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RESEARCH ARTICLE

CONTROL OF SOME FOOD-BORNE PATHOGENS ON SOME MEAT PRODUCTS USING DILL (*DISAMBIGUATION*) AND PARSLEY (PETROSELINUM CRISPUM*)* ESSENTIAL OILS

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ABSTRACT

Enhancement of food safety is the major interest by increasing interest in natural preservatives, which has antioxidant, antimicrobial properties and more healthy specially in meat which is highly susceptible to microbial growth, it is which can cause its spoilage and contributes to food borne diseases in human, resulting in serious health problems. The objective of the present study was to, (i)evaluate different uses of (dilland parsley) extracts,(coat, dipped, 1.5% and 3%) when they added to some popular meat productson pH and TBC, (ii)evaluate their inhibitory effect on some food spoilage microorganisms, (TSC, and coliforms) and some food-borne pathogens (*Staph. aureus*, *E. coli*, *salmonella spp*. and *Shigella spp*.) to ensure meat safety were investigated during storage at 4±1 °C for 14 days. The extracted oils from Dill and parsley added to meat products (minced meat, Burger and Kofta) by different methods, (control sampling, coating, dipping, 1.5% concentrations and 3% concentration). pH measured then different microbial examination. Results declared that with dill were more effective on pH and TBC, TSC, coli form, *Staphylococcus areus, Salmonella spp.*, *Shigella spp.* while parsley was more effective against *E. coli*. In conclusion, obtained results recommended usage of dill extract as safe and natural antimicrobial meat additives to prolong shelf life of meat product due to its high antibacterial activity against many meat spoilage microorganisms and meat pathogens. On the other hand, parsley extract showed mild to moderate effect against most meat spoilage and meat pathogens.

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INTRODUCTION

Recently, enhancement of food safety is the major interest by increasing interest in natural preservatives whichhas antioxidant and antimicrobial properties by their essential oils (EOs),for decreasing and eliminating pathogen and food spoilage microorganisms as well as the interactions among food-plant extract-microorganisms and possible combinations of antimicrobial agents and study the preservative properties from plant extract from fresh produce can be used as an alternative treatment and most consumers all over the world also are becoming increasingly conscious of the nutritional value and safety of their food and its ingredients. At the same time, there is an increase preference for natural food and food ingredients, which are generally believed to be safer, more healthy and less subject to hazards than food containing artificial food additives specially in meat which is highly susceptible to microbial growth, it is which can cause its spoilage and contributes to food borne diseases in human, resulting in serious health problems.

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The most important pathogenic bacteria associated with meat products are *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Escherichia coli* (Borch *et al.,* 1996, Oussalah *et al.,* 2007, Hyldgaard *et al.,* 2012, Komba *et al.,* 2012, Alsaiqali, *et. al.,* 2016 and Marín *et al.,* 2016). Chemical meat preservatives mainly not only causes a loss in food quality but also has correlated with oxidative damage which leading to fetal side effects such as, carcinogenesis, mutagenesis, ageing, and arteriosclerosis. While, aromatic plants oils (EOs) have antibacterial and antifungal activity when they are applied in meat products during storage beside their antioxidant properties.

One of the used methods to increase and improving the shelf life of meat and meat products is using of some of these aromatic plants which rich such as, dill (*Disambiguation*)) and parsley (*Petroselinum crispum*) (Baratta *et al.* 1998 and Karimi *et. al.,* 2014). Aromatic plants extract (EOs) mostly has antimicrobial effect mainly ongram-negative bacteria due to its contents of some antibacterial compounds such as, menthol, geraniol, cinnamyl alcohol, citronellol, linalool, carvacrol, eugenol, cinnamaldehyde, thymol, carvone, chavicol and estragole (Wangensteen *et al.,* 2004 and Trombetta *et al.,* 2005). Many antioxidant compounds, naturally occurring from plant sources such as, phenolic flavonoids, alkaloids, tannins and phenolic acids compounds, have been identified as free radical or active oxygen scavengers, which can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Velioglu *et al.,* 1998, Zheng and Wang, 2001 and Ayala-Zavala *et al.,* 2008). Dill *(disambiguation*) seed is a popular spice and finely ground seed is a major ingredient of curry powder. Volatile components in essential oil of dill leaves is stronger antioxidant and antimicrobial against wide range of micro-organisms than the seeds, (Delaquis *et al.,* 2002). Parsley *(Petroseliumcrispum)* is another herb which is very rich in vitamins, thiamin, carotene, organic minerals and phenolic compounds such as Cosmene, Limonene, Myristicin, -Pinene and –Pinene (Wong and Kitts, 2006 and Diez, 2015). Parsley also contains other antimicrobial compounds effects against *Salmonella*, *shigella*, *Staphylococcus aureus*and *E. coli* such as plus oleic, linoleic, palmatic and other fatty acids (Busatta *et al.,* 2008, Dostalova *et al.,* 2014 and Farah *et al.,* 2015). The objective of the present study wasto,(i)evaluate the uses of (dilland parsley) extracts (EOs) in different methods, (coat, dipped, 1.5% and 3%) when they added to some popular meat products, minced meat, beef burgers and koftaon pH and TBC, (ii)evaluate the uses of (dilland parsley) extracts (EOs) as inhibitory effect on some food spoilage microorganisms, (TSC, and coliforms) and some food-borne pathogens (*Staph. aureus*, *E. coli*, *salmonella spp*. and *Shigella spp*.) when they added to some popular meat products i.e. minced meat, beef burgers and kofta to ensure meat safety were investigated during storage at 4±1 °C for 14 days.

MATERIAL AND METHODS

Essential Oil Extraction (AOAC, 2005): The extraction of dill *Disambiguation* and parsley *Petroselinum crispum* essential oils by, 100 grams of dried leaves and seeds of dill and parsley dipped in 400ml cold distilled water in hydro-distilled for 3 hours using a Clevenger type apparatus. The collected oils dried by passing over anhydrous sodium sulfate on a filter paper (Whatman No.1) in a glass funnel. The oils were stored in a sealed glass bottle at 4˚c until subsequent tests. The extracted oils from dill and parsley added to meat products (minced meat, Burger and Kofta) at 5 different methods, ((i)control sampling, (ii)coating, coating with polyethylene page brushed on dill and parsley oils, (iii)dipping, by submersion of the meat in the extract for 5 minutes, (iv)1.5% concentrations and (v)3% concentration(v/w).

