STUDY ON THE EFFECTIVENESS OF ADDING SOME PLANT EXTRACTS WITH BHT ON MEAT QUALITY OF LAMB PATTIES DURING CHILLING STORAGE

Ibrahim , A. Baker
Assist. Prof
Dep. Animal Prod.Coll. Agric. Engin. Scie, University of Duhok, Kurdistan Region, Iraq
(Ibrahim.aswad@uod.ac)

ABSTRACT

This experiment was aimed to evaluate and compare the effects of adding natural extracts (clove, Mentha and thyme) with synthetic BHT on some physiochemical, microbial, and sensory characteristics of lamb patties stored at 4 C° for 12 days. Results indicated that there's a steady rise (P<0.01) in oxidative rancidity, microbial and pH with the increasing time of storage. Also, a significant (P<0.01) reduction in TBA, microbial and pH was observed in patties treated with clove, thyme, mentha and BHT as compared with untreated samples. It was found that adding natural extracts is more effective in inhibiting microbial count than did BHT. The highest score of overall acceptability was noticed in patties blended with thyme and mentha, and the lowest was recorded in control samples

Keywords: meat, clove, mentha, thyme extracts, antimicrobial, antioxidant

دراسة فاعلية اضافة بعض المستخلصات النباتية مع BHT على جودة اقراص لحم الحملان خلال الخزن بالتبريد

ابراهيم اسود بكر
استاذ مساعد

المستخلص

تهدف هذه الدراسة لتقييم تأثير اضافة ومقارنة بين BHT ومستخلصات القرنفل والنعناع والزعتر في بعض الصفات الفيزيوكيمياوية والحمل الميكروبي والصفات الحسية لاقراص لحم الحملان والمخزونة بالتبريد على (4م°) لمدة 12 يوم. تشير النتائج بوجود ارتفاع معنوي(أ < 0.01) في التزنخ التأكسدي والحمل الميكروبي و درجة الحموضة لاقراص اللحم بتقدم مدة الخزن. كما لوحظ انخفاض معنوي(أ< 0.01) في قيم كل من TBA والحمل الميكروبي و درجة الحموضة لاقراص اللحم المعاملة بالمستخلصات وBHT مقارنة بالسيطرة. كما وجد بأن فعالية المستخلصات الطبيعية كانت اعلى في تأثيرها على الحمل الميكروبي مقارنة ب BHT الصناعي بلغ اعلى درجة تقييم حسي لاقراص اللحم المعاملة بالزعتر والنعناع وادناه لاقراص لحم السيطرة.

الكلمات المفتاحية: لحم، مستخلصات القرنفل، النعناع الزعتر، مضادات االاكسدة والبكتريا

