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Anti-bacterial effect *of Rosmarinus officinalis Linn.* extract and *Origanum syriacum L.* essential oil on survival and growth of total aerobic bacteria and *Staphylococcus aureus* using cooked chicken meat

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Abstract

The effect of oregano essential oil (OE) and rosemary extract (RE) on the survival and growth of *Staphylococcus aureus*, and the total aerobic bacteria (TA) in cooked ground chicken meat stored at different temperatures had been evaluated. Five treatments including i) Control (no additives); ii) 150 ppm OE; iii) 350 ppm RE; iv) 150 ppm OE + 350 ppm RE; and v) 14 ppm of *butylatedhydroxyanisole* and *butylatedhydroxytolune* mixture (BHA/BHT) were prepared. After cooking, all samples were stored (7 days) at different temperature (10, 25, 43 °C) using oxygen permeable bags and tested for TA, and total viable count of *Staph. a*. In addition, all additives were showed significant ($P < 0.05$) antimicrobial effect during storage time compared to the control. Both OE and RE showed comparable antimicrobial effect compared to the synthetic (BHA/BHT) additive at all storage temperatures. However, the combination treatment (OE + RE) was the highest among other additives suppress bacterial growth (APC & *Staph. a*) during storage. Based on these results, it is concluded that both OE and RE were showed significant ($P < 0.05$) antimicrobial activity, but this effect could be higher if meat treated by their combined mixture.

Keywords: oregano essential oil; rosemary extract; cooked meat; *Staphylococcus aureus*; storage temperatures.

Practical Application: Combined plants extracts can improve both meat safety, and preventing food borne pathogens (FBPs) which may survive in different meat products.

1 Introduction

In developed countries poultry support many poor families through their food and livelihood (Scanes, 2007; Food and Agriculture Organization of the United Nations, 2008, 2020). For those, poultry meats consider one of the cheapest and easily available food protein sources (Food and Agriculture Organization of the United Nations, 2013; Kralik et al., 2017; Marangoni et al., 2015). Therefore, improving preservation technology is required, and highly supports food chain values to achieve better meat quality and safety (Scanes, 2007; Saranraj et al., 2016; Food and Agriculture Organization of the United Nations, 2020). In meat consumption, several food-borne diseases (FBD) may occur and increase the cost of hospitalization, illness, and death even in high income countries such as USA (Centers for Disease Control and Prevention, 2017). The major FBD (over 90% of food related illness) usually are caused by bacteria, as estimated by CDC-USA center recently. The US-CDC was reported two major group of food borne illnesses: 1) Known food-borne pathogens (FBP) (31 pathogens) and 2) Unspecified agents. However, *Staphylococcus aureus* consider one of the most important (top five germs) food-borne diseases which may transfer by meat and their products (Kadariya et al., 2014; American Meat Science Association, 2017). For instance, 241,000 illnesses per year caused by *Staph. aureus* contamination were estimated in the United States, and still consider challenging (Scallan et al., 2011; Al-Hijazeen & Al-Rawashdeh, 2019). Survival of *Staph. aureus* strains supressed by achieving optimum cooking and refrigeration temperatures, advanced packaging technology, good manufacturing practices, and perhaps by adding synthetic additives (Dinges et al., 2000; Kadariya et al., 2014; Saranraj et al., 2016). These synthetic additives extend meat shelf life, and improve their quality and safety (Al-Hijazeen, 2019; Al-Hijazeen & Al-Rawashdeh, 2019). Synthetic antimicrobial/antioxidant additive such as BHA, BHT, and nitrite/nitrate, are widely used in processed meat industry (Sebranek & Bacus, 2007; Ahn et al., 2007; Kumar et al., 2015; Majou & Christieans, 2018). However, these synthetic ingredients are questionable, and adversely may affect human health (Oostindjer et al., 2014; Saeed et al., 2019). Now days, researchers interested more in using natural food additives which consider safer alternative (Kumar et al., 2015; Al-Hijazeen, 2019). These plants essential oils and their varieties extracts are well tested, and documented of their positive antimicrobial effect (Kumar et al., 2015; Hintz et al., 2015; Quinto et al., 2019). For instance, oregano, rosemary, sage, thyme, garlic and many medicinal plants and their extracts showed positive antimicrobial effect in both raw and cooked meat products (Hintz et al., 2015; Al-Hijazeen et al., 2016; Kumar et al., 2015; Al-Hijazeen, 2019; Al-Hijazeen & Al-Rawashdeh, 2019; Quinto et al., 2019). Oregano essential oil (OE) antioxidant/antimicrobial action arising from their polyphenolic constituents: *Carvacrol* and *thymol* (78-82% of its composition), which are responsible on most of their preservation effect (Adam et al., 1998; Yanishlieva et al., 1999). In addition, they are main components of the OE responsible on