Meat Samples Preparation: 4.5 kilograms of fresh slaughtered meat purchased from the same carcasses from Elkharga abattoir in New Valley governorate, Egypt. Purchased Meat Samples kept in sterile impermeable polyethylene bag and shipped with sterile refrigerant reached the Food Hygiene laboratory, Faculty of Veterinary Medicine, New Valley, Egypt where all the meat minced and divided into 3 parts, 1st third part tested as minced meat products, 2nd third used to preparing beef burger and the 3rd part used to kofta preparing. Meat products prepared according to Egyptian standard specification (ESS 1688/1991).

Experimental Design: 1.5 kilograms from each meat products prepared and divided as following, (i)500gm control, (ii)250 gm coating with polyethylene page brushed on dill and parsley oils (125 gm for each), (iii)250 gm dippingin dill and parsley oils (125 gm for each),(iv)250 gm mixed with 1.5% concentrations of dill and parsley (125 gm for each), and (v)250 gm mixed with 1.5% concentrations of dill and parsley (125 gm for each). All samples kept at 2±0 °C for 14 days in sterile refrigerant then measurements were carried out on the days (0, 7 and 14) in the Food Hygiene laboratory, Faculty of Veterinary Medicine, in New Valley, Assuit University.

Physical Quality: (i)pH measured by pH meter (FAO, 2013), (ii)Odour, colour, and overall acceptability of different meat products samples were assessed by 3 members of Food Hygiene and Control Department (with past experience in meat products and evaluation) to evaluate their sensory characteristics. Sensory hedonic scheme, ranged from 0 (very bad) to 8 (very good) following the procedures of **AMSA (1995)**, was applied.

Microbiological Quality

Samples Preparation: Samples homogenized in a Seward stomacher (400R/UK) and serial dilutions (10^{-1}- 10^{-5}). The serial dilutions of each sample were investigated for count of total bacterial count (TBC) and Total Psychrophillic Count (TSC) according to (Valls, *et al.*, 2000) on plate count agar (Oxoid) using drop technique. The plates were incubated at 35°C for 24h.for TBC and incubated at 7°C/10 days for Total Psychrotrophic Count, Total *Coliform* Count on Violet Red Bile Agar (VRBA, Merk) using drop technique. The plate incubated at 35°C for 24hours.

E. coli: Detection Eosine Methylene Blue Agar (Nissui) using drop technique. The plate incubated at 37°C for 24 hours Colonies green sheen, showing nucleated centers were confirmed as typical E. coli, While mucoid and atypical colonies were confirmed as coliform organisms.

***Staphylococcus aureus*:** (APHA, 2002) on Baird Parker plate media with 5% egg yolk and 3.5% of Potassium Tellurite using spreading technique and incubated at 37°C/48 hours Suspected colonies were black and shiny convex colonies, greater than 1mm. with wide clear area with zone. Then 1-5 colonies inoculated into brain heart infusion broth and incubated over night at 37°C, and then streaked onto blood agar, Baird Parker and mannitol salt agar plates. Suspected colonies were picked up and examined microscopically by Gram's stain and pure cultures of the isolates were biochemically identified according to Quinn *et al.* (1994).

Microbial Inoculation: Three bacterial strains were (*Escherichia coli*, *Salmonella typhi* & *Shigella dysenteriae*) maintained on agar slant at 4 °Cuntil mixed with (25 gm minced meat, 25 gm burger & 25 gm kofta) for evaluating antibacterial activity of the dill and parsley extracts using nutrient agar media slants where the organisms were transferred from pure cultures then incubated at 37 °C/18-24 hours. Sensitivity test performed within 2-3 days using these fresh well-isolated colonies, thoroughly vortex, within 15 minutes a sterile cotton swab was dipped into the suspension, remove excess liquid, then streak three times the swab over the agar surface, punched filter paper and sterilized in an autoclave machine then soaked with sample solution then dried by dryer, then labeled into the sterile air tight vial and stored in refrigerator. Positive specific antibiotic discs and negative control disc prepare would be used also. Sample discs, negative control and positive antibiotics discs were placed on to the surface of the plates inoculated with the test organism within 15 minutes after inoculation. The discs placed individually with sterile forceps, and then gently press down onto the agar. No more than 5 discs on a 100 mm plate to prevented overlapping of the inhibition zones & error in measurement. The plates refrigerated at 4°C/1-2 hr. before incubated at 37 °C /18 hours. The accurate resulting inhibition zones were uniformly circular which measured to the margin of heavy growth with a ruler including the diameter of the disk Hudzicki, (2009). Isolation of *S. typhimurium*was performed according to USDA and FSIS, (2004) protocols using Rappaport-Vassiliadis broth (RV, Oxoid), incubated at 43°C/24 hr., followed by streaking a loopful of selective enrichment broth on Xylose lysine deoxycholate (XLD) agar (Merk) and Salmonella Shigellae (SS) agar media and incubated at 37°C/24 hr. All the isolates picked up, preserved on nutrient agar (Oxoid), for examined microscopically by Gram's stain to observe the morphological arrangement and staining reaction and pure cultures of the isolates were biochemically identified according to Quinn *et al.* (1994).

Statistical Analysis *(GraphPad Instant, 2009):* The statistical program, GraphPad Instant version 3 for window, was used for determination of means, the analysis of variance between the different data and treatment in this study were determined using standard error and analysis of variance ($P<0.05$).