Received:22/5/2021, Accepted:19/8/2021

INTRODUCTION

Meat is recognized to be a good source of protein, lipids, vitamins, and minerals (36). However, one of the most and greatest problems facing the processors of meat products is oxidation (33), particularly in minced meat due to the increase in surface area of meat exposed to air after grinding (28), as well as products containing high level of fat are more susceptible to oxidation which impact the quality of the product through loss of desirable odor, color, flavor, and shelf life (1). Oxidation occurs when the radical's reaction oxygen species (ROS) and reactive nitrogen species (RNS)are generated unevenly, causing oxidation and/or nitrosamine stress and damage to macromolecules including lipid and protein fractions (36). As a result, numerous synthetic food additives are employed to reduce oxidation changes and limit bacteria development such as BHT, BHA and TBH. However, as public concerns of potentially hazardous consequences and health risk grown, the usage of synthetic antioxidants has dropped, which the use of natural antioxidants has risen quickly (43). Thus, the use of natural antioxidants supplements was developed in recent years, which the evidence that they could be delay or prevent the oxidation of meat products (5). Clove (*Eugenia caryophyllus*) has been shown to be one of the most powerful antioxidants in preventing lipid oxidation (41). The high amount of eugenol (3%) and gallic acid (1.3%) in clove may explain its excellent effect (40). It has been reported that clove additives reduce lipid oxidation during storage in Chinese style sausage (51) row pork patties (18), silver carp fillet (22) and buffalo meat steaks (29). *Mentha spicata* L. is a polyphenol-rich plant belongs to the Lamiaceae family. There have been various studies on the antioxidants and antibacterial capacity of Mentha species essential oil and extracts (11, 27). Biswas et al (7) when examined the effects of several curries and mint leaf extracts on the oxidation stability and color of raw ground pig meat held at 4c^{o} were shown to have greater DPPH and ABTS activities. Thyme has been also used to flavor meat and meat products for centuries (21). Furthermore, the volatile oil components of thyme have been recognized to exhibit antibacterial action against several bacterial and fungal species (20), as well as antioxidant capacity owing to its content's flavonoids, Carvacrol, Thymol, 1-8-Cinote, PCymene, Borneol (6,12,31,38,44). This research was aimed to investigate whether natural antioxidants (i.e clove bud extract, Mentha and thyme leave extract) are powerful enough to replace synthetic antioxidants like BHT, which are presently utilized in lamb patties.

MATERIALS AND METHODS

Preparations of plant attracts: Thyme, Mentha leaves in this research was obtained from Doski area at Duhok province Kurdistan region of Iraq. Leaves were washed, dried at room temperature, followed by grinding. and Clove bud powder was obtained from local Duhok market province was used in this study. One hundred gram from each of crushed thyme, Mentha leaves, and clove bud powder were extracted in a closed conical flask with 1000 ml of 70 percent (v/v) aqueous ethanol and 30 percent distilled water, using a magnetic stirrer without a heat plate, for 24 hours at room temperature in the dark. The residue was removed three times using the same process then the extract was filtered through clean cheese cloth. The residual solution was dried and evaporated in a vacuum oven at 40 °C before frozen until use.

Preparation of meat patties

Fresh lamb loin muscles were chopped into small pieces after removing the visible fat and connective tissues. Chopped meat samples were minced using a meat mincer through a plate with 4- mm holes. then it was divided into five equal portions and assigned as follows: The first sample was untreated (control), while the others were blended with 200ppm BHT, 200ppm clove bud extract (CE), 200ppm Mentha leaves extract (ME) and 200ppm Thyme leaves extract (TE). After mixing, lamb patties of 50 g. were formed using a burger former placed on Styrofoam trays, wrapped with polyethylene film, and kept in refrigerator at 4 ºC for 12 days to evaluate chemical, microbial count, and sensory attributes at days (1, 4, 8, 12).

Determination MDA values (TBA): The MDA values were determined according to the method described by Witte *et al*. (47). Twenty grams of the minced meat were blended with

50 ml of cold solution containing 20% trichloroacetic acids in 2 M phosphoric acids. The resulting slurry was then transferred into a 100 ml with distilled water, homogenized by shaking and filtered through Whatman no.1 filter paper. Five ml of the filtrate was then pipette into a test tube while another 5 ml of fresh chilled 2-Thiobarbituric acid (0.005 M in distilled water) was added. The test tube was shaken well and placed in the dark at room temperature 25°C for 15- 17 h to develop the color reaction. The absorbance was measured using spectrophotometer (6400-JENWAY) at 530 nm to calculate the MDA values. The MDA value was expressed as mg malondialdehyde /kg meat, which was calculated by multiplying the absorbance by 5.2 factors as follows: MDA (mg malondialdehyde /kg meat) =A530 × 5.2

Microbial count

Microbial count was determined as recommended by the American Public Health Association for food stuff examination (4). Total plate count (TPC) was determined on nutrient agar medium, and the plates of different dilutions were incubated at 37°C for 48 h. The average number of colonies per countable plate as well as the total number of colonies per gram (CFU/g) was determined. MacConky agar medium was used for determination of coliform bacteria, and the inoculated plates were incubated at 37°C for 48 h. Psychrophilic bacteria (PSY) were determined on nutrient agar medium, and the plates, and the inoculated plates were inoculated at 7°C for 10 days.

pH Determination

pH of meat sample was carried out according to the method described by Mokhtar and Youssef, (25). Meat patty sample (10g) was homogenized in 90 ml distilled water for 1min in a blender and the pH was measured using a glass pH electrode (Bp 3001. Singapore pte. Ltd}.