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its antimicrobial activity (Ultee et al., 2002; Nostro et al., 2004, 2007). The antimicrobial activity of OE is well documented, and reported as effective natural antimicrobial for several FBP such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Vibrio parahaemolyticus, Listeria monocytogenes*, and *Escherichia coli* (Sivropoulou et al., 1996; Ozcan, 2001; Lin et al., 2004; Al-Hijazeen, 2018). In addition, rosemary is very popular and abundant plant grown in Jordan (Middle East), which reported as a powerful antimicrobial food additives (Alzoubi et al., 2014; Al-Hijazeen, 2018; Al-Hijazeen & Al-Rawashdeh, 2019). The antioxidant/antimicrobial activity of RE linked with their content of phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, ursolic acid, and caffeic acid (Aruoma et al., 1992; Basaga et al., 1997; Alzoubi et al., 2014). However, carnosic acid, carnosol, and rosmarinic acid reported as the main bioactive antimicrobial compounds present in RE (Del Campo et al., 2000; Moreno et al., 2006; Jiang et al., 2011; Issabeagloo et al., 2012). Similar to OE, the antimicrobial mechanism of RE is reviewed and explained as: 1) due to their interaction with bacterial cell membrane, which causing alteration in the calls genetic material and nutrients; 2) changing in electrons transportation; 3) loss of some cellular composition; 4) loss in membrane functionality (Nieto et al., 2018). The *wild* oregano and rosemary plants are widely available in Jordan, and their extraction found in a various concentration (Ibrahim et al., 2012a, b; Al-Hijazeen & Al-Rawashdeh, 2019). *Staphylococcus aureus* strains may cause FBD due to the effect of their heat stable enterotoxins toxin (Kadariya et al., 2014; Ibrahim et al., 2016). It does not compete well in raw meat, but have more ability to grow and survive on cooked meat (Casman et al., 1963; Normanno et al., 2007). In addition, it ranks the most prevalent causing gastroenteritis problems (Dinges et al., 2000; Hejazi, 2013), food contamination during food preparation, can tolerate salts up to 15%, and can survive in a wide range of temperatures (7 to 48.5 °C), pH (4.2 to 9.3), which make *Staphylococcus aureus* a common FBD (Chaibenjawong & Foster, 2011; Kadariya et al., 2014). Storage condition and temperature of cooked meat products also very important to decrease their growth and survival specially by cross contamination (Kadariya et al., 2014; Saranraj et al., 2016). In addition, Total Aerobic bacteria (TA) or aerobic plate count (APCs) also can be used to give general indication about bacterial population on the meat samples. TA count evaluates food microbiological status, manufacture system hygiene, quality and food safety (American Public Health Association, 1984) and estimate meat deterioration level (Kim et al., 2018; Saeed et al., 2019). The effect of direct adding essential oils and plants extract are extensively studied, and still need more investigation for a better applied methodology, dose, and best combination which can be consider by food industry (Kumar et al., 2015; Adelakun et al., 2016; Al-Hijazeen & Al-Rawashdeh, 2019). In addition, no research studies were conducted before to evaluate the combination effect of these plants supplements using cooked chicken meat. Therefore, this research was conducted to investigate: 1) effect of *Rosmarinus officinalis Linn.* extract and *Origanum syriacum L.* essential oil on survival and growth of TA, and *Staphylococcus aureus* using cooked chicken meat patties; 2) Comparing this effect with the synthetic antioxidant BHA/BHT currently uses in the meat industry.