RESULTS

Effect of dill (*Disambiguation*) and parsley (*Petroselinum crispum)* extract (EOs) during the refrigerator storage period on physical quality of different treatment of meat products

Effect on pH values: The results obtained in Figure 1 declared that the best treatment of minced meat is by coating the package with dill, while dipping of beef burger in dill or coating the beef burger or kofta samples package with parsley recoded the best reduction of pH values. As following, means of pH values of different treatments of meat products samples with dill and parsley as following, pH in minced meat were (6.9) in case of, (control, dill coating, dill 3%, parsley dipped, parsley 1.5% and parsley 3%) samples while, this value was (7.1) on (dill 1.5% and parsley coated minced meat), lower pH value were about (6.8) recorded in dill coated samples. pH in beef burger samples was (6.8) in case of, (control, dill coated samples) while, the majority of treatment recoded (6.7) in (dill 1.5%, dill 3%, parsley dipped, parsley 1.5% and parsley 3%) samples while, lowest pH value were about (6.6) recorded in (dill dipped and parsley dipped) samples. pH in kofta were (6.9) in case of, (control, dill 3%, parsley dipped, and parsley 3%) samples while, this value was (7) on dill coated samples and (7.1) on (dill dipped samples), lower pH value were about (6.8) recorded in dill 1.5% and parsley 1.5% samples whoever parsley coated samples was (6.6) in pH value. pH values were significantly difference ($P < 0.05$) in treated meat samples compare to control samples.

Effect on Sensory Characters: table (1) declared the Sensory Characteristics (Odour& Color) Changes in Types of Tested Meat after Parsley and Dill Different Treatments over Storage Period which showed that the sensory characters of tested samples which treated neither by dill or parsley decreased by prolonged storage period and all the treatments didn't improve these characters as following, all samples (odor and color) were excellent on zero day (8) while on 3rd day the quality decrease to (7) and lowest quality reported on 7th day about (6).

Effect of Different Treatment on Different Types of Tested Meat on Microbial Qualityover Storage Period:

Total Bacterial Counts (TBC) on Different Meat Products

Minced Meat Samples: The obtained results in figure 2 showed the effect of dill and parsley different treatments on minced meat samples respectively over storage period as following, the mean TBC of minced meat samples on zero day samples were, 7.05X10^4, 2.98X10^4, 2.87X10^4, 2.55X10^4, 6.32X10^4, 5.65X10^4, 4.55X10^4, 2.83X10^5 and 7X10^5, on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. While after 7 days of storage TBC means recorded, 9.18X10^4, 1.55X10^4, 4.43X10^3, 2.55X10^4, 4.90X10^4, 4.6X10^3, 7.32X10^3, 2.92X10^5& 7.72X10^5 on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. The highest TBC means recoded after 14 days as following, 7.14X10^5, 1.65X10^4, 1.27X10^4, 2.55X10^4, 4.68X10^4,8.14X10^4, Not Detected, 2.87X10^5& 2.88X10^5 on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. All samples were lower than the permissible limit although the best results recoded on dipped meat samples which were significantly different ($p<0.05$) than other samples followed by, (coat, 1.5%, 3%&control)dill minced meat treatments while in case of dipped meat sample the lowest TBC which were significantly different ($p<0.05$) than other samples followed by,(coated, control, 1.5% & 3%) parsley minced meat treatments respectively.

Burger Samples: The detected results in figure 3revealed the effect of dill and parsley different treatments on burger samples over storage period as following, the mean TBC of burger samples on zero day samples were, 7.46X10^3, 5.50X10^3,6.20X10^3, 9.60X10^3, 2.30X10^3, 6.38X10^3, 9.16X10^3, 1.87X10^4& 1.86X10^{4}on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. While after 7 days of storage TBC means recorded, 3.94X10^4, 2.18X10^4, 2.20X10^4, 2.52X10^4, 2.92X10^4, 3.30X10^4, 3.30X10^4, 2.60X10^4& 3X10^{4}on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. The highest TBC means recoded after 14 days as following, 2.15X10^4, 1.97X10^4, 2.04X10^4, 2.75X10^4, 3.16X10^4, 2.03X10^4, 1.80X10^4, 1.50X10^4&1.20X10^4 on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. All samples were lower than the permissible limit although the best results recoded on 3% dill burger meat samples followed by, (coat, dipped, control &1.5%)dill burger treatments samples while in case of parsley treatment, coated burger samples were the lowest TBC followed by dipped, 1.5% & 3% parsley treatments of burger samples, especially during (zero & 7 days) after treatments which were significantly different ($p<0.05$) than other samples.

Kofta Meat Samples: Figure 4 illustrated the effect of dill and parsley different treatments on kofta meat samples respectively over storage period as following, the mean TBC of kofta meat samples on zero day samples were, 4.86X10^4, 1.80X10^4, 1.89X10^4, 1.51X10^4, 3.52X10^4, 2.52X10^4, 3.35X10^4, 3.86X10^4 and 4.36X10^4, on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. While after 7 days of storage TBC means recorded, 5.18X10^4, 1.69X10^4, 1.59X10^4, 1.94X10^4, 2.14X10^4,

Figure 1. pH Changes in Types of Tested Meat after Parsley and Dill Different Treatments Over Storage Period

