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Effect of Adding Cumin, Oregano and Thyme Essential Oils on Microbial Load of Beef Burger during Cold Storage

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***Abstract*—** *Four batches of beef burger first as control, second, third and fourth groups contained cumin, thyme, oregano essential oils at concentration 1.5%, respectively. The effect of different treatments on aerobic plate count (APC), Staphylococcus count and Enterococcus count was studied during chilling days at 4±1°C. The mean values of APC, Staphylococcus and Enterococcus were (7.65±0.46, 5.03±0.35and 6.17±0.31 log10CFU/g), (6.92±0.47, 4.24±0.32 and 5.36±0.29 log10CFU/g), (6.67±0.44, 4.17±0.28 and 5.11±0.24 log10CFU/g) and (6.41±0.46, 4.21±0.27 and 5.17±0.25 log10CFU/g) in control, cumin, oregano and thyme treated groups, respectively on 13th day of chilling. Thyme essential oil at concentration 1% showed higher desirable effects in decreasing bacterial growth*.

I. INTRODUCTION

The essential oil is the product obtained from fresh citrus materials or herbal plants, either by steam concentrate or by mechanical processes from the epicarp of Citrus, or dry distillation. the essential oil is then unglued from the aqueous phase by physical means. this definition includes all products obtained from vegetable raw material, but using other extraction systems, like using cold absorption or non-aqueous solvents. (Dhifi *et al*., 2016)

Essential oil (EOs) are natural compounds obtained from plants that exhibit antimicrobial and antioxidant properties and attract attention as additives in food manufacturing. EOs from Oregano (*Origanum vulgare L*.) and Cumin (Cuminum cyminum L.*)* have the possibility as natural food antioxidants Few EOs are 'Generally Recognized as Safe' (GRAS) and accepted by the food and drug Administration (FDA). The use and optimal of EOs should consider the consumer sensory acceptability of the last product. (Vital *et al*., 2016).

Essential oil are mixes of 20–60 components at quite diverse concentrations, with some compounds at high levels (20–70%), and others in trace amounts. The ingredients at high levels (terpenes, terpenoids, molecules with an aromatic ring) showed a significant role in the antimicrobial/biological effect (Bakkali *et al*., 2008). Some vital compounds of EOs are mono and sesquiterpenes, carbohydrates, phenols, alcohols, ethers, aldehydes, and ketones. phenol composites have also been recognized as bioactive components Essential oil with aldehydes or phenols as principal components (cinnamaldehyde, citral, carvacrol, eugenol, or thymol) are the most effective, followed with Essential oil which containing terpene alcohols with ketones or esters (β-myrcene, α-thujone, or geranyl acetate) possess a lesser activity (Perricone *et al*., 2015).

In recent years, scholars and food producers have been gradually studying Oregano (*Origanum vulgare L., family Laminaceae*). Oregano is adding to dishes in the form of fresh and/or dried leaves. However, water, alcohol extracts of Oregano, and essential oil can be used in food processing. the main reason for numerous studies on the use of this spice in food is in height antioxidant Valverde and antimicrobial action. These Oregano properties have also been confirmed, entomb, in studies on prolonging the shelf life of meat and meat products (Hać-Szymańczuk *et al*., 2018).

Essential oil (EOs) of plant Oregano, were appraised for their ability to inhibit the growth of *Aspergillus niger*, *Penicillium chrysogenum*, *Aspergillus flavus*, *too Aspergillus parasiticus*. It was reported that a shared formulation of Oregano with thyme essential oil caused in a synergistic effect, thus viewing an enhancement in the efficacy against *A. flavus*, *A. parasiticus*, and *P. chrysogenum*. (Chouhan *et al*., 2017)

Oregano oil showed the highest potential of antimicrobial activity against all test organisms (10 molds, two yeasts, and five bacteria). The essential oil of Ocimum Basil at a concentration of 1.5 ml/l completely repressed the mycelia growth of 22 species of fungi counting as the mycotoxin producing strains of *A. flavus* and *A. parasiticus* (Macwan *et al*., 2016).

A primary constituent of Oregano is carvacrol (a phenolic monoterpenoid). EOs rich in carvacrol have been reported to own remarkable antimicrobial movement. Although the outer membrane is affected in carvacrol, the cytoplasmic membrane is thought to be its site of the act, causing passive transport of ions through the layer. as an adaptation mechanism to maintain optimum membrane function and structure, it has been proposed that cells exposed to carvacrol difference the fatty acid composition of the membrane as of the effect of carvacrol on fluidity it has been demonstrated that carvacrol affects the external layer of gram-negative bacteria (Chouhan *et al*., 2017).

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II. Materials and Methods

Extraction of EOs

Extraction had been done by hydro distillation of dried plant leaves followed by evaporation under vacuum according to Cosentino *et al*. (1999).

