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Impact of some essential oils on the quality of chilled minced beef

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Abstract

This study was carried out to evaluate the effect of the addition of some essential oil extraction from natural herbs (Clove, cinnamon, and thyme) on the stability and quality of raw chilled minced beef. Two factors were used in the research, namely: concentration of essential oil (0.25% and 0.5% and mixed with different extracted essential oils respectively) and shelf life at refrigerated temperatures (3°C ±1) (0, 3, 6, 9 and 12 days). The parameters analyzed were pH value, total volatile nitrogen (TVN), thiobarbituric acid (TBA) value, and microbial analysis. In general, obtained results showed that adding 0.25% and 0.5 % of essential oils of chilled minced beef samples had a positive effect on the physical properties, quality attributes and microbiological changes in minced beef meat during chilled storage. The samples contained thyme and cinnamon essential oils under levels 0.50 % recorded the best treatments. While the mixing from different essential oils showed low effect on the stability of chilled minced beef meat. In conclusion, using essential oils (Clove, cinnamon, and thyme) alone gave better results than mixed, as safe natural preservatives to improve the stability and quality of chilled minced beef.

Keywords: essential oils, clove oil, cinnamon oil, thyme oil, chilled minced beef.

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1. Introduction

Meat and meat products are generally recognized as good sources of high biological-value proteins, group B vitamins, minerals as well as some other bioactive compounds. The composition of meat products depends on their formulation (Ramos *et al*., 2013). However, a significant amount of meat and meat products is lost or wasted each year. These annual losses amount to roughly 20% of the original meat supply (Gram *et al*., 2002). The rate of deteriorating changes depends on meat composition, hygienic practices during cutting, grinding, preparation and storage conditions (Brooks *et al*., 2008). Meat has a complex physical structure and chemical composition (Rather *et al*., 2016). The chemical composition of meat is considered typical for the survival and growth of microbes during production, storage, and distribution due to the readily available proteins, free amino acids, lipids, vitamins, mineral salts and moisture (Muchenje *et al*., 2009). Minced meat is appreciated because of its convenience. Unfortunately, its shelf- life is limited because the large, exposed surface area facilitates spoilage. The most important factor in controlling meat spoilage is microbial contamination and its growth, which can affects on minced meat safety and its color (Brooks *et al*., 2008). The spoilage of meat can be done by chemical and enzymatic activities, which lead to the breakdown of protein, fat and carbohydrates of meat, which contribute to secondary defects in flavors and off-odors that make the meat unacceptable for human consumption (Pal and Devrani, 2018). Meat preservation ensures that the quality, nutritional value, and edibility of the meat are all preserved (Pal and Devrani, 2018). Therefore, the majority of current research has focused on identifying natural antioxidants from a novel of plant sources (Shah *et al.*, 2014). These natural antioxidants from plants have been extracted from various sources as natural antioxidants for preserving and increasing meat and meat products' overall quality (Das *et al.*, 2012). The antimicrobial activity of essential oils, similar to all natural extracts, is dependent on their chemical composition and the amount of the single components. Their antimicrobial activity is not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell (Pinto *et al*., 2007). Factors affecting antimicrobial activity in the antimicrobial action of essential oil components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance (Akhtar *et al*., 2013). Clove (*Syzygium aromaticum*) is one of the essential oils commonly used in seasoning of food. Antimicrobial activity is mainly attributable to eugenol, oleic acid and lipids present in it (Nzeako *et al*., 2006). The high levels of eugenol contained in clove essential oil are responsible for its strong biological and

antimicrobial activities. It is well known that both eugenol and clove essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast (Gupta and Prakash, 2021). The major component in the cinnamon (*Cinnamomum zeylanicum*) was cinnamaldehyde and this component was reported to inhibit ATPase enzymes and disrupt the outer cell membrane (Akhtar *et al*., 2013). Thyme (*Thymus sp*.) is commonly used in the meat industry application due to its capacity to extend the shelf life of minced meat as well as its impact on the chemical, microbiological, and sensory qualities of minced meat during storage (Shaltout *et al*., 2017). Therefore, the aim of the study was to evaluate the effect of addition some essential oil extraction from natural herbal (Clove, cinnamon and thyme) on the stability and quality of chilled minced.

2. Materials and methods

2.1 Materials

2.1.1 Meat

Twenty kg of fresh beef (bottom round) was purchased from private sector shops in local market at Assiut city, Egypt and transported under refrigeration to the laboratory within 30 min. Then, meat was cut and minced with a grinder through a 4 mm plate diameter (AC110V, China) just before analysis and treatments.