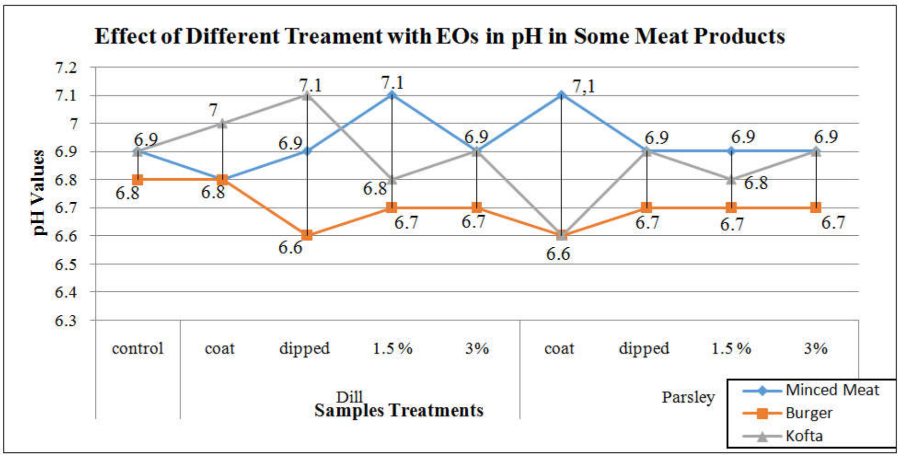
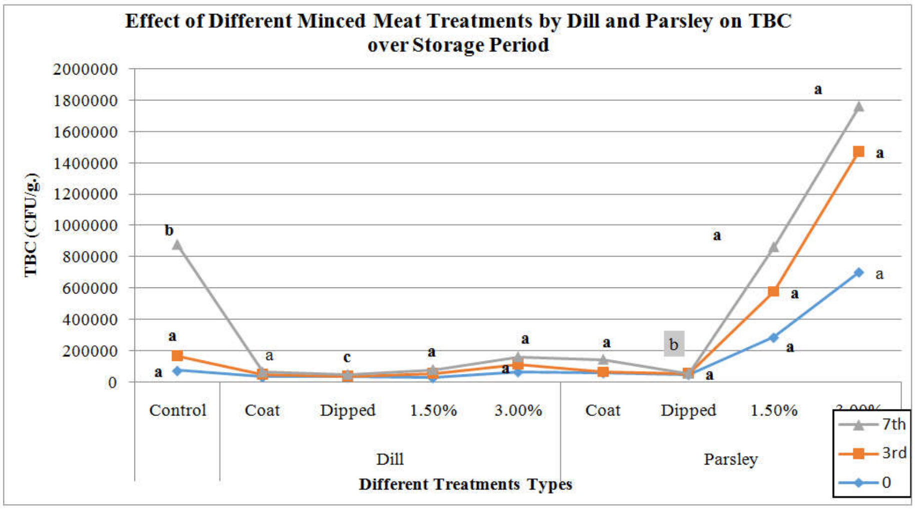
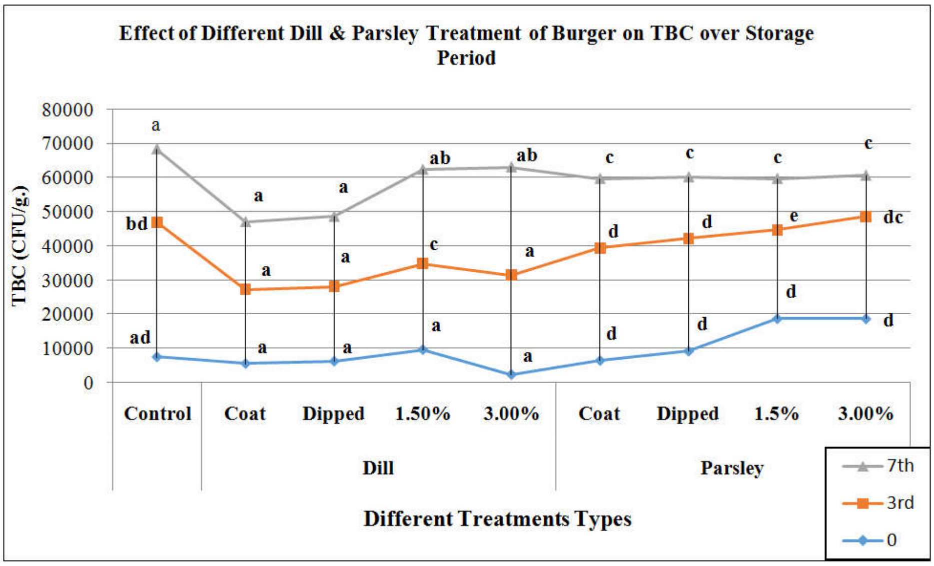


Figure 2. Total Bacterial Counts Changes in Minced Meat after Different Dill& Parsley Treatments over Storage Period



Means followed by a different letter in the line are significantly different (*p>0.05*)

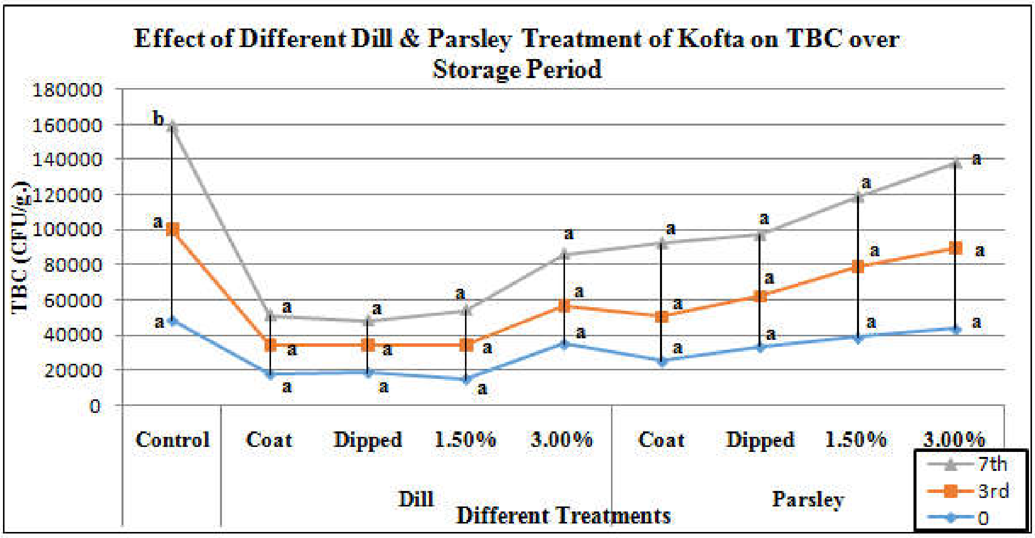
Figure 3. Total Bacterial Counts Changes in Burger after Different Dill& Parsley Treatments over Storage Period



Means followed by a different letter in the line are significantly different (*p>0.05*)