Sensory evaluation

The investigated samples were evaluated using a panel test according to Cross, et al., (9).

Statistical analysis

The Statistical computations were done using SAS software program (37) to explore the influence of treatment and period (factorial experiment: Interaction 5 x 4 in Completely Randomized Design-CRD) in studied parameters. Duncan's multiple range test (13) was used to compare between means. The statistical model was as follows:

$$\mathbf{Y_{ijk} = \mu + T_i + P_j + TP_{(ij)} + e_{ijk}}$$

Where:

Y_{ijk} = Dependent variable

μ = Overall mean

T_i= Effect of treatment (Control, BHT, CE, ME, and TE).

P_j = Effect of period (1, 4, 8 and 12 day).

$TP_{(ij)}$ = Effect of Interaction ($T_i \times P_j$)

e_{ij} = Error term

RESULTS AND DISCUSSION

TBA. The TBA is one of the main widely used method for measuring secondary oxidation products mainly malonaldehyde which are known as the cause of oxidation rancidity which may contribute to the off flavor of oxidized fat (50). In the current investigation, the TBA values for the patties treated with BHT, CE, ME and TE during storage at 4C° for 12 days are presented in Table (1). It appears from the table that TBA values for treated samples were significantly ($P<0.1$) lower than those observed in the control sample. Also, it seems that during storage at 4 c° for 12 days lipid oxidation in the control samples increased at day 1 from 0.401 to 1.367 mg MDA/Kg meat at day 12 of storage, while treated meat samples apparently retarded significantly ($P<0.01$) oxidative rancidity during storage by 42.6, 44.2, 45.3 and 44.7% for samples treated with BHT, CE, ME, and TE, respectively as compared to untreated control samples. Additionally, it appears from Table (1) that all natural additives used are almost similar in their effect to TBA values as ($P>0.05$) BHT did. Therefore, it's advisable to use such natural antioxidants instead of using synthetic antioxidant. The increase for TBARS value for the control could be due to the origination of increased MDA that has been considered as secondary product for lipid oxidation (50). While, the activity of cloves may be caused by the presence of eugenol the major constituent of cloves, which is known for its antioxidant activity (50). The capacity of EOs to act as antioxidant is attributed to the fact that phenols are chain-breaking antioxidants. They denote an H- atom from the phenolic hydroxyl group to peroxyl radicals

(ROO which are responsible for the propagation of the oxidative radical's chain (32). Also, mint leaves contain eugenol, caffiec acid, rosmarenic acid and alpha tocopherol (36). Similarly, the decrease for TBARS values was found in clover extract treated pork patties (18) chicken meat sample (50), buffalo patties (45) and beef burgers (25). Also, the antioxidant action of thyme has been shown in vitro (8), and from its application in a minced products (14,19) as well as veal (42), Pork (15) and lamb (26). On the same line, Raesi et.al (34), Tajik et.al (46) found that adding mint to meat resulted in reducing the TBA values as compared to control.