2 Materials and methods

2.1 Cooked patties preparation

Fresh chicken of thigh meat was purchased from a local market and ground twice a through a 8-mm plate then a 3-mm plate (*Moulinex*, Type *DKA1*, France). Five treatments were prepared into: 1) Control (no additive); 2) 150 ppm OE; 3) 350 ppm RE; 4) Combination (CM) of 150 ppm OE + 350 ppm RE; and 5) 14 ppm combination (50% each) of *butylatedhydroxyanisole/ butylatedhydroxytolune* (0.02% of BHA/BHT according to their fat content). Oregano essential oil (OE) and RE dose were selected according to previous meat quality studies which was conducted to evaluate antioxidant effect of plants extracts (Al-Hijazeen & Al-Rawashdeh, 2019; Al-Hijazeen, 2019). Oregano essential oil (*Origanum syriacum L.*) was obtained from a certified company in Jordan (*Green Fields Factory for oils*, Amman/ Jordan) using the most efficient purification, extraction, and steam distillation methods. The additives (OE & RE) composition analysis was done in the Royal Scientific Society (RSS) research institution in Amman, Jordan. The HPLC analysis of the OE showed that 76.39% of the essential oil was *carvacrol*. Rosemary pure extract (Cultivated in Jordan) was obtained from the previous company, and the HPLC analysis of the RE was measured in RSS (Method of Okamura et al., 1994), and it was containing 26 ± 3% as the average of phenolic diterpenes (4% carnosol and 6% carnosic acid and other phenolic compounds). The BHA/BHT powder, RE, and OE were dissolved in 10 mL of 100% ethanol, and then mixed with 50 mL mineral oil (Sant Cruz Biotechnology, Dallas, TX, USA), to prepare their stock working carrier. The ethanol was split out using a rotary evaporator (Heidolph, Model Laborota 4001-effecient) at (70 °C, 175 mbar vacuum pressure) before adding the stock to the meat mixture. Each treatment supplement was added to the ground meat, and mixed for 3 min in a bowl mixer. All treatments were added with the same quantity of mineral oil (carrier) to maintain the same condition. Chicken meat patties (*approx.* 100 g each) were individually packaged in separate oxygen-impermeable vacuum bags (Albalabki-Jordan, Malcom SRL, Italy), and were cooked gradually to a maximum temperature 90 °C using water bath (WNB 14, Memmert, Germany) until the internal temperature of meat samples reach 75°C. The current research was performed in the Microbiology Laboratory of Agriculture Collage, at Mutah University. Cooked meat samples were placed in a closed cold condition (4.5 °C) and used in experiments within 1-3 hours. All meat packages were removed and replaced by oxygen permeable bags for the inoculation part.

2.2 Proximate composition, cooking loss percentage, and ultimate pH.

Proximate analysis

Chicken thigh meat samples of the five batches were individually sampled to measure their protein, fat, water and ash percentage according to AOAC standard methods (Association of Official Analytical Chemists, 2000).

Cooking loss percentage

Ground chicken fresh meat samples (25 g) were prepared as the method described by Al-Hijazeen (2019).

Ultimate pH values of raw thigh meat

The pH value of raw ground meat samples were determined using a pH meter (PL-600, pH/mV/Temp Meter, Taiwan) (before cooking) after homogenizing the 1.0-g samples with 9 mL de-ionized distilled water (DDW) (Sebranek et al., 2001).