$2.52X10^4$, $2.86X10^4$, $4.05X10^4$&$4.58X10^4$on [control,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. The highest TBC means recoded after 14 days as following, $5.87X10^4$, $1.65X10^4$, $1.65X10^4$, $1.36X10^4$, $2.01X10^4$, $4.19X10^4$, $3.49X10^4$, $3.96X10^4$& $4.86X10^4$ on [control ,(coated, dipped, 1.5% and 3%) dill &(coated, dipped, 1.5% and 3%) parsley treatment samples] respectively. All samples

Figure 4. Total Bacterial Counts Changes in Kofta after Different Dill& Parsley Treatments over Storage Period



Means followed by a different letter in the line are significantly different ($p>0.05$)

Table 1. Sensory characteristics (Odor & Color) changes in types of tested meat after parsley and dill different treatments over storage period

Storage Period	Control	Dill Treatments				Parsley Treatments			
		Coat	dipped	1.5%	3%	Coat	dipped	1.5%	3%
0 day	8	8	8	8	8	8	8	8	8
3rdday	7	7	7	7	7	7	7	7	7
7th day	6	6	6	6	6	6	6	6	6

Sensory hedonic scheme, ranged from 0 (very bad) to 8 (very good) following the procedures.

were lower than the permissible limit although the best results recoded on, 1.5%, dipped & coated dill treated kofta samples respectively while coated parsley kofta samples were the lowest results followed by, dipped, 1.5% & 3% respectively.

Mean values of (TSC, Coliform, *Staphylococcus areus*) which detected on (minced meat, burger & kofta) over storage period were discussed in table (2) as following

Minced Meat Samples:7.9X10^{3}, 6.2X10^{3}, 2.9X10^{6} were the means of TSC of dill minced meat treatment samples on (zero, 3rd & 3rd) storage days respectively. While, coliform means of the same samples were, 2.5X10^{4}, 2.2X10^{4}, 1.8X10^{4}on (zero, 3rd, 7th) storage days respectively. *Staphylococcus areus* means results of dill minced meat treatment samples were, 2.9X10^{4}, 10^{4}, 290 on (zero, 3rd, 7th) storage days respectively. Higher TSC results recorded in parsley minced meat samples as following, 7.98X10^{3}, 3.03X10^{4}, 2.4X10^{6}were the means of TSC of parsley minced meat treatment samples on (zero, 3rd&7th) storage days respectively. While, the coliform means of the same samples were nearly similar to the dill minced meat samples as following, 2.5X10^{4}, 1.3X10^{4}, 2.7X10^{4}on (zero, 3rd, 7th) storage days respectively. *Staphylococcus areus* mean results of parsley minced meat treatment samples were lower than those detected in dill treatment samples, 2.9X10^{4}, 1.6X10^{3}, 1.3X10^{3}on (zero, 3rd, 7th) storage days respectively.

Burger Samples:1.4X10^{5}, 2.6X10^{5}, 2.7X10^{5} were the means of TSC of burger dill treatment samples on (zero, 3rd & 3rd) storage days respectively. While, coliform means of the same samples were, 4.9X10^{4}, 1.7X10^{4}, 1.5X10^{3}on (zero, 3rd, 7th) storage days respectively. *Staphylococcus areus* means results of dill treatment burger samples were, 4.9X10^{4}, 3.8X10^{4}, 1.3X10^{4} on (zero, 3rd, 7th) storage days respectively. Higher TSC results recorded in burger parsley samples as following, 1.4X10^{5}, 8.9X10^{5}, 6.1X10^{4} were the means of TSC of burger parsley treatment samples on (zero, 3rd&7th) storage days respectively. 4.9X10^{4}, 2.7X10^{4}, 2.8X10^{3}represented the coliforms mean values of burger parsley treated samples over (zero, 3rd, 7th) storage days respectively which consider slightly higher values than those detected by dill treatment. While, *Staphylococcus areus* means of the same samples were nearly similar to the dill treated burger samples as following, 4.9X10^{4}, 3.0X10^{4}, 1.7X10^{5}on (zero, 3rd, 7th) storage days respectively.

Kofta Samples:7.3X10^{3}, 1.9X10^{5}, 4.5X10^{5} were the means of TSC of dill treated kofta samples on (zero, 3rd&7th) storage days respectively. While, coliform means of the same samples were, 2.3X10^{4}, 6.9X10^{2}, 2.1X10^{3}on (zero, 3rd, 7th) storage days respectively. *Staphylococcus areus* means results of dill treated kofta samples were, 2.9X10^{4}, 2.3X10^{4}, 2X10^{4}on (zero, 3rd, 7th) storage days respectively. Higher TSC results recorded in parsley treated kofa samples as following, 7.3X10^{3}, 2.2X10^{6}, 2.4X10^{6} were the means of TSC of parsley treated kofta samples on (zero, 3rd&7th) storage days respectively. 2.3X10^{4}, 2.3X10^{4}, 1.7X10^{4}represented the coliforms mean values of kofta parsley treated samples over (zero, 3rd, 7th) storage days respectively. *Staphylococcus areus* mean results of parsley treated kofta samples were lower than those detected in dill treatment samples, 2.9X10^{4}, 1.6X10^{4}, 1.2X10^{4}on (zero, 3rd, 7th) storage days respectively.

Prevalence % of (*E.coli*, *Salmonella spp.*, *Shigella spp.*) which detected on (minced meat, burger & kofta) over storage period were discussed in table (3) as following

Minced Meat Samples: The incidence % of *E. coli* were,(20, 15, 5) in dill minced meat treatment samples on (zero, 3rd & 3rd) storage days respectively. While, in case of *Salmonella spp.* the incidence % were (15, 0, 0) % in dill minced meat treatment samples on (zero, 3rd& 7th) storage days respectively.