Beef Burger Production

Twenty kilo- grams of freshly beef chuck 24 hours' postmortem was purchased from local butcher shop at Gassim-Saudi Arabia and directly transported to the laboratory in an ice box to be minced in electrical mincer (4 mm). Minced meat 65%, fat 20%, soybean 5%, black pepper 0.3%, salt 1.8% and water 10% were thoroughly mixed for five minutes and divided into five portions. First portion was used as control, while the other portions were either mixed with TEO (Cumin 1%, Thyme 1% and Oregano 1%), respectively. The obtained pastes were formed into 50 g beef burger using cardboard meat box, packed in foam plates and stored at refrigerator shelf at 4ºC.

Bacteriological Examinations

The preparation of the burger samples and serial dilutions were performed according to ISO 6887-2 (2003). Briefly twenty-five grams of each sample were homogenized aseptically with mixing in 225 ml of 0.1 % Buffered Peptone Water (BPW, HIMEDIA, M614-500G) in a stomacher (Colworth, 400) for 2.5 min at room temperature (25ºCand then allowed to stand for 5 min to provide a homogenate which represents the dilution of 10-1). Quantity of 1 ml of the homogenate was transferred into a sterile test tube containing 9 ml of 0.1% BPW, then ten folds' serial dilutions were prepared up to the required dilution (10-9). Enumeration of APC was done according to ISO 4833-1: (2013) using pour plate method of 1ml onto plate count agar (Oxoid CM325) then incubated at 30O C. *Staphylococcus* count carried out Baired-Parker agar medium plates (Oxoid, CM 275) according to ISO, 6888-1: (1999). Enumeration of *Enterococcus* count carried out on Bile Esculin Azide ager (BEA, HIMEDIA, and M340). The BEA agar was inoculated by spreading 0.1 ml of the ready prepared serial dilutions onto the surface. The agar plates were incubated for 24 h at 37±0.5°C aerobically (ISO 7899-2, 2000). The medium inoculated by spreading 0.1 ml of the ready prepared serial dilutions onto the surface. The agar plates were incubated for 48 h at 37±0.5°C aerobically.

Statistical Analysis

All values of chemical and bacteriological analysis are presented as means ± standard error (SE). All microbial counts were converted to log10 CFU/g values. Kruskal- wallis H One-way analysis of variance (ANOVA) post hoc Bonferroni correction to estimate the differences in chemical and bacterial counts. P-values less than 0.05 were considered statistically significant. The difference between the treatments groups in the decontamination trial were analyzed by One-way ANOVA. p-values less 0.05 were considered statistically significant.

III. Results and Discussion

TABLE 1. Effect of essential oils on APC in beef burger stored at 4 ±1°C (N=5).

	Control	**Cumin 1.5%**	**Oregano 1.5%**	**Thyme 1.5%**
Zero time	5.54±0.42^{a}	5.15±0.45a	5.05±0.44^{a}	4.65±0.41^{b}
2nd day	5.78±0.36^{a}	5.32±0.28ab	5.21±0.31a^{b}	4.92±0.34^{b}
4th day	6.06±0.36^{a}	5.64±0.34ab	5.42±0.29a^{b}	5.09±0.42^{b}
6th day	6.45±0.39^{a}	5.93±0.42ab	5.66±0.44^{b}	5.36±0.47^{b}
9th day	6.84±0.45^{a}	6.12±0.41b	5.92±0.43^{b}	5.67±0.45^{c}
11th day	7.13±0.49^{a}	6.54±0.51b	6.23±0.44^{b}	6.12±0.43^{c}
13th day	7.65±0.46^{a}	6.92±0.47b	6.67±0.44^{bc}	6.41±0.46^{c}

(a,b,c)Means on the same raws carrying different superscript small letters are significantly different (P< 0.05).

Total bacterial count is a commonly used microbiological method for estimating shelf-life of food. The data in table 1 showed significant decrease (p< 0.05) in APC in thyme 1.5% treated group start from zero day at 4.65±0.41 and extend all over the storage days while it reached 6.41±0.46 at the end of storage period. On the other hand, significant decreases in oregano 1.5% treated group start from 5.05±0.44 till reached 6.67±0.44 at the end of storage period at 13 th day while, in cumin 1% start on zero time at 5.15±0.45 till 6.92±0.47 at the end of storage period when compared with control group. This result agreed with that reported by Arya *et al*., (2019) standard plate count value was not observed till one week in treatments with oregano oil. plants and seeds such as cumin (Cuminumcyminum) are very rich with natural compounds such ascuminaldehyde, limonene and linalool that have both antimicrobial and antioxidant activity (Derakhshan et al., 2008). The finding nearly similar to Uhart *et al*. (2005) indicated that after 3 days of storage of ground beef samples treated with garlic extract, 0.38-log10CFU/g reduction was observed. Essential oils contain active principles responsible for the bactericidal effects, garlic holds up the highest concentration of sulfur compounds like allicin and others that having biological activities. These are responsible for not only its pungent smell, but also for its medicinal value, with effective anti-microbial properties (Corzo-Martinez *et al*. 2007). Cumin oil are very rich with natural compounds such as cuminaldehyde, limonene and linalool that have both antimicrobial and antioxidant activity (Derakhshan *et al*. 2008).