2.3 Staphylococcus aureus culture preparation

Four-strains of *Staphylococcus aureus* were obtained from the culture collection of the Microbial Food Safety Laboratory at Mutah University, and JFDA (Jordan Food and Drug Administration) laboratory. Frozen stocks (in 10% glycerol at -80 °C) were thawed and cultured separately in tryptic soy broth (TSB; Biolab Zrt. 1141 BUDAPEST Öv u. 43, Hungary) supplemented with 0.6% yeast extract (TSBYE) at 35 °C for 24h. Two consecutive 24-h transfers of the each culture were prepared in TSBYE (35 °C) to make our working cultures. Before the inoculation part each working culture was individually grown in 10 mL of TSB supplemented with 0.6% yeast extract (TSBYE-Biolab Zrt. 1141 BUDAPEST Öv u. 43, Hungary) at 35 °C for 24h. These strains were adapted gradually in the TSBYE with different concentrations of added Naldixic acid (NA; antibiotic; M.W 232.24, 10 ug/mL, 30 ug/mL, and 50 ug/mL; Santa Cruz Biotechnology, Dallas, TX, USA). Two consecutive transfers 24-h for each Naldixic Acid Resistant strain (NAR) cultures were prepared. In addition, when starting, 6 mL of each individual NAR adapted culture in TSBYE was aseptically transferred to a sterilized centrifuge tube to give a total 24 mL of strain mixture culture (NARC).

Bacterial cells from current mixture strains were harvested by centrifugation at 10,000 x g for 10 min at 4 °C (Medical Centrifuge, TG16G; Hunan Kaida Scientefic Instruments Co., Ltd; China). The pelleted cells were re-suspended in a 24-mL sterilized saline (0.85% NaCl), washed, vortex, and then centrifuged again at the same speed and temperature conditions. After harvesting the cells from the second centrifugation, the collected cells were suspended in fresh saline (0.85% w/v NaCl) and diluted (10-fold) using tubes of saline to obtain 10^6 CFU/mL in a suspension of washed cells used for inoculating the ground chicken meat.

2.4 Preparation and inoculation of meat samples

Cooked meat samples were prepared (Meat Laboratory-Department of Animal Production) and transported to the Microbial Food Safety Laboratory (Dept. of Nutrition and Food Technology) for inoculation and microbial analysis. Meat patties were aseptically transferred to oxygen permeable bags before the inoculation. Each cooked meat patties was inoculated with a mixture of four nalidixic-acid resistant serotypes of *Staphylococcus aureus* (ATCC 6538, ATCC 25923, ATCC 10390, and ATCC 29213) to give an initial cell concentration of ~10^4 colony forming units (CFU)/g. All inoculated packages of ground chicken meat were closed, manually smashed and massaged for 40 s from outside of the bag, and held at different storage times and temperature. At different storage time, samples of cooked meat were analyzed for *Staphylococcus aureus* survivors. In addition, separate bags of non-inoculated ground cooked meat were stored at 10, 25 and 43 °C and analyzed for aerobic plate counts.

2.5 Microbial analysis

Ground chicken patties were aseptically opened from their packages, and two-25 g portions of meat were transferred into separate sterile filter-lined stomacher bags (Seward^{Tm}). Sterile 0.1% (w/v) peptone water (225 mL) was added to each bag. The meat mixture was homogenized (60 second) in a laboratory stomacher blender (Easy Mix, AESAP1068; 35172 BRUZ, France) programmed at medium speed. After that, serial dilutions of meat samples (10-fold) were prepared in sterilized tubes of 0.1% peptone and 0.1 mL aliquots of diluted samples were surface plated on appropriate selective agar media to enumerate pathogenic bacteria.

Baird-Parker Agar base were used to enumerate the facultative anaerobic gram positive *Staphylococcus aureus*. Inoculated agar plates were incubated at 35 °C and bacterial colonies total count were measured after 48 hours. The aerobic plate count (APC) was determined by surface plating aliquots (0.1 mL) of meat homogenate on tryptic soy agar (TSA), incubating the inoculated TSA plates at 35 °C, and counting bacterial colonies at 48 hours.

2.6 Statistical analysis

Completely randomized design (CRD) was conducted in this study. Two separate samples per treatment per replication were analyzed over 2 replications of each independent experiment. The statistical analysis was performed using the procedures of generalized linear model (Proc. GLM, SAS program, version 9.3, 2012). Mean values and standard error of the means (SEM) were reported. The significance was defined at $P < 0.05$ and *Tukey* test or *Tukey's Multiple Range* test were used to determine whether there are significant differences between the mean values.