Table 2. Mean of some food pathogens in different treatments meat products over storage period (cfu/g)

Storage period (days)	Minced Meat						Burger						Kofta					
	Dill			Parsley			Dill			Parsley			Dill			Parsley		
	TSC	Coliform	*S. aureus*	TSC	Coliform	*S. aureus*	TSC	Coliform	*S. aureus*	TSC	Coliform	*S. aureus*	TSC	Coliform	*S. aureus*	TSC	Coliform	*S. aureus*
Zero	7,9 X 10^3	2,5 X 10^4	2,9 X 10^4	7,98 X 10^3	2,5 X 10^4	2,9 X 10^4	1,4 X 10^5	4.9 X 10^4	4,9 X 104	1,4 X 10^5	4,9 X 10^4	4,9 X 10^4	7,3 X 10^3	2,3 X 10^4	2,9 X 10^4	7,3 X 10^3	2,3 X 10^4	2,9 X 10^4
3rd	6,2 X 10^3	2,2 X 10^4	1,0 X 10^4	3,03 X 10^4	1,3 X 10^4	1,6 X 10^3	2,6 X 10^5	1,7 X 10^4	3,8 X 104	8,9 X 10^5	2,7 X 10^4	3,0 X 10^4	1,9 X 10^5	6,9 X 10^2	2,3X 10^4	2,2 X 10^6	2,3 X 10^4	1,6 X 10^4
7th	2,9 X 10^6	1,8 X 10^4	2,9 X 10^2	2,4 X 10^6	2,7 X 10^4	1,3 X 10^3	2,7 X 10^5	1,5 X 10^3	1,3 X 104	6,1 X 10^4	2,8 X 10^3	1,7 X 10^5	4,5 X 10^5	2,1 X 10^3	2,0X 10^4	2,4 X 10^6	1,7 X 10^4	1,2 X 10^4

Table 3. Detected % of Some Food Pathogens in Different Treatments of Different Meat Types over Storage Period

Storage period (days)	Minced Meat						Burger						Kofta					
	Dill			Parsley			Dill			Parsley			Dill			Parsley		
	E. coli	*salmonella*	*Shigella*	*E. coli*	*salmonella*	*Shigella*	*E. coli*	*salmonella*	*Shigella*	*E. coli*	*salmonella*	*Shigella*	*E. coli*	*salmonella*	*Shigella*	*E. coli*	*salmonella*	*Shigella*
Zero day	20	15	25	20	15	25	15	10	28	15	10	28	30	00	25	30	00	25
3rd day	15	00	18	9	00	20	8	00	16	6	00	18	10	00	00	8	00	10
7th day	5	00	7	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00

Shigella spp. % of dill minced meat treatment samples were, (25, 18, 7) on (zero, 3rd, 7th) storage days respectively. Dill treatment very effective inhibition activity on *Salmonella spp.,*and moderate inhibition effect on (*E. coli* & *Shigella spp.*), parsley was more effective than dill in *E. coli* inhibition % in parsley minced meat treatment samples as following, (20, 9, 0) on (zero, 3rd, 7th) storage days respectively. While, it recorded the same inhibition % on *Salmonella spp.,* in parsley minced meat samples as following, (15, 0, 0) % on (zero, 3rd&7th) storage days respectively. Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley minced meat treated samples as following, (25, 20, 0) on (zero, 3rd) storage days respectively with total reduction % after (7th) storage days respectively.

Burger Samples: The incidence % of *E. coli* were,(15, 8, 0) in dill burger treated samples on (zero, 3rd&7th) storage days respectively. While, in case of *Salmonella spp.* the incidence % were (10, 0, 0) % in dill burger treated samples on (zero, 3rd&7th) storage days respectively. *Shigella spp.* % of dill burger treated samples were, (28, 16, 0) on (zero, 3rd, 7th) storage days respectively. Dill treatment has very effective inhibition activity on *Salmonella spp.,* and moderate inhibition effect on (*E. coli* & *Shigella spp.*) its effectiveness increasing with longer storage period, parsley was more effective than dill in *E. coli* inhibition % in parsley burger treated samples as following, (15, 6, 0) on (zero, 3rd, 7th) storage days respectively. While, it recorded the same inhibition % on *Salmonella spp.,* in parsley burger treated samples as following, (10, 0, 0) % on (zero, 3rd&7th) storage days respectively. Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley burger treated samples as following, (28, 18, 0) on (zero, 3rd) storage days respectively with total reduction % after (7th) storage days respectively.

Kofta Samples: The incidence % of *E. coli* were,(30, 10, 0) in dill kofta treated samples on (zero, 3rd & 3rd) storage days respectively. While, *Salmonella spp.* was absent in dill kofta treated samples during storage days. *Shigella spp.* % of dill kofta treated samples were, (25, 0, 0) on (zero, 3rd, 7th) storage days respectively. Dill treatment has very effective inhibition activity on *Salmonella spp., & Shigella spp.* and moderate inhibition effect on (*E. coli*) its effectiveness increasing with longer storage period, parsley was more effective than dill in *E. coli* inhibition % in parsley kofta treated samples as following, (30, 8, 0) on (zero, 3rd, 7th) storage days respectively. While, it recorded the same inhibition % on *Salmonella spp.,* in parsley kofta treated samples as it not detected all over the storage days. Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley kofta treated samples as following, (25, 10, 0) on (zero, 3rd) storage days respectively with total reduction % after (7th) storage days respectively.

DISCUSSION

There are some factors affect the antibacterial effect of herbs extract in meat such as, water, fats, antioxidants, salt, pH, packaging, temperature, and microorganism nature (Burt, 2004). Addition of herbs extract does not effect on the sensory properties of meat products (taste, odor and color) (Reglero *et al.,* 2008). The finding from obtained results reported that coating the package with dill considering the best treatment of minced meat, while dipping of beef burger in dill or coating the beef burger or kofta samples package with parsley recoded the best reduction of pH values which ranged from (6.9 – 7.1) in minced meat, (6.6 – 6.8) in burger and (6.6 – 7.1) in kofta. Similar results recoded byAlsaiqali *et al.,* (2016) who showed also the effect of essential oils on burger few days of cold storage in both treated and control samples the mean pH value of control were 6.56 at the 6.67 after few days of storage. While, the treated meat samples with 1.2 % of parsley oils decreased pH value to 6.63. This reduction of pHvalues in samples treated with essential oils may refer to the antimicrobial effect of herb oils phenolic compound and some other compounds, which retarded the autolysis of meat protein (Viuda-Martos *et al.,* 2011, Ashour *et. al.,* 2014 and Marín *et al.,*2016). Nalini *et al.,* (1998) recorded **s**imilar results in the pH values of treated minced beef samples with EOs, where the pH values of control samples were higher than treated samples. Generally, microorganism growth inside meat always increasing their pH value, which usually causes hydrolysis of protein and nitrogenous compounds of meat releasing while, pH, and temperature and oxygen reduction has antibacterial effect on meat (Burt, 2004).