2.1.2 Plant samples

The plant samples such as clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*) and thyme (*Thymus vulgaris*) were obtained from Al-Galaly market at Assiut city, Egypt.

2.1.3 Chemicals and reagent

All chemicals and reagents used in the analytical methods (analytical grade) were purchased from El-Gamhouria Trading Chemicals and Drugs Co., Assiut city, Egypt.

2.2 Methods

2.2.1 Extraction of the essential oil

Essential oils were obtained by steam distillation method as described by Guenther (1961). The plant material (about 300 g) was cut into small pieces and placed in a flask (4 L) together with doubly distilled water (1.5 L). The mixture was boiled for 3 h, collected essential oil were dried with anhydrous sodium sulfate and kept at 4°C until its use.

2.2.2 Preparation of minced beef treated with essential oils

Minced beef was mixed in sterile mixer with essential oils (0.25 and 0.5%) of

their weight, The samples were divided into three groups; the first group was treated with 0.25 or 0.5% essential oils, second group was mixed with different essential oils at concentration 0.25 or 0.5%, and the third groups was kept as a control group. Each sample was packed in polyethylene bags and stored at 3°C ±1, and all analysis was conducted at intervals of 0, 3, 6, 9 and 12 days.

2.2.3 pH value

The pH of minced meat sample was measured by homogenizing 10 gm of the sample with 100 ml distilled water for 30 seconds. The pH of the prepared sample was measured using a pH meter (OAKTON, pH/ mV/°C meter, USA) with a glass electrode at 20°C according to the method described by Turhan *et al*. (2005).

2.2.4 Determination of total volatile nitrogen (TVN)

The TVN content of prepared beef patties was determined by macro-distillation method as described by Egan *et al*. (1976).

2.2.5 Determination of thiobarbituric acid (TBA)

The value of thiobarbituric acid (TBA mg MDA/kg) was calculated using a distillation method as described by Tarladgis *et al*. (1960).

2.2.6 Determination of total bacterial count in minced beef meat

The total bacterial counts were determined using the plate counts technique on a nutrient agar medium according to procedures by APHA (1976) and Difco (1984). The plates were incubated at 37°C for 48 h.

2.2.7 Psychrotrophic bacterial count

Psychrotrophic bacterial count was carried out as described in typical procedure of the plat bacterial count methods, except, incubation was achieved at 8°C for 5 days according to APHA (1976).

2.2.8 Coliform group bacteria count

Coliform group bacteria were determined using VRBA according to the procedures described by APHA (1976) and Difco (1984). The plates were incubated at 37°C for 24 h.

2.3 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using a completely randomized factorial design. Basic statistics and ANOVA were performed to test the significance within replications and between treatments (SPSS, 2011). L.S.D tests were used to determine the differences among means at the level of 0.05%.

3. Results and Discussion

3.1 Changes in pH values of chilled minced beef meat treated with essential oils during storage at 3±1°C

Data given in Table (1) showed the pH values of minced beef treated with essential oils under levels 0.25 and 0.5% at zero time and during refrigerated storage at 3±1°C up to 12 days. From these results, it could be observed that untreated minced beef (control sample) was higher than that of the treatments recorded. Data showed pH values of control sample was 6.36 at zero time and 7.27 at the end of storage periods. Moreover, addition of essential oils led to noticeable decrease in pH value especially in case of thyme essential oil under level (0.50%) where the pH value at zero time was 6.32 and 6.36 at the end of storage periods. From these data, it could be observed that at zero time of storage the pH values were 6.36 to 6.32 for the mixed essential oils. While, at the end of storage the final pH values were (6.77 and 6.80) for mixed samples at levels 0.25 and 0.50 %, respectively. These results are consistent with Shaltout *et al*. (2017) who found that during different periods of evaluation, samples treated with thyme essential oil had lower pH values than control samples. Salem-Amany *et al*. (2010) who reported that this could be owing to the activation effect of thyme essential as an antibacterial agent producing protein breakdown and the development of alkyl groups.

Table (1): Changes in pH values of chilled minced beef meat treated with essential oils during storage at 3±1°C.