3 Results and discussion

In term of microbiology basis meat composition should be well known when studying the treatments inhibitory effect. In current study, there were no significant differences ($P > 0.05$) appeared among all treated meat batches of their fat, protein, water, and ash mean percentage (Table 1). These results are agreed with Keokamnerd et al. (2008) and Holland et al. (1991) of their proximate analysis using chicken thigh meat. In addition, all treatments samples showed similar cooking loss % values, and no significant differences ($P > 0.05$) among them were found when measuring their ultimate pH values. These data also confirm the univariate, and meat homogenizing starting point (initial count) which makes current study suitable to investigate all supplements antibacterial effect. In present study, storage temperatures of 10, 25, and 43 °C were all used to represent abnormal conditions which allowing aerobic bacteria and *Staphylococcus aureus* to grow in a poor refrigeration unit (10 °C), during transportation and handling of cooked meat products at ambient temperature (25 °C), and during unsafe (43 °C) hot-holding temperature that may often occur in foodservice area.

Among all treatments samples the APC was increased significantly ($P < 0.05$) during storage time (Table 2). At 10 °C, all treatments additives were showed significant ($P < 0.05$) inhibitory (APC) effect after day one of storage time compared to the control samples. Oregano essential oil (OE) was showed

Table 1. Cooked loss %, pA[1], and U- pH[2] values of chicken thigh of all treatment samples.

TRT*	Proximate Analysis				Ash%	pH Value
	Cooking Loss %	Fat %	Protein %	Water %		
Control	0.171[a]	6.87[a]	18.74[a]	73.43[a]	0.96[a]	6.17[a]
OE	0.178[a]	6.85[a]	18.45[a]	73.72[a]	0.97[a]	6.16[a]
RE	0.176[a]	6.87[a]	18.45[a]	73.74[a]	0.94[a]	6.11[a]
CM	0.177[a]	6.71[a]	18.60[a]	73.73[a]	0.96[a]	6.16[a]
BHA/BHT	0.173[a]	6.94[a]	18.48[a]	73.61[a]	0.97[a]	6.14[a]
SEM	0.011	0.073	0.078	0.085	0.04	0.065

[a-c]Different superscripts within a column differ significantly ($p < 0.05$). Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; 4) Combination of T2 & T3; 5) 14 ppm of BHA/BHT. N=4. [1]pA: Proximate analysis of fresh meat before cooking; [2]U-pH: pH values after 24h postmortem of the chicken thigh meat; *Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of T2 & T3: T5) 14 ppm BHA/BHT.

Table 2. Aerobic plate count of cooked chicken (thigh) meat patties during storage at 10 °C.

Treatments	Storage (days)				SEM
	0 day	1 day	3 days	5 days	
	---------- Log CFU/g meat ----------				
Control	2.32[az]	4.34[ay]	7.51[ax]	8.44[aw]	0.05
OE	2.31[az]	4.02[by]	5.74[bx]	6.37[bcw]	0.10
RE	2.20[az]	4.2[aby]	5.99[bx]	6.62[bw]	0.09
CM	2.11[az]	3.77[cy]	5.55[bx]	6.02[cw]	0.09
BHA/BHT	2.22[az]	4.08[by]	5.66[bx]	6.20[cw]	0.07
SEM	0.09	0.05	0.1	0.08	

[a-c]Different superscripts within a column differ significantly ($p < 0.05$). [w-z]Different superscripts within a row differ significantly ($p < 0.05$). n=4. Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of T2 & T3: T5) 14 ppm BHA/BHT.

higher antibacterial effect (APC test) compared to the RE, but this was not significant ($P > 0.05$) during storage time. However, the combination of OE & RE was showed the highest anti-bacterial effect compared to the other treatments additives. Furthermore, no significant differences ($P > 0.05$) appeared between OE, BHA/BHT, and combination treatments at day 5 of their APC at 10 °C. In addition, no synergistic effect was found when adding both OE & RE in combination (Table 2). Generally, bacteria grow faster in all treatments samples during storage when the meat affected by a higher temperature (Table 3). The control treatment samples were showed the highest total bacterial count at both storage temperatures of 25 and 43 °C.

