.3	60.1	54.6	57.2	47.2	39.8	**58.4**
	LS	315.9	184.6	199.7	209.7	217.8	162.4	150.4	**205.8**
	TEO	283.1	181.3	189.6	184.5	212.6	159.1	147.8	**194.1**
Mean		**226.5**	**70.8**	**149.8**	**149.6**	**162.6**	**122.9**	**112.7**	
F-test A(Var.)=n.s		**L.S.D0.05**	**B =2.67**	**AB=n.s**	**C (P)=4.07**	**AC=5.76**	**BC=7.06**	**ABC=9.98**	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

Table 7. Changes in total antioxidant activity (DPPH %) of chicken and turkey meatballs treated with LS or TEO during frozen storage up to 180days

Var.	Tre.	Storage periods (days)							Mean
		0	30	60	90	120	150	180	
Chicken	**C**	27.47	26.81	23.20	18.61	18.56	12.42	8.16	**19.32**
	LS	78.87	69.05	61.31	56.69	56.59	51.43	45.12	**59.87**
	TEO	76.40	65.91	60.81	54.82	56.39	49.29	43.76	**58.20**
Mean		**60.91**	**53.93**	**48.44**	**43.37**	**43.85**	**37.71**	**32.34**	**45.79**
Turkey	**C**	30.48	31.62	22.45	17.38	17.89	9.69	7.79	**19.61**
	LS	80.19	73.53	62.17	56.35	61.30	49.25	45.78	**61.22**
	TEO	74.15	69.97	60.28	56.09	56.05	46.95	43.43	**58.13**
Mean		**61.61**	**58.37**	**48.30**	**43.27**	**45.08**	**35.30**	**32.33**	**46.23**
Main effects	**C**	28.97	29.21	22.82	17.99	18.22	11.05	7.97	**19.46**
	LS	79.53	71.29	61.74	56.52	58.94	50.34	45.45	**60.54**
	TEO	75.27	67.94	60.54	55.45	56.22	48.12	43.59	**58.16**
Mean		**61.26**	**56.14**	**48.37**	**43.32**	**44.46**	**36.50**	**32.34**	
F-test A(Var.)=*		**L.S.D0.05**	**B = 0.51**	**AB=0.74**	**C (P)=0.79**	**AC=1.13**	**BC=1.38**	**ABC=****	

** C: control sample LS: liquid smoke TEO: thyme essential oil*

3.7 Effect of LS or TEO on Antioxidant Activity of Chicken and Turkey Meatballs

The change in total antioxidant activity of chicken and turkey meatballs during frozen storage up to 180 days are shown in Table 7. A significant increase (p≤0.05) in total antioxidant activity (DPPH) was observed in treatments when compared to control sample at zero time. Also, the treatments caused a proportional increase in the antioxidant activity. Thus, the highest levels were observed in LS then TEO at zero time (78.87, 76.40 and 80.19, 74.15%, respectively) for chicken and turkey meatballs. These increases were attributed to the content of LS and TEO from polyphenols and other compounds as a source of antioxidant activity. The antioxidant properties of phenolic compounds have been extensively studied, and there is a direct correlation between phenolic content and antioxidant activity. As a result, the high level of antioxidant activity in treated chicken and turkey meatballs was attributed to the high level of phenolic compounds in LS or TEO compared to the untreated sample. The antioxidant properties of phenolic compounds were very well documented and a significant relation between phenolic content and antioxidant activity. Thus, the high level of antioxidant activity was attributed to the high level of phenolic compounds found in these powders [67].

During frozen storage, antioxidant activity gradually decreased in all treatments. On the other hand, the treatments still had high levels of antioxidant activity at 180 days of frozen storage. The levels were observed in LS or TEO at the end of frozen storage (45.12, 43.76, and 45.78, 43.43%, respectively) for chicken and turkey meatballs. The decrease in antioxidant activity may be attributed to the retard oxidative process. This result agrees with [66]**,** who reported that during storage antioxidant activity decreased. Total phenolic compounds (TPC) and antioxidant activity were gradually decreased in all treatments and the lowest levels were observed at 6 months of storage those reported by [67]. The reduction in antioxidant activity might be attributable to effectively slowing down the oxidative process in treated meatballs products. The decrease in TPC and antioxidant activity may be attributed to the principal microconstituents contributing to antioxidant action being phenolic chemicals, which could be effectively used to retard the oxidative process in meatball products this results agree with[67,68].

4. CONCLUSION

The rate of change in the physical and quality characteristics was reduced in chicken and turkey meatballs that were treated with 1% LS or 0.1% TEO during frozen storage periods. The most significant effect was observed on samples treated with liquid smoke, followed by thyme essential oil, compared to the control samples. During frozen storage, the total phenolic compounds and total antioxidant activity were significantly reduced. The reason for the decrease in phenolic compounds and total antioxidant activity may be attributed to possible oxidation of antioxidant components under favorable conditions during storage. As a result, liquid smoke can be used as an alternative to preservatives in the food industry.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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