Table 1. Effect of some plant extracts on changes in MDA (mg malondialdehyde/kg meat) values of lamb meat patties during storage at 4c° for 12 days

Treatment	Storage Period (days)			
	1	4	8	12
Control	0.401 ±0.004 G	0.781 ±0.03 C	0.86 ±0.025 B	1.367 ±0.028 A
BHT	0.293 ±0.007 H	0.568 ±0.066 EF	0.608 ±0.0 ED	0.784 ±0.005 C
CE	0.265 ±0.03 H	0.539 ±0.005 F	0.579 ±0.001 EDF	0.762 ±0.034 C
ME	0.299 ±0.011 H	0.587 ±0.002 EDF	0.631 ±0.005 D	0.747 ±0.031 C
TE	0.283 ±0.016 H	0.564 ±0.025 EF	0.6 ±0.004 ED	0.755 ±0.001 C

For each trait, means with different letters within each column and each row differed significantly (p<0.01).C= control, BHT= 200 ppm butylated hydroxytoluene, CE= 200ppm Clove bud extract, ME= 200ppm Mentha leave extract, TE= 200ppm Thyme leave extract

Microbial changes

Microbial changes of lamb's patties were determined through estimating total plate count (TPC) psychrophilic bacteria (PSY) and Coliform bacteria, during storage at 4c° for 12 days. Results presented in Table (2) indicated that there was a significant (P<0.01) steady rise during storage at 4c° for 12 days in untreated control patties from their initial value in TPC (7.0 vs. $82x10^5$), PSY (5.0 vs. $72x10^5$) and Coliform (0.2 vs. $5.6x10^2$). The present results revealed that addition CE, TE, ME and BHT resulted in a significant (P<0.01) reduction in all count of studied bacteria as compared to untreated samples, and the highest reduction was observed in ME treated samples being (40.6%) for TPC (45.8%.)for PSY and (75.0%.) for Coliform treated samples. It is also worth to note that treated samples with the natural antioxidants resulted in a higher reduction of studied bacteria as compared with samples treated with BHT (Table2). It has been indicated that the use of plant extracts, essential oils and organic acid could be considered as a relevant strategy to inhibit spoilage in meat products (30). The plant extracts and essential oils showed a potential anti-microbial impact through the following mechanisms. A/ The phenolic compounds in these extracts and essential oils affect either enzyme activity or cause protein denaturation, B/ it causes changes in the permeability of microbial cells, and C/ it causes changes in the function of the normal activity of cell membranes, such as electron transfer, nutrient exchange, protein synthesis, nucleic acids and enzymatic activity. (2). This result was in agreement with Shan et al (41) Abd El Wahab et al (1) who showed that cloves have a powerful anti-microbial activity in meat products. Also, Zengin and Baysal (49) using clove and thyme, Backer et al (6) and Anwar and Baker (3) using Thyme. And Mkaddem et al (24) and shahabzi(39) using Mentha demonstrated that these additives have antibacterial capacity.

Table 2 Total plate count (T.P.C), psychrophilic bacteria count (PSY) and coliform bacteria as affected by some plant extracts on lamb patties stored for 12 days at 4 c°

TPC×10^5

Treatment	Storage Period (days)			
	1	4	8	12
Control	7.0 ±0.577 GH	33 ±1.52 E	61.33 ±9.4 B	82.0 ±2.309 A
BHT	4.0 ±0.577 H	22 ±1.52 F	50 ±2.88 CD	60 ±2.886 CB
CE	2.0 ±0.577 H	17.666 ±1.45 F	38 ±1.54 E	51 ±4.6 CD
ME	4.0 ±1.154 H	16.333 ±1.85 GF	38 ±4.04 E	49 ±0.577 D
TE	3.0 ±0.577 H	21.666 ±6.0 F	37.333 ±2.4 E	53 ±3.46 CB