In addition, the total number of bacteria (CFU/g meat) was increased significantly ($P < 0.05$) during the set interval storage time using both temperatures. There were no significant differences ($P > 0.05$) among all treatments samples at day 0 for all experimental study (Tables 2 and 3). The antibacterial effect of both OE and RE was very effective during storage time; however their effectiveness was depending on meat storage temperature. For instance, OE showed better antimicrobial effect at both 10 and 25 °C storage temperatures. On the other hand, RE antibacterial effect was significantly ($P < 0.05$) higher using 43 °C storage temperature during storage time (2-8 hr). It is not clear why RE showed better effect on a higher temperature, but this could be due to their chemical composition, and how these consistent affected and react to each other. Similar results were found in different meat study evaluating the antibacterial effect of OE & RE either in combination or separately (Ntzimani et al., 2011; Zhang et al., 2016; Nieto et al., 2018; Hać-Szymańczuk et al., 2019). The highest significant antibacterial effect was showed by the combination treatment (OE & RE) especially appeared after 6 hr of storage time using both storage temperatures. However, the antibacterial effect of the synthetic BHA/BHT was comparable to the OE and RE when it added separately. Furthermore, no synergistic effect was found by the CM treatment, but both RE and OE showed higher effect compared to their separate effect.

There were no significant differences ($P > 0.05$) of total viable number regarding *Staph .a* grown on cooked chicken meat among all treatments additives at day 0 of storage time. However, all treatments additives suppress this pathogen mixture strain (NARC) growth significantly ($P < 0.05$) compared to the control samples at 10 °C during storage. In addition, there were no significant differences ($P > 0.05$) between RE and OE treatments of their anti-*Staph .a* effect until day 5. However, the OE showed higher significant ($P < 0.05$) anti-*Staph* activity (7.17 Log CFU/g meat) compared to the RE (7.39 Log CFU/g meat) at day 7 of storage time using temperature of 10 °C (Table 4). In addition, the combination treatment were showed the highest significant ($P < 0.05$) effect suppress the *Staph. a* survival and growth compared to the other additives during the storage period (day 5 - day 7). However, the anti-*Staph .a* effect of synthetic BHA/BHT was similar and comparable to the effect of OE and RE when it was adding separately. Researchers reported that adding combinations of natural antimicrobial plant extracts may increase their effectiveness (synergistic effects) (Pol & Smid, 1999; Lin et al., 2004; Brewer., 2011; Abd-Kalek & Mohamed, 2012). However, in current study the combination of RE and OE dose not expose any synergistic effect against *Staph. a* and

Table 3. Aerobic plate counts of cooked meat patties held at 25 or 43 °C during storage.

Treatments	Storage (hrs)					
	0 hr	2 hr	4 hr	6 hr	8 hr	SEM
	---------- Log CFU/g meat ----------					
Storage at 25 °C						
Control	2.33^{az}	3.62^{ay}	4.86^{ax}	5.60^{aw}	7.21^{av}	0.04
OE	2.32^{az}	3.39^{by}	4.34^{bx}	5.05^{bcw}	6.51^{cv}	0.04
RE	2.33^{az}	3.40^{by}	4.45^{bx}	5.35^{abw}	6.75^{bv}	0.06
CM	2.31^{az}	2.72^{cy}	3.63^{cx}	4.22^{dw}	5.21^{ev}	0.07
BHA/BHT	2.27^{az}	2.80^{cy}	3.85^{cx}	4.81^{cw}	5.71^{dv}	0.05
SEM	0.03	0.04	0.04	0.09	0.04	
Storage at 43 °C						
Control	2.27^{az}	3.88^{ay}	5.11^{ax}	6.16^{aw}	8.32^{av}	0.04
OE	2.25^{az}	3.69^{by}	4.85^{bx}	5.82^{bw}	7.79^{bv}	0.03
RE	2.30^{az}	2.89^{cy}	3.67^{dx}	4.65^{cw}	6.67^{cv}	0.04
CM	2.20^{az}	2.68^{cy}	3.48^{ex}	4.31^{dw}	6.46^{dv}	0.02
BHA/BHT	2.20^{az}	2.86^{cy}	3.79^{cx}	4.67^{cw}	6.75^{cv}	0.03
SEM	0.04	0.04	0.02	0.02	0.03	

$^{a-e}$Different superscripts within a column differ significantly ($p < 0.05$). $^{x-z}$Different superscripts within a row differ significantly ($p < 0.05$). n = 4. Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of T2 & T3: T5) 14 ppm BHA/BHT.