Treatments		Storage periods by days					
		0	3	6	9	12	Mean
Control		6.36	6.18	6.44	6.80	7.27	6.61
ClO 0.25%		6.34	6.18	6.26	6.34	6.43	6.31
ClO 0.5%		6.35	6.19	6.27	6.30	6.43	6.31
Cin 0.25%		6.34	6.19	6.29	6.32	6.45	6.32
Cin 0.5%		6.28	6.19	6.28	6.33	6.43	6.30
Thy 0.25%		6.34	6.17	6.29	6.33	6.40	6.31
Thy 0.5%		6.32	6.19	6.29	6.33	6.36	6.30
MIX 0.25%		6.36	6.22	6.36	6.57	6.77	6.46
MIX 0.5%		6.32	6.21	6.36	6.49	6.80	6.44
Mean		6.34	6.19	6.32	6.42	6.59	6.37
L.S.D 0.05	A	0.05					
	B	0.04					
	AB	0.00					

ClO = clove oil, Cin = cinnamon oil, Thy = thyme oil, MIX = (clo + cin + thy) (1: 1: 1). A= treatments. B= storage periods. AB= interaction between treatments × storage periods.

3.2 Changes in total volatile nitrogen (TVN) of chilled minced beef treated with essential oils during storage at 3±1°C

The total volatile nitrogen (TVN) is a positive predictor of meat and meat products quality during storage (Baltić *et*

al., 2017). Data in Table (2) showed TVN of the studied samples treated with essential oils compared with the control during refrigerated storage periods. From these data, it could be observed that at zero-time storage the total volatile nitrogen value was 7.50 mg/100g for the control sample. Meanwhile, total volatile nitrogen for all samples contained clove, cinnamon and thyme essential oils under levels 0.25 and 0.50% ranged from 7.24 to 7.63 mg/100 g**.** At the end of storage period the control sample was recorded 23.87 mg TVN/ 100 g. While, for samples contained essential oils the TVN value ranged from 12.71 to 14.47 mg/100. Pasaraeng *et al*. (2013) reported that some active perishable bacteria activate a working enzyme and aid in protein breakdown, resulting in a reduction in meat quality. Degradation of protein compounds producing base-base volatile. The decrease in total volatile nitrogen value may be attributed to the antimicrobial effect of these spices which caused inhibiting the growth of all microorganisms. The finding could be supported by Abd El-Qader (2003) and Liu *et al*. (2009). Generally, TVN for samples contained thyme and cinnamon essential oils under levels 0.50% recorded the best treatments. Meanwhile, TVN for samples contained mixture essential oils under levels 0.25 and 0.50 % recorded (19.36 and 18.43 mg/100 g; respectively) that higher than essential oils alone. This may be due to the low synergic effect of the mixture essential oils. Koura and Hanan (2018) mentioned that treated minced meat with thyme oil at different concentrations showed decreasing in TVN values than those of control samples and by increasing concentration of Thyme was more effective than low concentration in decreasing TVN values during 12 days of storage.

Table (2): Changes in total volatile nitrogen (TVN mg/ 100 g sample) of chilled minced beef treated with essential oils during storage at 3±1°C.

Treatments		Storage periods by days					
		0	3	6	9	12	Mean
Control		7.50	10.56	16.37	20.41	23.87	15.74
ClO 0.25%		7.50	8.67	11.52	13.77	14.47	11.18
ClO 0.5%		7.63	8.66	11.55	13.34	14.40	11.12
Cin 0.25%		7.24	8.71	10.89	12.54	13.43	10.56
Cin 0.5%		7.60	8.84	10.28	11.92	13.12	10.35
Thy 0.25%		7.51	8.21	10.22	11.66	13.15	10.15
Thy 0.5%		7.49	8.11	10.03	11.38	12.71	9.94
MIX 0.25%		7.68	9.33	13.89	16.65	19.36	13.38
MIX 0.5%		7.37	9.18	13.90	15.57	18.43	12.89
Mean		7.50	8.92	12.07	14.14	15.88	11.70
L.S.D 0.05	A	0.58					
	B	0.43					
	AB	0.00					

ClO = clove oil, Cin = cinnamon oil, T = thyme oil, MIX = (Clo + Cin + Thy) (1: 1: 1).

3.3 Changes in thiobarbituric acid (TBA) values of chilled minced beef treated with essential oils during storage at 3±1°C

The change in TBA values for chilled minced beef samples were evaluated for 12 days during storage 3±1°C, and the data are summarized in Table (3) declared that there were not differences in TBA values between treated samples and untreated sample (control sample) at zero time, whereas there were significant ($P<0.05$) in TBA values among the treatments at throughout the storage periods. During storage periods TBA values of control sample showed continuous progressive increases to reach the highest value (1.49 mg malonaldhyde /kg sample) at the end of storage period. Although the other treatments showed slight increment in TBA values during refrigerated storage at 3±1°C up to 12 days. Abd El-Aziz (2001) reported that, TBA values of all meat samples increased with increasing storage periods. The slight increment of minced beef treated with different essential oils may be due to the effects of these oils on the microbial activity. Also, data showed that the minced beef with thyme and cinnamon essential oils had the lowest TBA values at the end of storage, this may be due to its essential oils contained more amount of antioxidant compounds compared to the other essential oils under study.