	PSY×10^5			
Control	5.0 ±0.577 G	30 ±1.5 E	51.33 ± 9.4 B	72 ±1.15 A
BHT	3.0 ±0.579 G	18 ±1.154 F	40 ±2.88 DC	50 ±2.886 B
CE	1.33 ±0.44 G	14 ±1.154 F	32.66 ±1.76 DCE	39 ±1.15 DCE
ME	3.33 ±0.88 G	13.33 ±1.333 F	31.33 ±2.33 DCE	39 ±0.577 DCE
TE	2.0 ±0.577 G	18.66 ±4.37 F	31 ±3.511 DE	40 ±1.763 C
	Coliform×10^2			
Control	0.2 ±0.02 F	0.4 ±0.057 F	3.0 ±0.57 B	5.6 ±0.23 A
BHT	0.1 ±0.011 F	0.25 ±0.028 F	1.8 ±0.115 D	2.5 ±0.288 CB
CE	0.1 ±0.023 F	0.2 ±0.057 F	1.5 ±0.173 ED	2.4 ±0.23 C
ME	0.11 ±0.015 F	0.23 ±0.017 F	1.3 ±0.173 ED	1.7 ±0.115 D
TE	0.086 ±0.017 F	0.18 ±0.011 F	1.1 ±0.057 E	1.4 ±0.115 ED

For each trait, means with different letters within each column and each row differed significantly (p<0.01).C= control, BHT= 200 ppm butylated hydroxytoluene, CE= 200ppm Clove bud extract, ME= 200ppm Mentha leave extract, TE= 200ppm Thyme leave extract

Ph

The effect of synthetic and natural antioxidants on the pH values of patties stored at 4c^o for 12 days is demonstrated in Table (3) At day 1, The initial values of control and samples submitted to various treatments was found to range from 5.54±0.01 to 5.66±0.10, and the difference among them was significant (P<0.01) (Table 3). The pH values of all patty's samples decreased slowly during the first 4 days of storage, whereas after day 4 there was a gradual increase. (Table 3). During storage for 12 days at 4c^o it, it was noticed that pH values of the control are significantly (P<0.01) higher (6.38±0.01) than the treated samples with BHT (6.26±0.01), CE (6.33±0.005), ME (6.17±0.005) and TE (6.2±0.01) Table 3. Similar findings have been reported in beef burger (25), in raw chicken meat (50), in minced meat (34) and on lamb meat (16). The pH increase (P<0.01) of the untreated control samples may have been caused by the utilization of amino acids by bacteria which are released during protein degradation because the stored glucose has been depleted. Accumulation of ammonia and the products of amino acids decomposition result in an increase in pH (23). However, the lower pH for treated samples with natural antioxidants is attributed to the inhibitory effect of anti-microbial ingredients found in extracts on the growth and proliferation of spoilage microorganisms that metabolize basic nitrogen compounds (Zhang et al (50).

Table 3. Effect of some plant extracts on change in pH values of lamb meat patties during storage at 4c^o for 12 days

Treatment	Storage Period (days)			
	1	4	8	12
Control	5.66 ±0.011 H	5.65±0.025 H	6.25 ±0.014 B	6.38 ±0.011 A
BHT	5.64 ±0.011 H	5.66 ±0.011 H	6.05 ±0.005 E	6.26 ±0.011 B
CE	5.64 ±0.011 H	5.65 ±0.025 H	6.01 ±0.005 F	6.23 ±0.005 CB
ME	5.54 ±0.011 J	5.64 ±0.0 I I	5.76 ±0.011 G	6.17 ±0.005 D
TE	5.57 ±0.013 JI	5.64 ±0.011 H	6.07 ±0.005 E	6.2 ±0.011 CD

For each trait, means with different letters within each column and each row differed significantly (p<0.01).C= control, BHT= 200 ppm butylated hydroxytoluene, CE= 200ppm Clove bud extract, ME= 200ppm Mentha leave extract, TE= 200ppm Thyme leave extract