Table 4. Numbers of viable *Staphylococcus aureus* of cooked chicken meat patties during storage at 10 °C.

Treatments	Storage (days)					
	0 day	1 day	3 days	5 days	7 days	SEM
	---------- Log CFU/g meat ----------					
Control	4.22^{az}	5.11^{ay}	6.58^{ax}	7.33^{aw}	8.18^{av}	0.035
OE	4.17^{az}	4.60^{bcy}	5.50^{bx}	6.47^{bcw}	7.17^{cv}	0.037
RE	4.21^{az}	4.63^{by}	5.59^{bx}	6.65^{bw}	7.39^{bv}	0.038
CM	4.11^{ay}	4.22^{dy}	5.18^{cx}	5.88^{dw}	6.50^{dv}	0.053
BHA/BHT	4.18^{az}	4.48^{cy}	5.26^{cx}	6.23^{cw}	7.02^{cv}	0.062
SEM	0.06	0.03	0.02	0.06	0.05	

$^{a-e}$Different superscripts within a column differ significantly ($P < 0.05$). $^{w-z}$Different superscripts within a row differ significantly ($P < 0.05$). n = 4. Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of T2 & T3: T5) 14 ppm BHA/BHT.

total aerobic bacteria during storage time. Several research studies were reported positive antimicrobial effect, and extend meat shelf-life by using OE in both raw (Al-Hijazeen, 2014; Khaled et al., 2016; Quinto et al., 2019) and cooked/ready to eat meat products (Yemiş & Candogan, 2017; Ulusoy et al., 2018) against different bacterial strains. For example, the antimicrobial effect of OE combined with other natural additives against the growth of *E. coli* O157:H7; *Listeria monocytogenes; Salmonella Enteritidis, and Staphylococcus aureus* was also reported (Yemiş & Candogan, 2017; Boskovic et al., 2015; Man et al., 2019). The antimicrobial effect of OE (*Origanum syriacum L.*) is based on their high carvacrol and other polyphenolic content (Xu et al., 2008; Masadeh et al., 2013; Alhijazeen, 2019). Other researchers (Ultee et al., 1998, 2002; Di Pasqua et al., 2010) reported that OE and its major component carvacrol changed of bacterial cell membrane potential, and their biological activities inside the cell. The antimicrobial effect of OE (Chouliara et al., 2007; Zhang et al., 2016; Yemiş & Candogan, 2017) and RE (Kahraman et al., 2015; Al-Hijazeen & Al-Rawashdeh, 2019) through meat system against several FBP are well documented. In addition, RE and it's essential oil have antimicrobial activity due to their polyphenolic composition (Azizkhani & Tooryan, 2015; Zhang et al., 2016). The antimicrobial action of RE explained by its phenolic constituents such as rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol as reviewed by Nieto et al. (2018). This positive antimicrobial effect found in current study were connected to the improvement in the meat quality, sensory attributes, color stability reported in previous research studies (Part-I) (Al-Hijazeen & Al-Rawashdeh, 2019; Al-Hijazeen, 2019). In addition, the antimicrobial effect of this combination (OE & RE) against survival and growth of *Salmonella enterica, Listeria monocytogenes*, *Escherichia coli* O157:H7, *Enterobacteriaceae* (ENT), and the aerobic plate count (APC) in raw ground chicken meat stored at different temperatures were reported by Al-Hijazeen (2018).

All cooked meat samples were having same initial number (CFU/g meat) of *Staph. a* count after the inoculation step at 0 hr of storage time (Table 5). This will confirm the univariate concept of having the same total viable number of the *Staph. a* at the beginning of this study. *Staph. a* colonies was increased dramatically, and very fast compared to the previous experiments which were used lower storage temperatures. For both storage temperatures (25 and 43 °C) OE was showed higher anti-*Staph* effect compared to the RE during storage time. The effect of all

Table 5. Numbers of viable *Staphylococcus aureus* in cooked ground meat patties held at 25 or 43 °C.