TABLE 2. Effect of essential oils on *Staphylococcus* count in beef burger stored at 4 ±1°C (N=5).

	Control	**Cumin 1.5%**	**Oregano 1.5%**	**Thyme 1.5%**
Zero time	3.22±0.26^{a}	3.04±0.21a	2.83 ±0.19a	2.54±0.22b
2nd day	3.56±0.24^{a}	3.25±0.25a	3.17 ±0.21a	2.85±0.23b
4th day	3.85±0.23^{a}	3.41±0.23ab	3.37±0.22ab	3.21±0.21b
6th day	4.04±0.32^{a}	3.66±0.25ab	3.49±0.26b	3.35±0.29b
9th day	4.33±0.27^{a}	3.78±0.23b	3.61±0.28b	3.42±0.23c
11th day	4.55±0.25^{a}	3.91±0.21b	3.75±0.26b	3.51±0.24c
13th day	5.03±0.35^{a}	4.24±0.32b	4.17±0.28bc	4.21±0.27c

Presence of high number of *Staphylococcus*/g of burger may indicate a tendency towards presence of *S. aureus* and predication of health hazard. The data in table 2 showed

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significant decrease ($p< 0.05$) in *Staphylococcus* count in thyme 1% treated group start from zero day and extend all over the storage days. On the other hand, significant decreases in oregano 1.5% treated group start from 6th day and in cumin 1.5% start from 9th day of storage when compared with control group.

Microorganisms connected, by meat can be in general classified into three major groups: spoilage microorganisms, pathogenic microorganisms, and beneficial microorganisms (Davies and Board, 1998). The activities of microorganisms in meat might have different effects both on human health and the meat itself. Meats and another animal food are generally contaminated by microbes living in it naturally or entering it from the surroundings, as those resulting from processing operations, and their spoilage is quick if it is not kept adequately because of microbial activities (Onuoha *et al*., 2015). Meat and meat products are highly perishable commodities. So, they must be appropriately stored, processed, and packed and distributed to prevent microbial growth in the level of microorganisms present in meat products can be decreased only when they are further handled. The meat will spoil from Pathogens, as *Aeromonas*, Bacillus *cereus*, *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Salmonellas* (Pal *et al*., 2018).

The results were in accordance with Burt, (2004) who found that cumin showed an antimicrobial effect against Staphylococcus aureus, Rassooli *et a*l. (2006) and Sienkiewicz *et al*. (2011) who found that thyme essential oil is noted to possess bactericidal properties against S. aureus. Enterococci have been considered harmless commensals.

TABLE 3. Effect of essential oils on *Enterococcus* count in beef burger stored at 3 ±1°C (N=5).

	Control	**Cumin 1.5%**	**Oregano 1.5%**	**Thyme 1.5%**
Zero time	4.32±0.25a	4.11±0.23a	3.75 ±0.21a	3.63±0.24b
2nd day	4.75±0.25a	4.18±0.21a	3.95 ±0.23a	3.74±0.22b
4th day	4.98±0.23a	4.46±0.22ab	4.26±0.24ab	3.98±0.23b
6th day	5.21±0.27a	4.74±0.25ab	4.49±0.23b	4.24±0.25b
9th day	5.48±0.25a	4.95±0.25b	4.64±0.27b	4.49±0.29c
11th day	5.67±0.25a	5.01±0.24b	4.94±0.28b	4.71±0.26c
13th day	6.17±0.31a	5.36±0.29b	5.11±0.24bc	5.17±0.25c

The data in table 3 declared significant decrease ($p< 0.05$) in *Enterococcus* count in thyme 1.5% treated group start from zero day at 3.63±0.24 and extend all over the storage days while, the number reached 5.17±0.25 afterb13 th day. On the other hand, significant decreases in oregano 1.5% treated group start from 4.11±0.23 at zero time to 5.36±0.29 at the end of the storage period and in cumin 1.5% start from 9 th day of storage when compared with control group. The reduction of bacterial counts may be attributed to the antimicrobial effect of different compounds in the essential oils. This finding agreed with Sienkiewicz *et al*., (2013) who found that Basil oil showed inhibitory activity against *E. coli* and Boskovic *et al*., (2019) who found films obtained from alginate-based nano emulsions loaded with TEO exhibited strong antibacterial effects against *E. coli* and reduced the count of these bacteria by 4.71 logs within 12 h. Also, Sakkas and Papadopoulou, (2017) reported that the Oregano oil showed the greatest antibacterial effect against *Escherichia coli* O157:H7.

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