Table (3): Changes in TBA values (mg. malonaldhyde per kg sample) of chilled minced beef treated with essential oils during storage at 3±1°C.

Treatments		Storage periods by days					
		0	3	6	9	12	Mean
Control		0.15	0.39	0.80	1.07	1.49	0.78
ClO 0.25%		0.15	0.26	0.29	0.38	0.59	0.33
ClO 0.5%		0.14	0.24	0.27	0.36	0.52	0.30
Cin 0.25%		0.15	0.24	0.27	0.34	0.51	0.30
Cin 0.5%		0.15	0.24	0.27	0.34	0.51	0.30
Thy 0.25%		0.15	0.23	0.25	0.31	0.48	0.29
Thy 0.5%		0.15	0.22	0.25	0.29	0.47	0.28
MIX 0.25%		0.15	0.26	0.42	0.74	0.92	0.50
MIX 0.5%		0.48	0.23	0.40	0.73	0.89	0.55
Mean		0.19	0.26	0.36	0.51	0.71	0.40
L.S.D 0.05	A	0.07					
	B	0.05					
	AB	0.00					

ClO = clove oil, Cin = cinnamon oil, T = thyme oil, MIX = (Clo + Cin + Thy) (1: 1: 1).

These results were also similar to those found by Krishnan *et al*. (2014) who reported that 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.

3.4 Changes in total bacterial count of chilled minced beef treated with essential oils during storage at 3±1°C

The result given in Table (4) showed the changes in total bacterial counts in chilled minced beef samples formulated with essential oil of clove, cinnamon, thyme and mixture under levels 0.25 and 0.50 % during refrigerated storage at 3±1°C up to 12 days was ranged between 5.39 and 6.72 log cfu/g at zero time. At the end of storage periods the final total bacterial counts in samples contained essential oil of clove were (7.30 and 6.22), cinnamon (7.06 and 5.23), thyme (5.31 and 4.59) and mixture (8.05 and 8.05) log cfu/g under levels 0.25 and 0.50 %, respectively. On the other side, total bacterial counts for treated samples were lower than the control sample (9.93 log cfu/g) at the end of storage periods Also, from these results, it could be observed that the addition of thyme essential oil under level 0.25 and 0.50% to the minced meat caused highest significant ($P<0.05$) decrease in total bacterial count, this decrease might be attributed to the effect of the antimicrobial compounds in these essential oil. Mohamed *et al*. (2011) reported that thyme showed a stronger inhibitory effect among the used spices and herbs on microbial growth.

Table (4): Changes in total bacterial count (log cfu/g) of chilled minced beef treated with essential oils during storage at 3±1°C.

Treatments		Storage periods by days					
		0	3	6	9	12	Mean
Control		5.44	5.99	7.36	8.47	9.93	7.44
ClO 0.25%		5.41	5.69	6.45	6.24	7.30	6.22
ClO 0.5%		5.39	5.35	6.25	6.04	6.22	5.85
Cin 0.25%		6.05	6.23	6.49	6.79	7.06	6.52
Cin 0.5%		6.16	5.89	6.18	5.65	5.23	5.82
Thy 0.25%		6.10	5.82	5.56	5.52	5.31	5.66
Thy 0.5%		6.72	6.09	5.53	5.03	4.59	5.59
MIX 0.25%		5.54	5.84	6.49	7.29	8.05	6.64
MIX 0.5%		5.54	5.84	6.49	7.29	8.05	6.64
Mean		5.82	5.86	6.31	6.48	6.86	6.26
L.S.D 0.05	A	0.16					
	B	0.12					
	AB	0.00					

ClO = clove oil, Cin = cinnamon oil, T = thyme oil, MIX = (Clo + Cin + Thy) (1: 1: 1).

3.5 Changes in psychrotrophic bacterial count of chilled minced beef treated with essential oils during storage at 3±1°C

From the results in Table (5), it could be observed that the psychrotrophic bacterial count in control sample was 5.80 log cfu/g at zero time and significant ($P<0.05$) difference increased to 8.76 log cfu/g at the end of storage periods. Meanwhile, the psychrotrophic bacterial count of samples contained essential oil of clove, cinnamon, thyme and mixture under levels 0.25 and 0.50 % had ranged

from 5.72 to 6.14 log cfu/g at zero time, while at the end of storage periods showed little changes in psychrotrophic bacterial count under the same condition. From the same data it could be stated that minced beef samples treated with essential oils had little number of psychrotrophic bacterial count compared with control sample. Moreover, the increasing rate of psychrotrophic bacterial count was slow in minced beef samples treated with thyme essential oils. On the other hand, the increasing rate of psychrotrophic bacterial count was observed in minced beef samples treated with mixture essential oils than control sample during storage periods at 4±1°C up to 12 days. Oke *et al*. (2009), reported that plant extracts and essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries. Essential oils and purified components are used as natural prevent the growth of food borne bacteria and molds antimicrobials in food systems, as well as to resulting in extension of the shelf- life of processed foods.