The obtained TBC results ranged from, $4.43X10^3$ -$7.14X10^5$ in different dill treated minced meat samples over the storage period and from (Not Detected- $7.72X10^5$) in different parsley treated minced meat samples over the storage period. The mean values of TBC in burger samples ranged from, $2.30X10^3$-$3.94X10^4$ in different dill treated burger samples over the storage period and from ($6.38X10^3$- $3.30X10^4$) in different parsley treated burger samples over the storage period. The mean values of TBC in kofta samples ranged from, $1.36X10^4$-$5.87X10^4$ in different dill treated kofta samples over the storage period and from ($2.52X10^4$- $4.86X10^4$) in different parsley treated kofta samples over the storage period. The recorded results declared that different treatments of minced meat, burger &kofta with dill were more antibacterial effect than parsley treatments of the meat products over storage period. All samples were lower than the permissible limit although the best results recoded on dipped minced meat samples which were significantly different ($p<0.05$) than other samples followed by, (coat, 1.5%, 3% & control) dill treated minced meat samples, In burger samples, 3% dill burger samples recorded the lowest mean value followed by, (coat, dipped, control & 1.5%) dill burger samples. In kofta samples, 1.5%, dipped & coated dill treated kofta samples were the lowest mean values respectively. In case of parsley treatment, the lowest TBC recoded in dipped parsley treated minced meat, burger samples followed by, (coated, control, 1.5% & 3%) parsley treated minced meat, respectively. While, coated parsley kofta samples were the lowest results followed by, dipped, 1.5% & 3% respectively. The reported reduction of TBC rates might referred to the presence of some antibacterial compounds on dill & parsley oils such as, (flavonoids) which affect most micro-organisms and bacteriostatic compounds such as, phenolics similar results reported by, (Wong and Kitts, 2006, Ashour *et al.,* 2014, Farah *et al.,* 2015 and Alsaiqali *et al.,* 2016). Similar result noted by Farah *et al.,* (2015) who observed that the dill and parsley extract has significant reduction of TBC. However, dill was more effective than parsley extract. Alsaiqali *et al.,* (2016) examined the parsley extract effect on refrigerated meat. Their results concluded that parsley extract had a strong antimicrobial effect against TBC, *E. coli*, *S. aureus* during the first storage period however this effect reduced by time. Herbs containing various antimicrobial chemical compounds such as, alkaloids, tannins, volatile oils, and lipids. Dill containing antimicrobial volatile oils. Our results declared that, the mean values of TSC of dill treated minced meat samples over the storage period were, $7.9X10^3$, $6.2X10^3$, $2.9X10^6$ While, coliform means of the same samples were, $2.5X10^4$, $2.2X10^4$, $1.8X10^4$ and *Staphylococcus areus* mean values of the same results were, $2.9X10^4$, 10^4, 290. On the other hand, the mean values of TSC of parsley treated burger samples over the storage period were, $7.98X10^3$, $3.03X10^4$, $2.4X10^6$, however the coliform mean values of the same samples were, $2.5X10^4$, $1.3X10^4$, $2.7X10^4$ and the mean values of *Staphylococcus areus*of them were, $2.9X10^4$, $1.6X10^3$, $1.3X10^3$. The mean values of TSC of dill treated burger samples over the storage period were, $.4X10^5$, $2.6X10^5$, $2.7X10^5$ While, coliform means of the same samples were, $4.9X10^4$, $1.7X10^4$, $1.5X10^3$ and *Staphylococcus areus* mean values of the same results were, $4.9X10^4$, $3.8X10^4$, $1.3X10^4$. On the other hand, the mean values of TSC of parsley treated burger samples over the storage period were, $1.4X10^5$, $8.9X10^5$, $6.1X10^4$, however the coliform mean values of the same samples were, $4.9X10^4$, $2.7X10^4$, $2.8X10^3$ and the mean values of *Staphylococcus areus*of them were, $4.9X10^4$, $3.0X10^4$, $1.7X10^5$. The mean values of TSC of dill treated kofta samples over the storage period were, $7.3X10^3$, $1.9X10^5$, $4.5X10^5$ While, coliform means of the same samples were, $2.3X10^4$, $6.9X10^2$, $2.1X10^3$ and *Staphylococcus areus* mean values of the same results were, $2.9X10^4$, $2.3X10^4$, $2X10^4$. On the other hand, the mean values of TSC of parsley treated kofta samples over the storage period were, $7.3X10^3$, $2.2X10^6$, $2.4X10^6$, however the coliform mean values of the same samples were, $2.3X10^4$, $2.3X10^4$, $1.7X10^4$ and the mean values of *Staphylococcus areus* of them were, $2.9X10^4$, $1.6X10^4$, $1.2X10^4$. Obtained results declared that dill was more effective in inhibition of TSC than parsley minced meat, burger & kofa samples. While, the dill and parsley has nearly inhibition similar effect on coliform mean values in minced meat samples. In addition, Dill was more effective in inhibition of coliforms than parsley burger &kofa samples. *Staphylococcus areus* mean results of parsley (minced meat & kofta) treatment samples were lower than the dill treated samples, however it recorded nearly similar to the dill treated burger samples. Similar dill antimicrobial inhibitory results observed by Şimonaţi and Mihuţa (2009) whom recorded a variable degree on different microorganisms such as, *S. aureus,* which consider one of the most important food-poisoning pathogen it has been identified as the most sensitive strain against dill. Lower results recorded by Seyyednejad *et al.* (2008) who reported that parsley extract hadn't any antimicrobial effect at different concentration, (0.1 to 0.4 gr.ml) against *S.ureus*. El Astal *et al.,* (2005) also found that parsley extract has not any antimicrobial effect at different concentrations on *S.aureus*. Wahba *et al.,* (2010) clarified the antibacterial parsley extract effect against *S. aureus.