Treatments	Storage (hrs)					
	0 hr	2 hr	4 hr	6 hr	8 hr	SEM
	---------- Log CFU/g meat ----------					
Storage at 25 °C						
Control	4.22^{az}	4.76^{ay}	5.45^{ax}	5.81^{aw}	6.59^{av}	0.029
OE	4.22^{az}	4.52^{cy}	4.82^{bx}	5.26^{cw}	5.63^{bv}	0.022
RE	4.29^{az}	4.61^{by}	4.97^{bx}	5.45^{bw}	5.70^{bv}	0.051
CM	4.19^{az}	4.39^{dy}	4.56^{cx}	4.88^{dw}	5.20^{dv}	0.038
BHA/BHT	4.24^{az}	4.44^{cdy}	4.63^{cx}	5.01^{dw}	5.43^{cv}	0.028
SEM	0.045	0.021	0.036	0.039	0.029	
Storage at 43 °C						
Control	4.24^{az}	5.44^{ay}	6.62^{ax}	7.74^{aw}	8.55^{av}	0.036
OE	4.21^{az}	4.71^{bcy}	5.73^{cx}	6.77^{bw}	7.34^{bv}	0.028
RE	4.19^{az}	4.82^{by}	5.88^{bx}	6.88^{bw}	7.53^{bv}	0.035
CM	4.21^{ay}	4.55^{dy}	5.29^{ex}	6.22^{dw}	7.16^{bv}	0.110
BHA/BHT	4.23^{az}	4.64^{cdy}	5.43^{dx}	6.47^{cw}	7.20^{bv}	0.023
SEM	0.051	0.026	0.024	0.033	0.11	

$^{a-e}$Different superscripts within a column are differ significantly ($p < 0.05$). $^{x-z}$Different superscripts within a row are differ significantly ($p < 0.05$). n = 4. Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of T2 & T3: T5) 14 ppm BHA/BHT.

treatments additives was very comparable, especially at 43 °C to each other. However, the CM treatment was always had the lower viable numbers compared to the other treatments additives for both storage temperatures (25 and 43 °C). The effect of RE and OE was higher than using them individually during storage time (Table 5). No synergistic effect was found in current study between both OE and RE. This possible synergistic activity between the RE and other natural antioxidants may not achieve in some cases (Nieto et al., 2018).

The inhibitory effect of RE incorporated into different meat system against *E. coli, Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Bacillus cereus, Brochothrix thermosph -acta, and Enterobacteriaceae* is well documented (Pandit & Shelef, 1994; Burt, 2004; Ahn et al., 2007; Sirocchi et al., 2013). In addition, OE and their extract inhibitory effect was already discussed, and their antibacterial effect against several FBP through varieties of meat mixture preparation is also reported (Chouliara et al., 2007; Boskovic et al., 2015; Khaled et al., 2016; Man et al., 2019). Similar results were found by Al-Hijazeen (2014) who reported that OE (*Origanuim vulgare subsp. hirtum*) at level 200 ppm showed a potential antimicrobial activity in both raw and cooked chicken meat. Finally, in order to achieve this effect in the practical meat industry these findings still need more investigation. For instance, method of adding, dose, products composition, carrier type, and their interaction with meat mixture of each individual product always needs evaluation.

4 Conclusion

All additives were showed significant ($P < 0.05$) antimicrobial activity during storage time against both *Staph. a* and aerobic bacteria. Both OE (150 ppm) & RE (350 ppm) were suppress bacterial growth and survival at all storage temperatures (10, 25, 43 °C). However, it is clearly appeared that combination (OE + RE) treatment effect is better than using them alone. Overall, their antibacterial activities may consider effective as the synthetic antimicrobial (BHA/BHT); however no synergistic effect appeared by using them in combination. The combination of these two plants extract gives more hope to use them in the future of processed meat, and their products. Finally, it is still need more investigation to use this substation in the meat industry such as mixing method, level, and effectiveness after processing, their effect on other food-brone pathogens.

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