Table (5): Changes in psychrotrophic bacterial count (log cfu/g) of chilled minced beef treated with essential oils during storage at 3±1°C.

Treatments		Storage periods by days					
		0	3	6	9	12	Mean
Control		5.80	6.31	6.78	7.68	8.76	7.07
ClO 0.25%		5.72	5.30	5.94	6.25	6.56	5.95
ClO 0.5%		5.75	5.36	5.15	5.89	6.37	5.70
Cin 0.25%		6.10	5.88	5.47	6.12	6.44	6.00
Cin 0.5%		6.14	5.85	5.55	5.51	6.23	5.86
Thy 0.25%		5.92	5.56	5.45	5.74	6.09	5.75
Thy 0.5%		5.84	5.66	5.23	5.58	5.98	5.66
MIX 0.25%		5.91	5.87	6.10	6.33	7.39	6.32
MIX 0.5%		5.79	5.70	5.96	6.33	7.18	6.19
Mean		5.89	5.72	5.74	6.16	6.78	6.06
L.S.D 0.05	A	0.17					
	B	0.13					
	AB	0.00					

ClO = clove oil, Cin = cinnamon oil, T = thyme oil, MIX = (Clo + Cin + Thy) (1: 1: 1).

3.6 Changes in coliform group counts of chilled minced beef treated with essential oils during storage at 3±1°C

Data given in Table (6) showed the coliform group in chilled minced beef samples formulated with essential oil of clove, cinnamon, thyme and mixture under levels 0.25 and 0.50 % during refrigerated storage at 3±1°C up to 12 days was ranged between 3.29 and 4.18 log cfu/g at zero time. Whereas control sample had significantly ($p \geq 0.05$) difference the higher counts coliform group compared to other treatments which formulated with essential oils under same levels at the end of storage periods. From the results it could be noticed that gradually decrease in coliform group counts during storage

periods. Moreover, the essential oil of thyme under level 0.25 and 0.50 % was more active of inhibiting effect against coliform group bacteria which disappear after 9 days of storage periods compared with other essential oils.

Table (6): Changes in coliform group counts (log cfu/g) of chilled minced beef treated with essential oils during storage at 3±1°C.

Treatments		Storage periods by days					
		0	3	6	9	12	Mean
Control		3.23	3.91	4.34	5.13	6.41	4.60
ClO 0.25%		3.46	3.14	2.10	1.46	0.00	2.03
ClO 0.5%		4.01	3.30	1.76	1.06	0.00	2.03
Cin 0.25%		3.77	2.68	2.26	1.09	0.00	1.96
Cin 0.5%		4.05	3.41	2.06	0.00	0.00	1.90
Thy 0.25%		3.55	2.48	2.03	0.00	0.00	1.61
Thy 0.5%		4.18	3.21	2.15	0.00	0.00	1.91
MIX 0.25%		3.44	3.81	4.46	5.30	5.87	4.58
MIX 0.5%		3.29	4.20	4.71	5.12	5.20	4.50
Mean		3.67	3.35	2.87	2.13	1.94	2.79
L.S.D 0.05	A	0.14					
	B	0.11					
	AB	0.00					

ClO = clove oil, Cin = cinnamon oil, T = thyme oil, MIX = (Clo + Cin + Thy) (1: 1: 1).

Meanwhile, the coliform groupdisappear in most chilled minced beef samples formulated with essential oil after 12 days. These results indicated that the essential oils at both concentrations inhibited the growth of coliform group bacteria. These results are similar as mentioned by (Mahmoud, 2013) who observed that coliform group disappear during cold storage in most minced beef samples treated with essential oils. (Mahmoud, 2022) reported that the coliform group disappear in turkey meatballs samples treated with thyme essential oil after 12 days.

4. Conclusion

The treatments significantly had a positive effect on the physical properties, quality attributes and microbiological changes. The minced beef meat treated with 0.25 and 0.50 % of essential oils demonstrated higher enhancement compared to the control samples during chilled storage periods. The highest significant effect was observed on samples treated with thyme essential oil, which reduced the rate of microbial damage and improved shelf life. While the mixed from different essential oils showed low effect on stability raw minced beef meat.

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