* While, Farah *et al.,* (2015) indicated that parsley has more antibacterial effect against *S. aureus* which reached to 18% while dill green extract against *S. aureus*reach to 9%. Dill contains high amount of, flavonoids, Phenolic acids and sterols, (Wallis, 2005). According to (Darughe *et al.* 2012 and Bhat *et al.,* 2014) antioxidant effects of dill extract may referred to terpenoid components (α-pinene, limonene, camphor, and geraniol) which has antioxidant and antibacterial activity specially against *Salmonella* spp. phenolic compounds in herbal extracts has decrease bacterial activity through its effect on bacterial enzymes specially energy producing enzymes which may leading to protein denaturation. Phenolics also can changes the microbial cell permeability which causing the loss of macro compounds of bacterial cell such as ribosome, phenolics can interfere the normal bacterial cell membrane activity such as, (nutrient exchange, protein synthesis, electron transfer, nucleic acids and enzymatic activity (Gutierrez *et al.,* 2008). The antibacterial activity of dill against some food pathogens was clear in our results as following, dill treatment of meat products samples reduced incidence % of *E. coli* from(20 - 5)% , (15 – ND) % & (30 – ND)% in minced meat, burger and kofta samples respectively. Dill treatment leading to complete reduction of *Salmonella spp.* in all meat products samples. Dill treatment reduce *Shigella spp.* incidence % of minced meat samples from (25–ND)% while it leading to complete reduction of the same organism in burger and kofta samples. Parsley was more effective than dill in *E. coli* inhibition % in minced meat, burger &kofta treated samples from (25-7)%, (15 - 0)% & (30-0)% respectively. While, it leading to complete reduction of *Salmonella spp.* in parsley minced meat, burger & kofta samples. Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley minced meat, burger & kofta treated samples from (25, - ND), (28 – ND)% & (25 – ND)% respectively. Dill treatment of minced meat samples had very effective inhibition activity on *Salmonella spp.,*and moderate inhibition effect on (*E. coli* & *Shigella spp.*), parsley was more effective than dill in *E. coli* inhibition % in parsley minced meat treatment samples. While, it recorded the same inhibition % on *Salmonella spp.,* inparsley minced meat samples. Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley minced meat treated samples. Burger dill treated samples had very effective inhibition activity on *Salmonella spp.,* and moderate inhibition effect on (*E. coli* & *Shigella spp.*) its effectiveness increasing with longer storage period, parsley was more effective than dill in *E. coli* inhibition % in parsley burger treated samples. While, it recorded the same inhibition % on *Salmonella spp.,* inparsley burger treated samples. Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley burger treated samples. Dill kofta treated samples was very effective against *Salmonella spp., & Shigellaspp.*and moderate inhibition effect on (*E. coli*) its effectiveness increasing with longer storage period, parsley was more effective than dill in *E. coli*inhibition %. While, it recorded the same inhibition % on *Salmonella spp.,* Parsley recorded lower inhibition % than dill on *Shigella spp.* in parsley kofta treated samples. Similar results have been observed in Şimonaţi and Mihuţa (2009) studied whom assayed the dill inhibitory effect, *E. coli*, *Sal. Typhimurium.* Karimi *et al.,* (2014) tested antimicrobial effect of parsley and observed its effect against, *Staphylococcus aureus*, Salmonella, *E. coli* which were nearly equal to effect of antibiotics currently used against the microorganisms without any side effects. Seyyednejad *et al.,* (2008) reported that parsley extract had antimicrobial effect at different concentration, (0.1 to 0.4 gr.ml) against Gram negative e.g., (*E.coli*) and Gram positive microorganisms, its better effect against *Salmonella typhi* was when used on(0.4gr.ml) concentration. Lower results reported by El Astal *et al.,* (2005) whoshowed that parsley extract hasn't any antimicrobial effect at different concentrations on (*Salmonella typhi&E.coli*). Dostalova *et al.,* (2014) showed that parsley has relatively strong antimicrobial activity against *E. coli*. Gyawali and Salam (2012) stated that parsley extract should be used as food products additives due to its antimicrobial activity against*Salmonella.* Mau *et al.,* (2001) observed that parsley extract has inhibitory effect against *E. coli.* Wahba*et. al.,* (2010) confirmed antibacterial parsley extract effect against coliforms. Delaquis *et al.,* (2002) detected the antibacterial effect of dill extract against some Gram-negative and Gram-positive food pathogens (*Salmonella typhimurium*, *Staphylococcus aureus*). Teshale *et al.,* (2013) determined the dill inhibition zone against *Salmonella typhi* about 18 mm. while Keskin and Toroglu (2011) measured the dill inhibitory zone against *Escherichia coli*. which was about (15 mL/disc). Alsaiqali *et al.,* (2016) examined the parsley extract effect on refrigerated meat. Their results concluded that parsley extract had a strong antimicrobial effect against TBC, *E. coli*, *S. aureus* during the first storage period however this effect reduced by time, they recorded the inhibitory effect on *E. coli* about (1.2 %). Similar results reported by Wong and Kitts (2006) and Dostalova *et al.,* (2014) who noted parsley extracts inhibitory effect against *E. coli.*

In conclusion, obtained results found that dill extract has a strong antimicrobial activity on some meat spoilage and meat pathogens. On the other hand, parsley extract showed mild to moderate effect against most meat spoilage and meat pathogens. We recommended to using dillextract as safe and natural antimicrobial meat additives to prolong shelf life of meat product.

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