Sensory evaluation

It's known that alterations in food quality because of lipid oxidation range from color variations to changes in appearance, odor, texture, and taste. Sensory evaluation is the most significant method for predicting oxidative stability, product shelf life and consumer acceptance (35). However, a spice extracts could be exhibiting an excellent antioxidants power, but if it fails sensory test, it will not be accepted (10). The results on sensory evaluation in terms of color, flavor and general acceptance of the lamb patties treated with BHT, CE, ME, TE, and untreated control stored at 4c^o for 12 days are demonstrated in (Table 4). Results indicated that treatment, period and their interaction affected significantly (P<0.01) general acceptance. It appears from Table (4) that the highest overall acceptability was noticed in samples treated with ME and TE (3.66±0.33). While the lower score was recorded in

untreated samples (1.66±0.33). A similar trend was noticed in both color and flavor as well. Similarly, it was demonstrated that addition of thyme resulted in significant improvement on overall acceptability in beef burger (17) and in lamb patties (3). On the same time, it was demonstrated that addition of clove (25,48) resulted in an increase in overall acceptability as compared with control samples

Table 4. Effect of some plant extracts on changes in color, flavor and acceptance of lamb meat patties during storage at 4c° for 12 days

Treatment	Storage Period (days)			
	1	4	8	12
Control	**3.66 ±0.33 A**	**3.0 ±0 BA**	**2.66 ±0.333 BA**	**2.0 ±0 B**
BHT	**3.66 ±0.33 A**	**3.66 ±0.333 A**	**3.0 ±0.0 BA**	**2.66 ±0.666 BA**
CE	**3.66 ±0.33 A**	**3.66 ±0.666 A**	**3.33 ±0.333 BA**	**3.0 ±0.00 BA**
ME	**3.33 ±0.333 BA**	**3.33 ±0.333 BA**	**3.0 ±0.0 BA**	**3.0 ±0.577 BA**
TE	**3.66 ±0.333 BA**	**3.66 ±0.666 A**	**3.33 ±0.333 BA**	**3.33 ±0.666 BA**
		Flavor		
Control	**4.66 ±0.33 A**	**4.0 ±0.577 BA**	**3.0 ±0.577 BC**	**2.0 ±0.57 C**
BHT	**4.66 ±0.333 A**	**4.0 ±0.577 BA**	**4.0 ±0.0 BA**	**3.0 ±1.00 BC**
CE	**4.33 ±0.333 BA**	**4.33 ±0.667 BA**	**4.0 ±0.0 BA**	**3.33 ±0.333 BAC**
ME	**4.33 ±0.333 BA**	**4.33 ±0.333 BA**	**4.0 ±0.577 BA**	**3.66 ±0.333 BA**
TE	**4.66 ±0.333 A**	**4.33 ±0.666 BA**	**4.0 ±0.0 BA**	**3.66 ±0.333 BA**
		Acceptance		
Control	**4.0 ±0.0 BAC**	**3.33 ±0.33 BDC**	**2.66 ±0.33 D**	**1.66 ±0.33 E**
BHT	**4.33 ±0.33 BA**	**3.66 ±0.666 BDAC**	**3.333±0.33 BDC**	**3.0 ±0 DC**
CE	**4.0 ±0.0 BAC**	**3.66 ±0.333 BDAC**	**3.66 ±0.333 BDAC**	**3.33 ±0.333 BDC**
ME	**4.66 ±0.333 A**	**4.33 ±0.333 BA**	**4.0 ±0.0 BAC**	**3.66 ±0.333 BDAC**
TE	**4.66 ±0.333 A**	**4.33 ±0.333 BA**	**4.0 ±0.0 BAC**	**3.66 ±0.333 BDAC**

For each trait, means with different letters within each column and each row differed significantly (p<0.01).C= control, BHT= 200 ppm butylated hydroxytoluene, CE= 200ppm Clove bud extract, ME= 200ppm Mentha leave extract, TE= 200ppm Thyme leave extract

Conclusion

From the results of this work, it can be concluded that adding natural extracts resulted in a significant reduction in oxidation and microbial count and it's advisable to use such natural extracts instead of synthetic antioxidants.

Acknowledgment

The author wishes to express his deep thanks for Prof. Dr. Jalal E. Alkass for his valuable help in reading the manuscript, and to Prof. Dr. Nasr Al-Anbari for his help in analyses of the data.

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